

**(12) SOLICITUD INTERNACIONAL PUBLICADA EN VIRTUD DEL TRATADO DE COOPERACIÓN
EN MATERIA DE PATENTES (PCT)**

**(19) Organización Mundial de la Propiedad
Intelectual**
Oficina internacional



PCT

**(43) Fecha de publicación internacional
22 de Mayo de 2009 (22.05.2009)**

**(10) Número de Publicación Internacional
WO 2009/063111 A1**

**(51) Clasificación Internacional de Patentes:
C12Q 1/68 (2006.01)**

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(21) Número de la solicitud internacional:

PCT/ES2008/000707

(22) Fecha de presentación internacional:

14 de Noviembre de 2008 (14.11.2008)

(25) Idioma de presentación:

español

(26) Idioma de publicación:

español

(30) Datos relativos a la prioridad:

P200703071

14 de Noviembre de 2007 (14.11.2007) ES

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(81) Estados designados (a menos que se indique otra cosa, para toda clase de protección nacional admisible): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(84) Estados designados (a menos que se indique otra cosa, para toda clase de protección regional admisible): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Publicada:

- con informe de búsqueda internacional
- antes de la expiración del plazo para modificar las reivindicaciones y para ser republicada si se reciben modificaciones
- con la parte de lista de secuencias de la descripción publicada separadamente en forma electrónica y disponible por medio de la Oficina Internacional previa petición

(54) Title: METHOD AND KIT FOR PREDICTING THE CHANCES OF SURVIVAL OF PATENTS WITH MANTLE CELL LYMPHOMA

(54) Título: MÉTODO Y KIT PARA PREDECIR LA SUPERVIVENCIA DE PACIENTES CON LINFOMA DE CÉLULAS DEL MANTO

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WO 2009/063111 A1

(57) Abstract: Mantle cell lymphoma (MCL) is a distinct subtype of B-cell non-Hodgkin's lymphoma (NHL-B). The invention provides a method and a kit for predicting the chance of survival of a patient suffering from MCL, based on measuring the level of expression of at least four genes selected from the group comprising: MYC, RAN, POLE2, SLC29A2 and TNFRSF10B in a sample taken from the patient. The method and kit are simple as they involve a minimum number of genes, are quantitative, can be used in routine diagnosis on a wide variety of samples, and offer a greater capacity for predicting the chances of survival of patients with MCL than current methods.

(57) Resumen: El linfoma de células del manto (LCM) es un subtipo diferenciado de linfoma no-Hodgkin de células B (LNH-B). La invención proporciona un método y un kit para la predicción de la supervivencia de un paciente que sufre un LCM, basado en la medida del nivel de expresión de al menos cuatro genes seleccionados del grupo que consiste en: MYC, RAN, POLE2, SLC29A2 y TNFRSF10B en la muestra del paciente. El método y kit son simples por implicar un número mínimo de genes, cuantitativos, aplicables en el diagnóstico de rutina a una gran variedad de muestras, y proporcionan una capacidad de predicción de la supervivencia de los pacientes con LCM más elevada que los métodos actuales.

Método y kit para predecir la supervivencia de pacientes con linfoma de células del manto

La presente invención se relaciona con métodos de diagnóstico y pronóstico de tumores malignos, en particular, linfomas malignos, y más particularmente con métodos de diagnóstico y pronóstico del linfoma de células del manto.

ESTADO DE LA TÉCNICA ANTERIOR

El linfoma de células del manto (LCM) es un subtipo diferenciado de linfoma no-Hodgkin de células B (LNH-B) caracterizado por la translocación t(11;14)(q13;32) y la sobreexpresión de la Ciclina D1. El curso clínico de estos enfermos puede ser muy variable. Generalmente, el LCM muestra un comportamiento clínico agresivo con poca respuesta a los tratamientos existentes en la actualidad, y la supervivencia media de los pacientes de LCM después del diagnóstico se sitúa entre los 3 y 5 años. De todos modos, algunos pacientes mueren por la enfermedad en menos de 6 meses, mientras que otros sobreviven más de 10 años. Las aproximaciones terapéuticas actuales para los pacientes de LCM son bastante uniformes y raramente reflejan este comportamiento clínico ampliamente variable.

En los últimos años, se han llevado a cabo muchos intentos para identificar marcadores clínicos, histológicos y moleculares que permitan estratificar los pacientes de LCM en diferentes grupos de riesgo. Morfológicamente, se han identificado dos variantes de LCM, llamadas variante típica o clásica y blastoide. Los casos de LCM blastoides muestran un comportamiento clínico peor. Genéticamente, las alteraciones que afectan la maquinaria del ciclo celular y las vías de respuesta al daño del ADN parecen ser características del LCM. Las alteraciones genéticas secundarias en LCM afectan frecuentemente reguladores clave de estas vías y también se asocian con un comportamiento clínico inferior.

El predictor más fuerte de la supervivencia en los pacientes con LCM se identificó mediante el análisis de los perfiles de expresión génica de una serie grande de pacientes con LCM (Rosenwald A. et al., "The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma", Cancer Cell, 2003, vol. 3, p. 185-

197). En particular, se construyó un predictor molecular basado en la expresión génica de 20 genes asociados con la proliferación que permitió la definición de subgrupos pronóstico de pacientes con LCM en que la supervivencia media difería en cerca de 6 años. Este predictor de la
5 supervivencia demostró ser superior a otros marcadores moleculares pronóstico tanto solos como en combinación y por lo tanto se vio como un integrador de múltiples eventos oncogénicos (Rosenwald et al., arriba). La plataforma técnica basada en microarrays actualmente no es factible para su aplicación en la rutina clínica.

10

El marcador indirecto potencial Ki-67 medido por inmunohistoquímica ha atraído mucha atención recientemente. En diversos estudios, índice elevados de Ki-67 en células tumorales de LCM se relacionaron con menor supervivencia de los pacientes y la medida de la proliferación de las células
15 tumorales a través de Ki-67 es ahora parte del procedimiento de rutina diagnóstica en muchos centros de linfoma. De todos modos, las técnicas inmunohistoquímicas son sólo semicuantitativas y, en un reciente estudio multicéntrico de inmunohistoquímica, la concordancia entre los procedimientos de tinción y valoración de Ki-67 en diversos laboratorios con
20 experiencia fue menos que satisfactoria.

EXPLICACIÓN DE LA INVENCIÓN

Los presentes inventores han desarrollado un modelo preciso y cuantitativo de la supervivencia de los pacientes de LCM basado en la expresión génica que implica un mínimo número de genes y es aplicable en la rutina diagnóstica tanto en tejidos frescos congelados como en tejidos fijados en formol e incluidos en parafina (FFIP) de pacientes con LCM.

30

Así, en un aspecto la presente invención proporciona un método de predicción de la supervivencia de un paciente que sufre un linfoma de células del manto, comprendiendo los pasos de (a) obtener una muestra de dicho paciente; (b) medir el nivel de expresión de al menos cuatro genes seleccionados del grupo que consiste en: *MYC*, *RAN*, *POLE2*, *SLC29A2* y
35 *TNFRSF10B* en dicha muestra; y (c) comparar el nivel de expresión de cada uno de los genes medidos con la expresión de uno o más genes control endógenos. El test de la presente invención permite una predicción

cuantitativa del curso clínico de los pacientes de LCM en el momento del diagnóstico, basado en las medidas de expresión génica de al menos cuatro de los cinco genes referenciados más arriba. Ventajosamente el test de la invención es aplicable en la mayoría de casos de LCM con tejido congelado 5 y/o FFIP disponible. La alta expresión de RAN, MYC, POLE2 y SLC29A2 se relaciona con un peor pronóstico, mientras que la expresión incrementada de. El test de la presente invención permite una predicción cuantitativa del curso clínico de los pacientes de LCM en el momento del diagnóstico, basado en 10 las medidas de expresión génica de al menos cuatro de los cinco genes referenciados más arriba. Ventajosamente el test de la invención es aplicable en la mayoría de casos de LCM con tejido congelado y/o FFIP disponible. La alta expresión de RAN; MYC, POLE2 y SLC29A2 se relaciona con un peor pronóstico, mientras que la expresión incrementada de TNFRSF10B 15 correlaciona con un curso clínico más favorable.

Por otro lado, como se ha mencionado arriba el índice Ki-67 es capaz de definir grupos de riesgo en muestras FFIP de pacientes con LCM. La evaluación del índice Ki-67 se basa en observaciones microscópicas individuales hechas por un patólogo. La mayoría de las series publicadas 20 representan investigaciones llevadas a cabo en una única institución. Por ejemplo, en un reciente estudio multicéntrico de inmunohistoquímica, se encontró que la variación entre laboratorios en los procedimientos de tinción y valoración de Ki-67 era sorprendentemente alta.

El test de la presente invención proporciona una medida cuantitativa de los niveles de expresión de genes asociados a la proliferación mejorando la capacidad predictiva de las técnicas inmunohistoquímicas existentes que en su mayoría son semicuantitativas (Rosenwald et al., arriba). Como se ilustra más abajo, el test parece superior, ya que discrimina mejor la supervivencia 25 de los pacientes, y es más objetivo que la medida inmunohistoquímica del índice Ki-67, siendo posible su aplicación usando una plataforma técnica automatizada. Mediante la integración de esta aproximación en estudios futuros de LCM, parece posible la realización de un paso adelante hacia una terapia de LCM adaptada al riesgo a sufrir la enfermedad. Esto es de gran 30 importancia, ya que con el método de acuerdo con la presente invención es posible determinar cómo de agresivo es un linfoma y, por lo tanto, es posible establecer el tratamiento apropiado para el paciente. Por lo tanto, con el 35

método de invención, se pueden probar en procesos clínicos prospectivos estrategias más individualizadas y adaptadas al riesgo.

En una realización del primer aspecto de la invención el tejido de la muestra
5 es fresco, congelado o fijado en formol e incluido en parafina.

Las técnicas inmunohistoquímicas conocidas necesitan que la muestra esté en fresco. Los ácidos nucleicos del material FFIP están frecuentemente degradados debido al proceso de fijación con formol. Además, la fijación
10 química modifica la estructura del ARN en tejidos tumorales impidiendo el análisis subsiguiente por técnicas moleculares como la RT-PCR cuantitativa. Esto es debido a que estas técnicas se basan en ciertos genes (genes marcadores) que se pueden romper en fragmentos si la muestra se procesa (es decir, congelada, fijada en formol o incluida en parafina). Si los genes
15 marcadores se rompen en fragmentos, la información obtenida de estos tests no refleja la situación real del paciente. Esto significa que cuando el médico tiene que determinar la supervivencia del paciente, el tiempo pasado entre la extracción de la muestra (tejido) y el análisis tiene que ser cuanto más corto mejor para evitar la fragmentación de los genes marcadores presentes en la
20 muestra del paciente y, por tanto, para evitar la pérdida de información que se necesita para hacer la estimación real del tiempo de supervivencia del paciente.

Sorprendentemente, el test de la presente invención puede llevarse a cabo
25 usando muestras de los pacientes procesadas. Esto se debe al hecho que los amplicones diseñados para los fragmentos de los genes que componen el test de la invención no se encuentran afectados cuando se someten a técnicas de conservación como la congelación, la fijación en formol e inclusión en parafina, entre otras. Esto es muy importante ya que la información proporcionada por el test de la invención (por la medida
30 cuantitativa de los niveles de expresión génica de al menos cuatro de los genes mencionados más arriba) refleja con gran precisión la situación del paciente. En los experimentos incluidos más abajo, el éxito del análisis de qRT-PCR de ARNm derivado de tejidos FFIP dependió en gran parte de los
35 tamaños pequeños de los amplicones (óptimamente hasta 80 pb). Usando ensayos optimizados de qRT-PCR fue posible amplificar ARN de cerca del 75% de las muestras de archivo examinadas. Más importante, el porcentaje

de éxito entre los casos más recientes recogidos como parte de estudios clínicos prospectivos llegó al 86% que está en el rango de las tinciones de Ki-67 analizables en un estudio reciente.

- 5 En otra realización del primer aspecto de la invención, se mide el nivel de expresión de cuatro genes seleccionados del grupo que consiste en: *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B*. Preferiblemente, se mide el nivel de expresión de *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B*.
- 10 *RAN* es una pequeña proteína de unión a GTP que pertenece a la superfamilia de RAS y se requiere para la translocación del ARN y proteínas a través del complejo del poro nuclear, así como para el control de la síntesis del ADN y la progresión del ciclo celular. La secuencia del gen se encuentra disponible en la base de datos Genbank con el número de referencia
- 15 NC_000012.10.

MYC es un factor de transcripción que juega un papel clave en la progresión del ciclo celular y la apoptosis. La desregulación de *MYC* por mutación, sobreexpresión o reordenamientos cromosómicos son eventos comunes en

- 20 malignidades hematológicas. La secuencia del gen se encuentra disponible en la base de datos Genbank con el número de referencia NC_000008.9.

TNFRSF10B es un miembro de la superfamilia de receptores TNF que media una señal apoptótica mediante su activación por la citoquina TRAIL. La

- 25 secuencia del gen se encuentra disponible en la base de datos Genbank con el número de referencia NT_023666.17.

POLE2 es la subunidad epsilon B de la ADN polimerasa y, por consiguiente, está implicada en la replicación del ADN, su reparo y recombinación y las

- 30 funciones de control del ciclo celular. La secuencia del gen se encuentra disponible en la base de datos Genbank con el número de referencia NC_000014.7.

SLC29A2 es un miembro de la familia SLC29 de transportadores de

- 35 nucleósidos que transportan un rango diverso de nucleósidos de la purina y la pirimidina. La secuencia del gen se encuentra disponible en la base de datos Genbank con el número de referencia NC_000011.8.

En una realización del primer aspecto de la invención, los niveles de expresión génica se miden por la reacción en cadena de la polimerasa cuantitativa a tiempo real.

5

En otra realización del primer aspecto de la invención, el tamaño del amplicón para cada uno de los genes *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B* es igual o menor que 80 pares de bases.

- 10 Los genes control endógenos son bien conocidos en la materia como genes internos usados cuando la expresión de un gen particular se tiene que medir. Estos genes no cambian sus niveles de expresión durante el desarrollo de la célula, el tratamiento o el estado del linfoma de células del manto.
- 15 En otra realización del primer aspecto de la invención, el gen control endógeno es *B2M*.

En un segundo aspecto, la presente invención proporciona un kit para llevar a cabo el método de acuerdo con el primer aspecto de la invención, que comprende medios adecuados para medir el nivel de expresión de al menos cuatro de los genes seleccionados del grupo que consiste en: *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B* y de uno o más genes control endógenos. Preferiblemente, los medios adecuados comprenden dos pares de oligonucleótidos adecuados como cebadores para la amplificación de al menos cuatro de los genes seleccionados del grupo que consiste en: *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B* y de uno o más genes control endógenos. Más preferiblemente, el kit comprende dos pares de oligonucleótidos adecuados como cebadores para la amplificación de los genes *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B*. Aún más preferiblemente, el kit comprende substancias requeridas para llevar a cabo la reacción en cadena de la polimerasa en tiempo real. Aún más preferiblemente, el gen control endógeno es *B2M*.

El método y kit de acuerdo con la presente invención son simples por implicar un número mínimo de genes, cuantitativos, aplicables en el diagnóstico de rutina a una gran variedad de muestras, y proporcionan una capacidad de

predicción de la supervivencia de los pacientes con LCM más elevada que los métodos actuales.

A lo largo de la descripción y las reivindicaciones la palabra "comprende" y
5 sus variantes no pretenden excluir otras características técnicas, aditivos,
componentes o pasos. Para los expertos en la materia, otros objetos,
ventajas y características de la invención se desprenderán en parte de la
descripción y en parte de la práctica de la invención. Los siguientes ejemplos
y dibujos se proporcionan a modo de ilustración, y no se pretende que sean
10 limitativos de la presente invención.

BREVE DESCRIPCIÓN DE LOS DIBUJOS

La FIG. 1 representa el valor del índice C de acuerdo con el número de genes
15 incluidos en el predictor de la supervivencia. Un modelo de cuatro o cinco
genes de acuerdo con la presente invención predice la supervivencia con
gran precisión. Los predictores de supervivencia compuestos por 4/5 genes
son superiores comparados con el valor predictivo del índice de proliferación
de Ki-67.

20 La FIG. 2 representa las curvas de Kaplan-Meier para visualizar los grupos de
supervivencia de acuerdo con el predictor de cinco genes en A) muestras
tumorales de tejidos frescos congelados de 73 pacientes con LCM; y B)
muestras FFIP de 42 pacientes con LCM. Cada línea representa un subgrupo
25 de pacientes de LCM definido por el valor del predictor de 5 genes y los datos
de supervivencia. La aplicación del modelo de 5 genes resultó en la definición
de subgrupos de MCL que diferían en una supervivencia media de más de 5
años en el caso de muestras tumorales frescas congeladas (FIG. 2A), y 3
años en el caso de muestras FFIP (FIG. 2B).

30

EJEMPLOS

A) Selección de casos

35 Muestras tumorales congeladas de 73 pacientes de linfoma de células del
manto (LCM) recién diagnosticados se obtuvieron del Instituto de Patología
de la Universidad de Würzburg y del Hospital Clínico de Barcelona. 15 de las

73 muestras de LCM habían sido incluidas en un estudio previo basado en la expresión génica. Muestras tumorales fijadas en formol e incluidas en parafina (FFPI) correspondientes a 13 de los 73 pacientes de LCM así como muestras en FFPI de 57 casos de LCM independientes, incluyendo 22 casos
 5 del Instituto de Patología de Kiel, se obtuvieron para la validación de los resultados. De estos, 42 tumores tenían ADNc amplificable. El diagnóstico se estableció de acuerdo con los criterios de la OMS, y el estudio se aprobó por el Comité Ético de cada institución. Entre los 73 casos de LCM con material congelado disponible, 54 mostraban una morfología típica y 19 correspondían
 10 a la variante blastoide. Entre el grupo independiente de 42 casos de MCL con muestra FFIP, 35 eran variantes típicas y 7 blastoides. La tinción inmunohistoquímica para Ki-67 y la evaluación de la fracción de proliferación se realizó como ya se había descrito previamente (Tabla 1, Tabla Suplementaria 1).

15

Tabla 1

Características de los casos con LCM	Muestras congeladas	Muestras FFIP
Edad media (años)	64	66
Género		
Varón	58 (80%)	31 (74%)
Mujer	15 (20%)	11 (26%)
Histología		
Típica	54 (74%)	35 (83%)
Blastoide	19 (26%)	7 (17%)
Supervivencia media (meses)	34.5	36.3
Índice Medio de Proliferación (tinción Ki-67)	30%	22%
Número total de casos	73	42

Tabla Suplementaria 1: Características clínicas de los pacientes con LCM

Pacientes LCM	Edad (años)	Género	Histología	Supervivencia (meses)	Estado	Ki-67 (%)	Tejido
LCM1	63	V	blastoide	24	muerto	40	congelado
LCM2	58	M	típico	52.5	muerto	10	congelado
LCM3	74	V	blastoide	64.1	muerto	30	Congelado /FFIP
LCM4	n.d.	V	típico	69.4	muerto	10	congelado
LCM5	63	V	típico	29.5	Vivo	10	congelado
LCM6	60	V	típico	81.3	muerto	15	congelado
LCM7	39	V	típico	22.9	Vivo	20	congelado
LCM8	57	M	típico	80.1	muerto	10	congelado
LCM9	50	V	típico	21.2	muerto	n.d.	congelado
LCM10	61	V	típico	11.7	Vivo	10	Congelado /FFIP
LCM11	59	V	típico	37.4	muerto	30	congelado
LCM12	62	V	blastoide	16.8	muerto	50	congelado
LCM13	51	M	típico	136	muerto	20	congelado
LCM14	75	V	típico	67.1	muerto	20	congelado
LCM15	69	M	blastoide	9.1	muerto	50	congelado
LCM16	64	M	típico	48	muerto	3	congelado
LCM17	n.d.	V	típico	122.4	muerto	10	congelado
LCM18	70	V	típico	81.1	Vivo	30	congelado
LCM19	60	V	típico	10.6	Vivo	60	Congelado /FFIP
LCM20	71	M	blastoide	20	muerto	70	congelado
LCM21	51	V	típico	81.2	Vivo	10	congelado
LCM22	61	M	típico	32	muerto	40	congelado
LCM23	71	M	típico	14	Vivo	40	congelado
LCM24	n.d.	M	típico	24.9	muerto	50	congelado
LCM25	40	V	típico	96.4	vivo	10	congelado
LCM26	67	V	típico	6.8	muerto	60	congelado
LCM27	60	V	blastoide	97.4	muerto	15	Congelado /FFIP
LCM28	80	V	blastoide	40.6	muerto	90	congelado
LCM29	58	V	típico	34	muerto	100	Congelado /FFIP
LCM30	52	V	típico	39.3	vivo	30	congelado
LCM31	58	V	típico	14	muerto	50	Congelado /FFIP
LCM32	72	V	blastoide	1.6	muerto	60	Congelado /FFIP
LCM33	53	V	típico	7.8	muerto	90	Congelado /FFIP
LCM34	42	V	típico	81.6	vivo	30	congelado
LCM35	70	V	típico	7.4	muerto	20	congelado
LCM36	64	V	típico	69.4	muerto	25	Congelado

								/FFIP
LCM37	66	M	blastoide	1	muerto	100	congelado	
LCM38	64	V	típico	30.6	muerto	25	congelado	
LCM39	78	V	típico	4.7	muerto	30	Congelado	/FFIP
LCM40	61	V	típico	106.3	muerto	4	Congelado	/FFIP
LCM41	72	V	típico	49	muerto	50	congelado	
LCM42	61	V	típico	12.9	muerto	40	congelado	
LCM43	77	V	típico	30	vivo	10	congelado	
LCM44	64	M	blastoide	8.2	muerto	60	congelado	
LCM45	71	V	blastoide	42.5	muerto	50	congelado	
LCM46	51	V	blastoide	61.9	muerto	5	congelado	
LCM47	81	M	típico	6.3	muerto	40	Congelado	/FFIP
LCM48	54	V	típico	19.5	muerto	30	congelado	
LCM49	85	V	blastoide	51.9	vivo	20	congelado	
LCM50	52	M	blastoide	49.1	muerto	10	congelado	
LCM51	67	V	blastoide	34.5	muerto	30	congelado	
LCM52	69	V	típico	32.6	muerto	35	congelado	
LCM53	78	V	típico	36.6	muerto	30	congelado	
LCM54	67	M	típico	26.5	muerto	60	congelado	
LCM55	81	V	blastoide	16	muerto	10	congelado	
LCM56	71	V	típico	53	muerto	n.d.	congelado	
LCM57	71	V	blastoide	37	muerto	50	congelado	
LCM58	37	V	típico	126.1	vivo	30	congelado	
LCM59	64	V	típico	49	muerto	10	congelado	
LCM60	82	V	típico	62	vivo	30	congelado	
LCM61	81	M	blastoide	8	muerto	50	congelado	
LCM62	75	V	blastoide	9.8	muerto	70	congelado	
LCM63	53	V	típico	20	vivo	n.d.	congelado	
LCM64	67	V	típico	142	vivo	20	congelado	
LCM65	67	V	tipico	61.6	muerto	20	congelado	
LCM66	64	V	típico	76.2	muerto	15	congelado	
LCM67	67	V	tipico	31.9	muerto	15	congelado	
LCM68	55	V	tipico	15.3	muerto	70	congelado	
LCM69	61	V	típico	8.6	muerto	70	congelado	
LCM70	72	V	típico	58	muerto	n.d.	congelado	
LCM71	80	V	tipico	1	muerto	60	congelado	
LCM72	40	V	típico	246.7	muerto	20	congelado	
LCM73	56	V	típico	94.2	vivo	15	Congelado	/FFIP
LCM74	63	V	blastoide	10.3	vivo	80	FFIP	
LCM75	73	M	típico	52	vivo	5	FFIP	
LCM76	68	V	típico	14.2	muerto	30	FFIP	
LCM77	69	V	típico	10.2	muerto	10	FFIP	
LCM78	57	V	típico	102.5	vivo	20	FFIP	
LCM79	53	V	típico	53.1	vivo	10	FFIP	

LCM80	67	V	típico	57.7	muerto	10	FFIP
LCM81	46	V	típico	14.9	muerto	50	FFIP
LCM82	69	V	blastoide	7.5	muerto	90	FFIP
LCM83	74	V	típico	3.7	muerto	10	FFIP
LCM84	72	V	típico	7.3	muerto	20	FFIP
LCM85	64	V	típico	77	vivo	20	FFIP
LCM86	59	V	típico	47.6	muerto	20	FFIP
LCM87	63	V	típico	66.3	muerto	10	FFIP
LCM88	55	M	típico	52.5	muerto	40	FFIP
LCM89	69	V	típico	38.6	muerto	n.d.	FFIP
LCM90	52	M	típico	3.3	muerto	40	FFIP
LCM91	77	M	típico	2.8	muerto	40	FFIP
LCM92	69	V	típico	42.3	muerto	40	FFIP
LCM93	64	V	blastoide	5	muerto	70	FFIP
LCM94	54	V	blastoide	6.4	muerto	90	FFIP
LCM95	73	M	blastoides	60.8	muerto	40	FFIP
LCM96	40	V	típico	44.8	vivo	30	FFIP
LCM97	71	V	típico	44.8	muerto	20	FFIP
LCM98	64	V	típico	53.8	muerto	n.d.	FFIP
LCM99	72	V	típico	5.8	vivo	20	FFIP
LCM100	68	M	típico	86.2	muerto	10	FFIP
LCM101	49	V	típico	81.3	muerto	30	FFIP
LCM102	66	V	típico	17.5	muerto	10	FFIP
LCM103	58	V	típico	71.6	muerto	10	FFIP
LCM104	56	V	blastoide	52.7	muerto	70	FFIP
LCM105	71	M	típico	47.9	muerto	10	FFIP
LCM106	62	V	típico	39.4	muerto	20	FFIP
LCM107	73	M	típico	38.7	muerto	20	FFIP
LCM108	64	V	típico	17.8	muerto	40	FFIP
LCM109	69	V	típico	27.5	vivo	20	FFIP
LCM110	61	M	típico	34	muerto	3	FFIP
LCM111	76	M	típico	4.3	Vivo	20	FFIP
LCM112	68	V	blastoide	2.2	muerto	90	FFIP
LCM113	65	M	típico	23.5	Vivo	30	FFIP
LCM114	57	V	típico	30.3	muerto	30	FFIP
LCM115	67	V	típico	4.6	muerto	30	FFIP

Abreviaturas: LCM, Linfoma de Células del Manto; FFIP, Fijado en formol, incluido en parafina; M: mujer; H: varón; n.d., no disponible

5 B) Extracción de ARN y PCR de transcripción reversa

El ARN de las muestras congeladas se obtuvo usando el reactivo TRIzol® y siguiendo las instrucciones del fabricante (Invitrogen Life Technologies®, Carlsbad, CA, USA). El ARN de las muestras FFIP se obtuvo como se había

descrito anteriormente con pequeñas modificaciones. El protocolo detallado se encuentra en la información suplementaria. La integridad del ARN se examinó con el Agilent 2100 Bioanalyzer (Agilent Technologies®, Palo Alto, CA, USA). La síntesis del ADN complementario se llevó a cabo a partir de 1 µg de RNA total con el reactivo High Capacity cDNA Archive Kit (Applied Biosystems®, Foster City, CA, USA) y siguiendo las instrucciones del fabricante.

C) RT-PCR cuantitativa a tiempo real (qRT-PCR)

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Los inventores seleccionaron 33 genes con un potencial impacto pronóstico y patogénico en LCM. Estos genes se analizaron usando una plataforma de arrays de baja densidad TaqMan® de diseño propio (Micro Fluidic Cards, Applied Biosystems®). En particular, la selección incluyó genes implicados en la signatura de proliferación (Rosenwald et al., arriba), ciclo celular, apoptosis, transporte de nucleósidos y procesos metabólicos. Los ensayos prediseñados escogidos se encuentran en la Tabla 2. La qRT-PCR se realizó usando el sistema ABI Prism®7900 HT Sequence Detection en condiciones estándares (Applied Biosystems®). Los datos primarios se analizaron con el

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programa Sequence Detector System (SDS) versión 2.1 (Applied Biosystems®). Dos genes control se incluyeron en el estudio: 18S (obligatorio para la técnica) y β2-microglobulina (*B2M*, escogido de acuerdo con trabajos anteriores (Rosenwald et al., arriba). Los niveles de expresión génica se calcularon más adelante mediante el método de $2^{-\Delta\Delta Ct}$ usando 18S y *B2M*

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como genes control endógenos (Tabla Suplementaria 2). Un caso de LCM de la serie se utilizó como calibrador matemático. Para una realización óptima de la qRT-PCR en material FFIP se redujo el tamaño del amplicón de cuatro genes a menos de 80 pb, porqué el ARN del material FFIP está muy degradado debido a los procesos de fijación en formol. El éxito del análisis de

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qRT-PCR con ARNm derivado de tejidos FFIP normalmente depende de los tamaños cortos del amplicón (óptimamente hasta 80 pb). De este modo, se escogieron ensayos prediseñados optimizados para *SLC29A2*

(Hs01546959_g1) y *TNFRSF10B* (Hs01043164_m1), y se modificaron sondas/pares de cebadores (utilizando el programa Primer Express®, Applied Biosystems®) para *MYC* (Sonda: 5'-CTTAGAGGTTGCTCAGACAA-3' (SEQ ID NO: 1), Cebador sentido: 5'GGCAGCACAGTATGAGCACG-3' (SEQ ID NO: 2), Cebador antisentido: 5'-ATCCTCATCCGGGAGAGCA-3' (SEQ ID

NO: 3)) y *RAN* (Sonda: 5'-AGGAGGAACAAGAAGAT-3' (SEQ ID NO: 4), Cebador sentido: 5'-CACCAACCAGCAGCGACTCT-3' (SEQ ID NO: 5), Cebador antisentido: 5'-ACAGAAACAAACATCGATTCTTCCT-3' (SEQ ID NO: 6)). Los niveles de expresión génica se calcularon como se ha descrito anteriormente usando *B2M* como gen control endógeno. El amplicón de *POLE2* ya tenía menos de 80 pb (Tabla 2).

Tabla 2

Gen	Ensayo	Tamaño del amplicón (pb)	Función
ASPM	Hs00411505_m1	77	Proliferación
BCL2L11	Hs00708019_s1	92	Apoptosis
BMI1	Hs00180411_m1	105	Ciclo celular
CCND1	Hs00277039_m1	94	Ciclo celular
CDC2	Hs00176469_m1	101	Proliferación
CDC20 *	Hs00415851_g1	108	Proliferación
CDK4	Hs00175935_m1	65	Ciclo celular
CDKN2A	Hs00233365_m1	117	Ciclo celular
CDKN3 *	Hs00193192_m1	83	Proliferación
CEBPB	Hs00270923_s1	75	Proliferación
CENPF	Hs00193201_m1	99	Proliferación
DBF4	Hs00272696_m1	77	Proliferación
GSTP1	Hs00168310_m1	54	Metabolismo
GTF2B	Hs00155315_m1	66	Proliferación
HMGB2 *	Hs00357789_g1	99	Proliferación
HPRT1 *	Hs99999909_m1	100	Proliferación
LCM1	Hs00172036_m1	124	Apoptosis
MCM2	Hs00170472_m1	82	Proliferación
MDM2	Hs00170472_m1	82	Ciclo celular
MKI67 *	Hs00606991_m1	137	Proliferación
MYC *	Hs00153408_m1	107	Múltiple
NEIL3	Hs00157387_m1	82	Proliferación
PCNA *	Hs00427154_g1	138	Proliferación
PIF1	Hs00228104_m1	104	Proliferación
POLE2 *	Hs00160277_m1	70	Proliferación
RAN *	Hs00741099_g1	146	Proliferación
SLC29A1	Hs00191940_m1	77	Transporte nucleosidos
SLC29A2	Hs00155426_m1	110	Transporte nucleosidos
TNFRSF10A	Hs00269491_m1	83	Apoptosis
TNFRSF10B	Hs00187196_m1	114	Apoptosis
TOP2A *	Hs00172154_m1	125	Proliferación
TUBA1B	Hs00744842_sH	110	Proliferación
UHRF1	Hs00273589_m1	105	Proliferación
18S	4342379	-	Gen control
B2M	Hs00187842_m1	64	Gen control

Tabla Suplementaria 2: Datos de expresión de los 5 genes incluidos en el predictor de los pacientes con LCM:

Paciente LCM	RAN*	MYC*	TNFRSF10B*	POLE2*	SLC29A2*	Valor pronóstico# &
LCM1	2.06	-0.66	0.10	2.72	-0.92	3.10
LCM2	-0.30	0.26	-0.58	0.38	-2.00	-1.08
LCM3#	0.83/-0.26	-1.74/1.19	-0.78/0.5	1.71/0.35	-2.08/1.65	-0.5/2.43
LCM4	1.45	1.08	0.73	-0.02	-0.02	1.76
LCM5	0.80	-0.79	0.18	0.73	-2.59	-2.03
LCM6	0.03	0.16	-0.55	0.28	-0.84	0.18
LCM7	0.92	-0.71	0.11	1.35	-1.68	-0.23
LCM8	0.80	-0.88	-0.26	1.32	0.16	1.66
LCM9	1.39	0.43	0.29	1.23	1.22	3.98
LCM10#	0.04/0.00	0.33/1.07	-0.52/-0.94	1.03/-1.64	-2.17/0.07	-0.25/0.44
LCM11	0.96	-0.87	-0.90	2.07	-3.51	-0.45
LCM12	2.05	1.32	-0.15	2.61	-2.15	3.98
LCM13	0.59	0.16	0.24	1.76	-1.51	0.76
LCM14	-0.79	-1.32	1.61	2.05	1.18	-0.49
LCM15	0.69	-1.43	-0.93	1.35	-2.26	-0.72
LCM16	0.40	-0.18	-1.46	1.28	-1.50	1.46
LCM17	1.29	-0.63	0.31	1.40	-3.05	-1.30
LCM18	0.55	-2.06	-2.11	0.04	-4.88	-4.24
LCM19#	0.89/-0.01	0.61/1.56	-0.65/0.11	2.79/0.75	0.55/1.60	5.49/3.79
LCM20	1.15	-0.72	-1.91	3.07	1.92	7.33
LCM21	0.90	-0.11	0.29	-0.50	-0.33	-0.33
LCM22	1.06	-0.33	-0.33	1.70	0.24	3.00
LCM23	1.10	-0.44	-0.51	1.07	-2.01	0.23
LCM24	1.20	0.54	-0.14	0.18	-0.01	2.05
LCM25	0.06	-0.38	0.05	-0.30	-2.25	-2.92
LCM26	0.91	0.00	-0.91	1.85	-0.90	2.77
LCM27#	1.35/-0.23	-0.02/2.76	-0.32/0.15	2.47/0.47	-1.61/0.73	2.51/3.58
LCM28	1.36	0.17	-0.43	2.12	0.01	4.09
LCM29#	0.62/0.02	-0.16/0.95	-0.78/-0.04	0.32/-0.02	-2.30/0.41	-0.74/1.40
LCM30	0.52	-1.19	-0.64	1.51	-1.16	0.32
LCM31#	1.55/0.79	0.25/0.97	-1.25/-0.22	2.53/-0.14	-1.04/0.22	4.54/2.06
LCM32#	2.08/0.38	1.62/2.14	0.71/0.54	2.45/0.43	0.69/1.67	6.13/4.08
LCM33#	1.42/-0.31	2.58/2.82	-0.10/-0.41	2.24/0.02	0.51/1.47	6.85/4.41
LCM34	1.15	-0.49	-0.59	1.10	-1.46	0.89
LCM35	1.52	1.71	0.00	1.34	1.84	6.41
LCM36#	0.97/-0.01	-0.19/2.47	-0.30/0.47	1.45/0.56	-0.10/1.81	2.43/4.36
LCM37	0.87	0.64	-0.63	1.89	-1.44	2.59
LCM38	1.32	-0.44	0.09	2.10	-2.35	0.54
LCM39#	1.06/0.30	-0.77/-0.21	0.03/0.24	1.86/0.18	-1.43/0.60	0.69/0.63
LCM40#	0.62/1.24	0.18/4.12	0.32/1.43	1.13/0.38	-3.22/-0.29	-1.61/4.02
LCM41	1.78	-1.42	-0.68	1.65	-2.87	-0.18

LCM42	1.15	0.53	-0.24	1.26	-0.74	2.44
LCM43	0.17	-1.85	-0.82	0.60	-2.43	-2.69
LCM44	1.39	1.10	-0.88	1.52	0.17	5.06
LCM45	0.17	-1.12	-0.44	1.97	-3.52	-2.06
LCM46	-0.49	-2.03	-1.08	-0.50	-3.11	-5.05
LCM47 [#]	1.68/1.07	1.20/3.26	0.22/0.32	3.33/1.31	0.88/1.51	6.87/6.83
LCM48	1.62	-0.41	0.44	3.23	-1.44	2.56
LCM49	1.55	-1.09	-0.39	2.12	0.18	3.15
LCM50	-1.30	0.81	0.32	-0.61	3.05	1.63
LCM51	1.22	-1.26	-0.87	2.36	0.34	3.53
LCM52	0.50	-1.01	-0.92	2.17	-2.56	0.02
LCM53	1.72	-0.80	-1.25	2.74	-4.00	0.91
LCM54	0.22	-0.16	-0.76	0.15	-1.26	-0.29
LCM55	0.58	-1.67	-0.71	1.12	-2.87	-2.13
LCM56	0.41	-1.06	-0.68	1.48	-2.23	-0.72
LCM57	1.47	0.34	-0.69	2.17	-3.01	1.66
LCM58	-1.61	-0.60	1.10	2.64	2.46	1.79
LCM59	1.26	-1.57	-0.88	1.20	-4.02	-2.25
LCM60	0.14	0.57	-0.98	-0.30	-3.53	-2.14
LCM61	1.95	1.83	-0.23	2.43	0.56	7.00
LCM62	1.46	1.66	1.06	2.86	-1.28	3.64
LCM63	0.52	-1.73	-1.54	1.57	-3.13	-1.23
LCM64	1.02	-0.22	-0.09	1.82	-3.19	-0.48
LCM65	-1.10	0.60	0.62	2.13	1.48	2.49
LCM66	0.34	-1.01	-0.36	0.92	-2.06	-1.45
LCM67	2.08	-0.72	0.90	3.18	-1.16	2.48
LCM68	1.78	-0.44	0.70	2.38	0.65	3.67
LCM69	1.55	1.45	-0.77	2.11	-0.64	5.24
LCM70	0.75	-1.46	-0.93	1.29	-3.53	-2.02
LCM71	1.11	-0.35	-1.43	2.09	-0.50	3.78
LCM72	0.25	-0.10	0.74	2.20	1.08	2.69
LCM73 [#]	0.48/-0.60	-1.04/0.09	0.31/0.75	0.87/-0.81	-1.78/0.54	-1.78/-1.53
LCM74	1.74	-0.46	1.07	0.39	0.00	0.60
LCM75	0.56	0.05	1.46	-1.14	-1.07	-3.06
LCM76	0.89	0.43	1.01	-1.24	2.09	1.16
LCM77	0.35	1.96	1.61	-0.64	0.59	0.65
LCM78	2.01	1.16	2.45	-1.56	-1.49	-2.33
LCM79	2.86	3.26	2.77	-0.61	2.36	5.10
LCM80	0.08	2.47	2.46	-1.30	2.49	1.28
LCM81	1.86	2.88	-0.77	0.68	2.16	8.35
LCM82	0.86	1.87	0.01	-0.29	-3.43	-1.00
LCM83	2.14	1.07	2.97	0.72	3.18	4.14
LCM84	3.24	-0.13	1.05	0.46	0.62	3.14
LCM85	0.49	0.70	1.60	-0.04	1.26	0.81
LCM86	0.50	-2.12	-1.46	-0.12	1.31	1.03
LCM87	0.71	-0.76	0.47	-1.01	1.39	-0.14
LCM88	-0.03	-0.82	1.38	0.71	1.21	-0.31
LCM89	2.82	3.27	3.23	0.09	3.94	6.89

LCM90	1.28	4.46	0.42	1.10	3.22	9.64
LCM91	-0.27	1.27	2.14	-1.02	2.27	0.11
LCM92	3.51	1.46	3.03	0.89	3.19	6.02
LCM93	0.74	3.40	1.34	-0.20	2.40	5.00
LCM94	2.43	3.74	2.08	-0.20	0.93	4.82
LCM95	0.60	1.73	0.85	0.36	1.39	3.23
LCM96	1.16	0.13	1.21	-0.68	0.82	0.22
LCM97	0.55	2.69	1.30	0.12	0.45	2.51
LCM98	0.11	1.56	0.56	-0.27	1.13	1.97
LCM99	-0.11	1.92	0.32	-1.04	2.06	2.51
LCM100	-0.24	1.42	0.65	-0.89	1.47	1.11
LCM101	-0.35	1.23	0.45	-0.42	-0.15	-0.14
LCM102	1.45	1.65	1.11	-0.61	0.53	1.91
LCM103	1.53	2.07	1.77	0.58	1.26	3.67
LCM104	0.15	1.53	-1.63	1.74	-1.83	3.22
LCM105	0.10	-2.42	-0.56	-1.15	-0.64	-3.55
LCM106	2.36	-0.18	0.43	0.48	0.61	2.84
LCM107	0.38	1.73	0.96	0.59	2.13	3.87
LCM108	0.42	3.2	-0.27	0.77	2.82	7.48
LCM109	-0.53	3.48	0.66	2.09	1.35	5.73
LCM110	0.68	0.03	0.36	-1.28	-0.41	-1.34
LCM111	0.30	0.64	0.26	0.43	-0.76	0.35
LCM112	0.05	2.14	0.68	0.13	1.02	2.66
LCM113	0.62	1.91	1.51	-0.59	2.15	2.58
LCM114	0.20	2.08	0.32	-0.53	2.34	3.77
LCM115	-0.20	-0.25	0.05	-0.61	0.52	-0.59

* :Valor - $\Delta\Delta CT$ calculado de acuerdo con el gen control B2M.

:Valor del material congelado (tarjetas microfluídicas)/Valor del FFIP

&: Suma de los datos de expresión para los genes, cambiando el signo para

5 TNFRSF10B.

D) Análisis estadístico

La supervivencia se estimó con el método Kaplan-Meier y se comparó por

10 tests logrank. Se utilizaron los modelos de riesgos proporcionales de Cox

multivariados para identificar los mejores subgrupos de genes con valor

pronóstico. Las ratios de riesgo y los intervalos de confianza del 95% se estimaron a partir de los modelos y los tests de las ratios de semejanza se utilizaron para obtener significación estadística. La precisión predictiva se

15 calculó usando el área bajo la curva ROC (Receiver Operating Characteristic)

(C-index) derivada del estadístico D de Sommers adaptado para las

observaciones censuradas. Se utilizó la validación interna por bootstrap del

procedimiento de selección stepwise para obtener la precisión predictiva corregida para un mejor ajuste. Los p-valores menores de 0.05 se consideraron significativos.

5 E) Resultados

E.1. Características de los pacientes con LCM

Los detalles clínicos e histológicos, los datos de supervivencia e información del índice Ki-67 para cada paciente se proporcionan en la Tabla 1 y en la Tabla Suplementaria 1. De forma importante, no se observaron diferencias entre las muestras de LCM congeladas y de FFIP en relación a la distribución de la media de edad, género, características histológicas, media de la supervivencia e índice medio de Ki-67 (Tabla 1). La gran mayoría (78%) de los pacientes con LCM recibieron una terapia basada en antraciclina (Protocolos CHOP o similares) como tratamiento de primera línea, el 10% recibieron un régimen de quimioterapia intensificada (en su mayoría hiperCVAD), el 6% no recibieron quimioterapia y en el 6% la terapia era desconocida. No se observaron diferencias entre los grupos de tratamiento en la distribución de géneros, características histológicas y supervivencia global. El grupo con tratamiento intensificado incluía pacientes jóvenes ($p=0.002$) y estaba enriquecido para los tumores con ratios de proliferación incrementadas ($Ki-67 \geq 40\%$), aunque esta característica no era estadísticamente significativa ($p=0.18$).

25
E.2. Desarrollo de la RT-PCR cuantitativa a tiempo real basada en el predictor de la supervivencia en muestras de LCM congeladas.

Primero, se investigaron los niveles de expresión génica de los 33 genes seleccionados (Tabla 2) en muestras tumorales de tejido fresco congelado de 30 73 pacientes con LCM por qRT-PCR para construir un predictor cuantitativo de la supervivencia en muestras congeladas. En un análisis univariado usando *B2M* como gen control, 10 de los 33 genes se asociaban significativamente con la supervivencia, y estos genes se encuentran marcados con un asterisco en la Tabla 2 ($p\text{-value} < 0.05$). Cuando se utilizó 35 18S como gen control, los niveles de expresión de estos 10 genes también se asociaban significativamente con la supervivencia demostrando la robustez

de los genes de referencia escogidos y la plataforma de arrays. Dos genes adicionales mostraron una significación límite (*CDC2*, p=0.053 y *TUBA1B*, p=0.054). Más importante, todos estos genes están implicados en el proceso biológico de la proliferación y 10 de los 12 genes se incluyeron en la
 5 signatura de proliferación basada en la expresión génica descrita anteriormente (Rosenwald et al., arriba). Este hallazgo subraya el valor pronóstico de la signatura de proliferación en muestras tumorales de LCM y confirma su valor en una serie grande e independiente de casos.

10

Tabla 3

	Genes	Índice-C
Modelo de 1 gen	<i>RAN</i>	0,690
Modelo de 2 genes	<i>RAN, MYC</i>	0,721
Modelo de 3 genes	<i>RAN, MYC, TNFRSF10B</i>	0,730
Modelo de 4 genes	<i>RAN, MYC, TNFRSF10B, POLE2</i>	0,754
Modelo de 5 genes	<i>RAN, MYC, TNFRSF10B, POLE2, SLC29A2</i>	0,762

Cuando los métodos estadísticos stepwise se aplicaron a los datos usando replicados de muestras bootstrap, los genes más frecuentemente seleccionados en los modelos pronóstico que comprendían entre uno y cinco genes eran los siguientes: *MYC* (45%), *RAN* (35%), *SLC29A2* (31%), *HPRT1* (28%), *POLE2* (26%), *TNFRSF10B* (25%) y *CDKN3* (24%). Usando los datos de expresión génica de estos genes, se ajustaron todos los modelos posibles y se estudiaron aquellos con un menor error de predicción. El mejor predictor de la supervivencia se componía de los cinco genes *RAN*, *MYC*,
 15 *TNFRSF10B*, *POLE2* y *SLC29A2* (Índice-C= 0.762, Índice-C corregido para el ajuste usando bootstrap= 0.71, 95%CI= 0.65-0.76). Es remarcable el hecho que un predictor compuesto por cuatro genes también proporciona una información precisa (Índice-C= 0.754) (Tabla 3, FIG. 1 y Tabla Suplementaria 2). En el modelo, niveles de expresión aumentados de *RAN*, *MYC*, *POLE2* y
 20 *SLC29A2* se correlacionaron con menor supervivencia, mientras que altos niveles de *TNFRSF10B* se asociaron con mayor supervivencia. Ya que todos los cinco genes incluidos en el modelo tenían coeficientes similares, se calculó un valor pronóstico simplificado usando coeficientes idénticos (+1; -1
 25

para *TNFRSF10B*). La aplicación de este modelo resultó en la definición de subgrupos de LCM que diferían en una supervivencia media de más de 5 años (FIG. 2A, logrank p-valor=1.1e⁻⁰⁶). Ya que los tumores con índices elevados de Ki-67 se encontraban enriquecidos (pero no eran

5 estadísticamente diferentes) en los pacientes con LCM y tratamiento intensificado, el ajuste del modelo en función del tratamiento resultó en un p-valor de significación similar (logrank p-valor=1.7e⁻⁰⁷). El predictor de cinco genes y el índice Ki-67 demostraron estar correlacionados (Correlación de Pearson=0.58). De todos modos, los dos proporcionaron un valor pronóstico 10 independiente en un modelo multivariado con los dos factores. Más importante, el predictor de cinco genes era superior comparado con el poder predictivo del índice de proliferación Ki-67 (p-valor <0.0001 para el predictor de cinco genes ajustado para Ki-67).

15 E.3. Aplicación del predictor de cinco genes en tejido de rutina fijado en formol e incluido en parafina (FFIP)

Para evaluar el potencial del modelo de cinco genes para su aplicación general en la rutina diagnóstica, se probó el predictor basado en qRT-PCR en 20 una serie de muestras de LCM de rutina fijadas en formol e incluidas en parafina (FFIP) (Tabla Suplementaria 2). Para 4 de los 5 genes del modelo, el tamaño del amplicón de los ensayos prediseñados escogidos para la plataforma de arrays de baja densidad (Micro Fluidic Cards) era mayor de 100 pb. Estos amplicones no mostraban amplificación o bien muy tardía en 25 las muestras de FFIP. Por esta razón, los ensayos se modificaron levemente para reducir el tamaño de los amplicones y optimizar el rendimiento de la qRT-PCR, como se describe en la sección de Material y Métodos. En un primer paso, un grupo de 22 muestras congeladas de LCM seleccionado al azar, que eran parte de la serie inicial, se utilizaron para probar los ensayos de qRT-PCR modificados. Este análisis demostró una correlación excelente entre los ensayos originales prediseñados y los modificados (p-valor<0.0001). En un segundo paso, se seleccionaron 18 casos de la serie inicial que tenían 30 tejidos tumorales en FFIP obtenidos de la misma biopsia al diagnóstico y se analizaron subsecuentemente usando los ensayos optimizados. 13 de las 18 muestras (72%) tuvieron éxito en la amplificación de todos los cinco genes 35 usando los ensayos de qRT-PCR modificados. Aunque las muestras de FFIP generalmente amplificaron a valores C_T mayores, los niveles de expresión

génica normalizados obtenidos del ARN extraído de los tejidos en FFIP eran comparables a los obtenidos de los tejidos congelados (Correlación de Pearson=0.77, p=0.002).

5 E.4. Validación del predictor de supervivencia de cinco genes en una serie independiente de muestras tumorales de LCM en FFIP

Para una posterior validación en un grupo de datos independientes, se examinó el funcionamiento del predictor de cinco genes en una serie 10 adicional de 57 muestras de LCM con datos clínicos y bloques de tejido en FFIP disponibles. 42 de las 57 muestras FFIP (74%) amplificaron para todos los cinco genes del modelo (Tabla 1, Tabla Suplementaria 1 y Tabla Suplementaria 2). Más importante, la ratio de éxito entre los casos más recientes (1998-2003) obtenidos como parte de procesos clínicos 15 prospectivos fue del 86%. En esta serie independiente de casos, el modelo predijo con éxito la supervivencia de los pacientes con LCM (logrank p-valor=0.011, ajustado para el tratamiento 0.023). Como ilustración, las curvas de Kaplan-Meier se muestran en la FIG. 2B mostrando tres subgrupos que difieren en una supervivencia media de más de 3 años (FIG. 2B). De todos 20 modos es destacable que el modelo de cinco genes predice la supervivencia de forma lineal con un tiempo de supervivencia predicho definido para cada paciente individual. En la serie de validación, el índice de proliferación Ki-67 también era un predictor significativo de la supervivencia (p-valor=0.008).

REIVINDICACIONES

1. Método para predecir la supervivencia de un paciente que sufre un linfoma de células del manto, comprendiendo los pasos de (a) obtener una muestra de dicho paciente; (b) medir el nivel de expresión de al menos cuatro genes seleccionados del grupo que consiste en: *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B* en dicha muestra; y (c) comparar el nivel de expresión de cada uno de los genes medidos con la expresión de uno o más genes control endógenos.
5
- 10 2. Método según la reivindicación 1, donde si el nivel de expresión de los genes *RAN*, *MYC*, *POLE2* y *SLC29A2* es mayor que el nivel de expresión del gen control endógeno y el nivel de expresión de *TNFRSF10B* es menor que el nivel de expresión del gen control endógeno se correlacionan con un peor pronóstico.
15
3. Método según cualquiera de las reivindicaciones 1-2, donde la muestra es una muestra de tejido que es fresca, congelada o fijada en formol e incluida en parafina.
20
4. Método según cualquiera de las reivindicaciones anteriores, donde se mide el nivel de expresión de cuatro genes seleccionados del grupo que consiste en: *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B*.
25
5. Método según cualquiera de las reivindicaciones 1-4, donde se mide el nivel de expresión de *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B*.
30
6. Método según cualquiera de las reivindicaciones anteriores, donde los niveles de expresión génica se miden por la reacción en cadena de la polimerasa cuantitativa a tiempo real.
35
7. Método según la reivindicación 6, donde el tamaño del amplicón para cada uno de los genes *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B* es igual o menor que 80 pares de bases.
35
8. Método según cualquiera de las reivindicaciones anteriores, donde el gen control endógeno es *B2M*.

9. Kit para llevar a cabo el método definido en la reivindicación 1, que comprende medios adecuados para medir el nivel de expresión de al menos cuatro de los genes seleccionados del grupo que consiste en: *MYC*, *RAN*,
5 *POLE2*, *SLC29A2* y *TNFRSF10B* y de uno o más genes control endógenos.
10. Kit según la reivindicación 9, donde los medios adecuados comprenden dos pares de oligonucleótidos adecuados como cebadores para la amplificación de al menos cuatro de los genes seleccionados del grupo que
10 consiste en: *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B* y de uno o más genes control endógenos.
11. Kit según cualquiera de las reivindicaciones 9-10, que comprende dos pares de oligonucleótidos adecuados como cebadores para la amplificación
15 de los genes *MYC*, *RAN*, *POLE2*, *SLC29A2* y *TNFRSF10B*.
12. Kit según cualquiera de las reivindicaciones 9-10, que comprende substancias requeridas para llevar a cabo la reacción en cadena de la polimerasa en tiempo real.
20
13. Kit según cualquiera de las reivindicaciones 9 a 12, donde el gen control endógeno es *B2M*.
14. Uso del kit como se define en cualquiera de las reivindicaciones 9-13, en
25 la predicción de la supervivencia de un paciente que sufre un linfoma de células del manto.

FIG. 1

Índice C

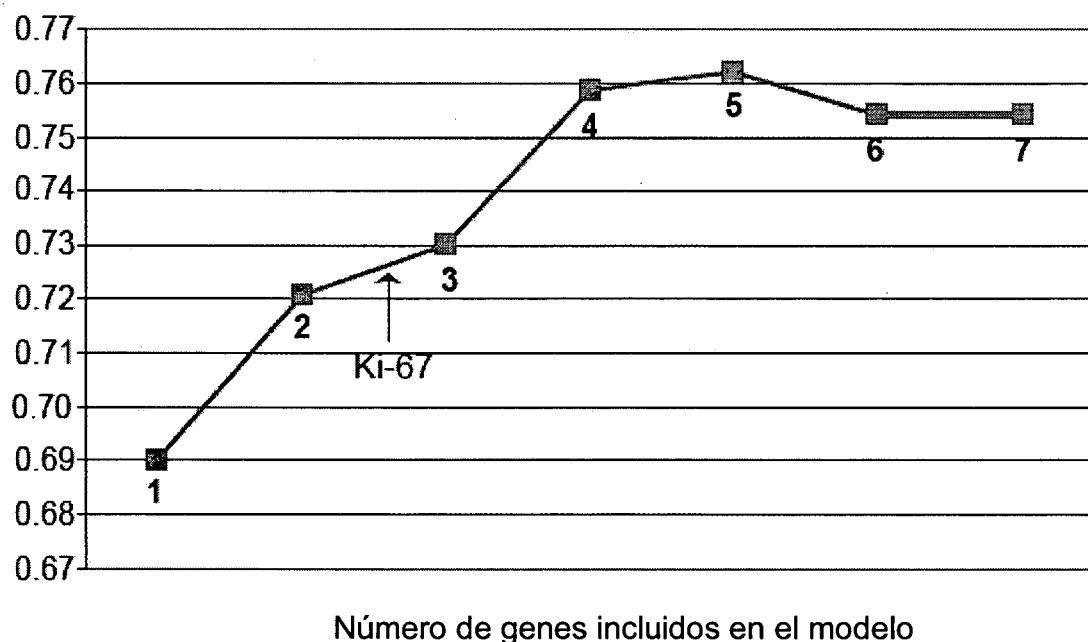
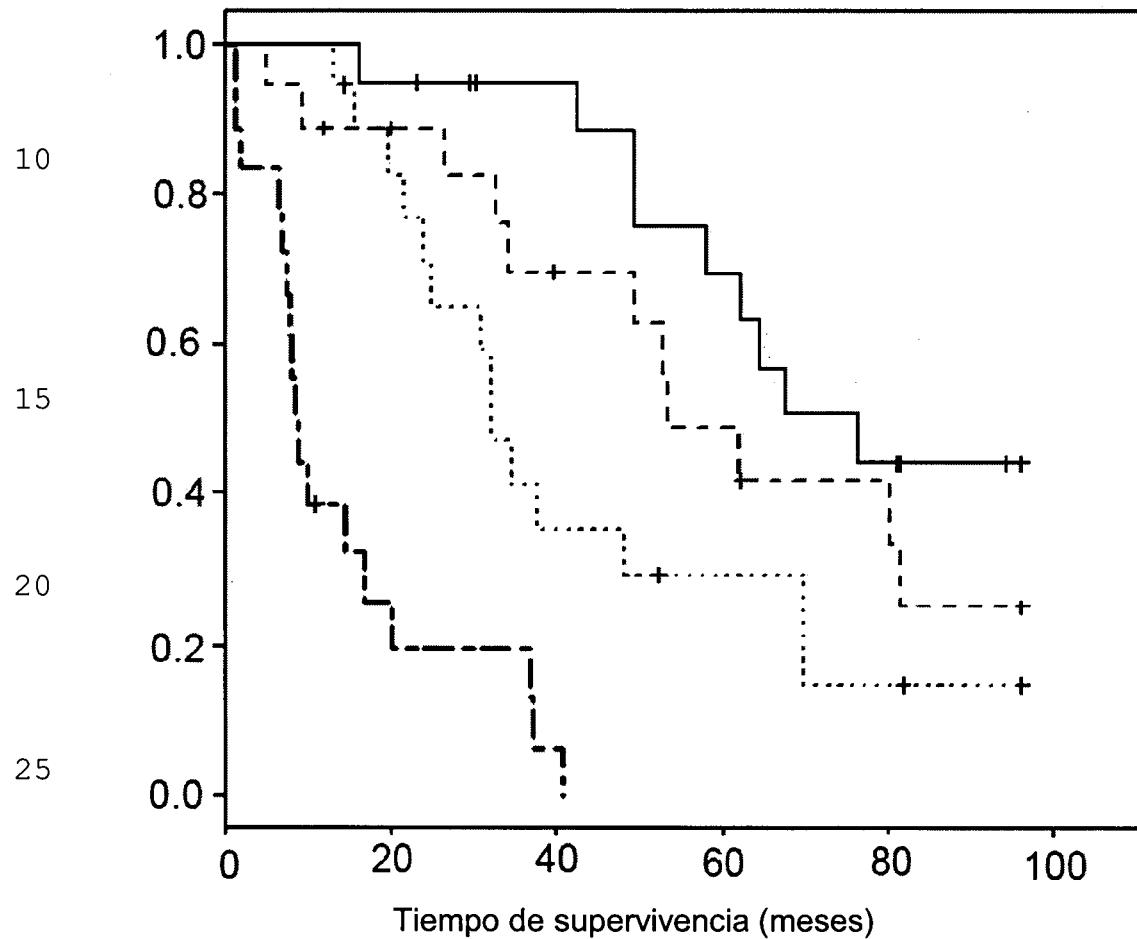


FIG. 2A

5 Probabilidad de supervivencia



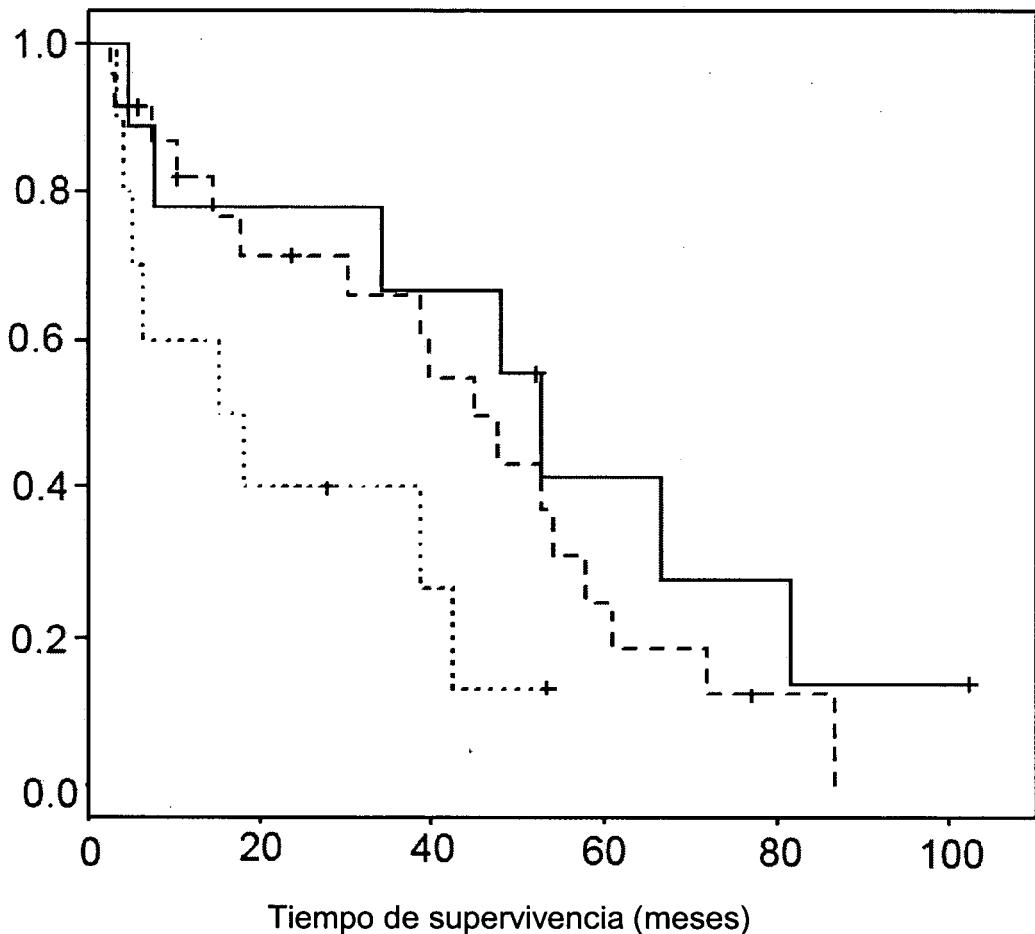
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FIG. 2B

Probabilidad de
5 supervivencia



INTERNATIONAL SEARCH REPORT

International application No.

PCT/ ES 2008/000707

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

INVENES, EPODOC, WPI, BIOSIS, EMBASE, MEDLINE, EMBL-EBI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROSENWALD, A. et al., "The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma.", CANCER CELL, 2003, Vol. 3, No. 2, pages 185-197, the whole document.	1-14
A	KIENLE, D. et al., "Quantitative gene expression deregulation in mantle-cell lymphoma: correlation with clinical and biologic factors.", JOURNAL OF CLINICAL ONCOLOGY, 2007 Jul, Vol. 25, No.19, pages 2770-2777, the whole document.	1-14
A	HOFMANN, W.K. et al., "Altered apoptosis pathways in mantle cell lymphoma detected by oligonucleotide microarray.", BLOOD, 2001, Vol. 98, No. 3, pages 787-794, the whole document.	1-14

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance.		
"E" earlier document but published on or after the international filing date		
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"P" document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search

02.March.2009 (02.03.2009)

Date of mailing of the international search report

(10/03/2009)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/ES 2008/000707

C (continuation).		DOCUMENTS CONSIDERED TO BE RELEVANT
Category*	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	RUBIO-MOSCARDO, F. et al., "Mantle-cell lymphoma genotypes identified with CGH to BAC microarrays define a leukemic subgroup of disease and predict patient outcome.", BLOOD, 2005, Vo.105, No.11, pages 4445-4454, the whole document.	1-14
A	KATZENBERGER, T. et al., "The Ki67 proliferation index is a quantitative indicator of clinical risk in mantle cell lymphoma.", BLOOD, 2006, Vol. 107, No.8, page 3407, the whole document.	1-14
A	LEICH, E. et al., "Diagnostic and prognostic significance of gene expression profiling in lymphomas.", APMIS, 2007 Oct, Vol. 115. No. 10, pages 1135-1146, the whole document, in particular, pages 1142-1143.	1-14
P,X	HARTMANN, E. et al., "Five-gene modthe to predict survival in mantle-cell lymphoma using frozen or formalin-fixed, paraffin-embedded tissue.", JOURNAL OF CLINICAL ONCOLOGY, 2008 Oct, Vol. 26, No. 30, pages 4966-4972, Epub: 07.07.2008, the whole document.	1-14

INFORME DE BÚSQUEDA INTERNACIONAL

Solicitud internacional N°

PCT/ ES 2008/000707

A. CLASIFICACIÓN DEL OBJETO DE LA SOLICITUD

C12Q 1/68 (2006.01)

De acuerdo con la Clasificación Internacional de Patentes (CIP) o según la clasificación nacional y CIP.

B. SECTORES COMPRENDIDOS POR LA BÚSQUEDA

Documentación mínima buscada (sistema de clasificación seguido de los símbolos de clasificación)

C12Q

Otra documentación consultada, además de la documentación mínima, en la medida en que tales documentos formen parte de los sectores comprendidos por la búsqueda

Bases de datos electrónicas consultadas durante la búsqueda internacional (nombre de la base de datos y, si es posible, términos de búsqueda utilizados)

INVENES, EPODOC, WPI, BIOSIS, EMBASE, MEDLINE, EMBL-EBI

C. DOCUMENTOS CONSIDERADOS RELEVANTES

Categoría*	Documentos citados, con indicación, si procede, de las partes relevantes	Relevante para las reivindicaciones N°
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En la continuación del Recuadro C se relacionan otros documentos

Los documentos de familias de patentes se indican en el Anexo

* Categorías especiales de documentos citados:	"T"	documento ulterior publicado con posterioridad a la fecha de presentación internacional o de prioridad que no pertenece al estado de la técnica pertinente pero que se cita por permitir la comprensión del principio o teoría que constituye la base de la invención.
"A" documento que define el estado general de la técnica no considerado como particularmente relevante.		
"E" solicitud de patente o patente anterior pero publicada en la fecha de presentación internacional o en fecha posterior.		
"L" documento que puede plantear dudas sobre una reivindicación de prioridad o que se cita para determinar la fecha de publicación de otra cita o por una razón especial (como la indicada).	"X"	documento particularmente relevante; la invención reivindicada no puede considerarse nueva o que implique una actividad inventiva por referencia al documento aisladamente considerado.
"O" documento que se refiere a una divulgación oral, a una utilización, a una exposición o a cualquier otro medio.	"Y"	documento particularmente relevante; la invención reivindicada no puede considerarse que implique una actividad inventiva cuando el documento se asocia a otro u otros documentos de la misma naturaleza, cuya combinación resulta evidente para un experto en la materia.
"P" documento publicado antes de la fecha de presentación internacional pero con posterioridad a la fecha de prioridad reivindicada.	"&"	documento que forma parte de la misma familia de patentes.

Fecha en que se ha concluido efectivamente la búsqueda internacional. 02.Marzo.2009 (02.03.2009)	Fecha de expedición del informe de búsqueda internacional 10 de Marzo de 2009 (10/03/2009)
Nombre y dirección postal de la Administración encargada de la búsqueda internacional O.E.P.M. Paseo de la Castellana, 75 28071 Madrid, España. Nº de fax 34 91 3495304	Funcionario autorizado J.L. Vizán Arroyo Nº de teléfono +34 91 349 85 73

INFORME DE BÚSQUEDA INTERNACIONAL

Solicitud internacional Nº

PCT/ ES 2008/000707

Recuadro I Secuencia(s) de nucleótidos y/o de aminoácidos (continuación del punto 1.c de la primera hoja)

1. En lo que se refiere a **las secuencias de nucleótidos y/o de aminoácidos** divulgadas en la solicitud internacional y necesarias para la invención reivindicada, la búsqueda se ha llevado a cabo sobre la base de:
 - a. Tipo de material
 - una lista de secuencias
 - tabla(s) relativas a la lista de secuencias
 - b. Formato del material
 - en papel
 - en formato electrónico
 - c. Fecha de presentación/entrega
 - contenido en la solicitud internacional tal y como se presentó
 - presentado junto con la solicitud internacional en formato electrónico
 - presentado posteriormente a esta Administración a los fines de la búsqueda
2. Además, en caso de que se haya presentado más de una versión o copia de una lista de secuencias y/o tabla relacionada con ella, se ha entregado la declaración requerida de que la información contenida en las copias subsiguientes o adicionales es idéntica a la de la solicitud tal y como se presentó o no va más allá de lo presentado inicialmente.
3. Comentarios adicionales:

INFORME DE BÚSQUEDA INTERNACIONAL

Solicitud internacional N°

PCT/ES 2008/000707

C (continuación).		DOCUMENTOS CONSIDERADOS RELEVANTES
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US 20090023149A1

(19) United States

(12) Patent Application Publication
Knudsen

(10) Pub. No.: US 2009/0023149 A1

(43) Pub. Date: Jan. 22, 2009

(54) METHODS, KITS AND DEVICES FOR IDENTIFYING BIOMARKERS OF TREATMENT RESPONSE AND USE THEREOF TO PREDICT TREATMENT EFFICACY

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(21) Appl. No.: 12/151,949

(22) Filed: May 9, 2008

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/IB2006/004048, filed on Dec. 1, 2006.

Foreign Application Priority DataDec. 1, 2005 (DK) PA2005/01696
May 11, 2007 (DK) PA2007/00714
Jul. 11, 2007 (DK) PA2007/01023**Publication Classification**(51) Int. Cl.
C12Q 1/68 (2006.01)

(52) U.S. Cl. 435/6

ABSTRACT

The present invention features methods, kits, and devices for predicting the sensitivity of a patient to a compound or medical treatment. The invention also features methods for identifying gene biomarkers whose expression correlates to treatment sensitivity or resistance within a patient population or subpopulation.

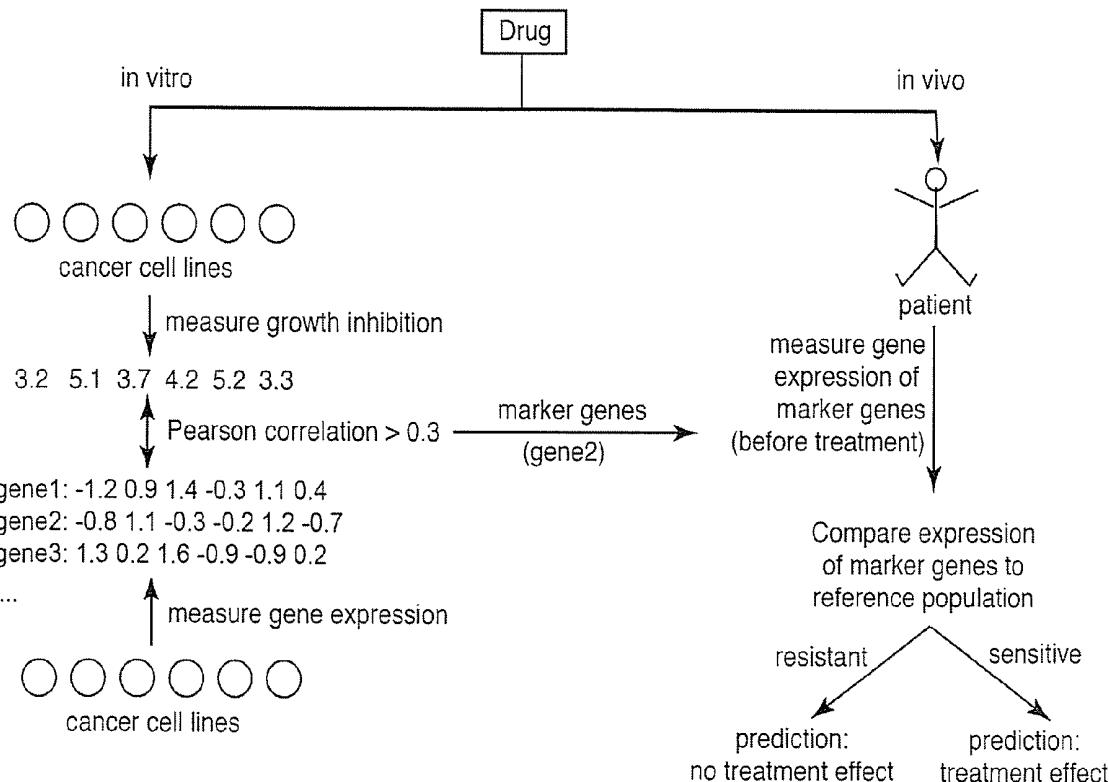


Figure 1

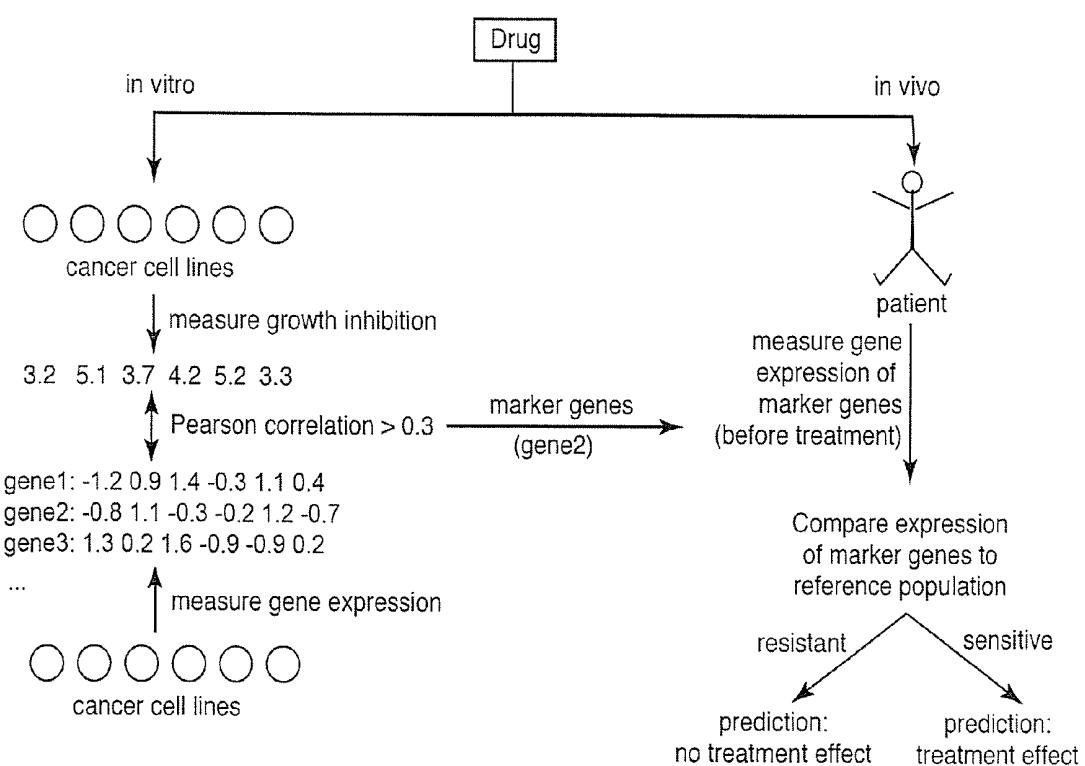


Figure 2:

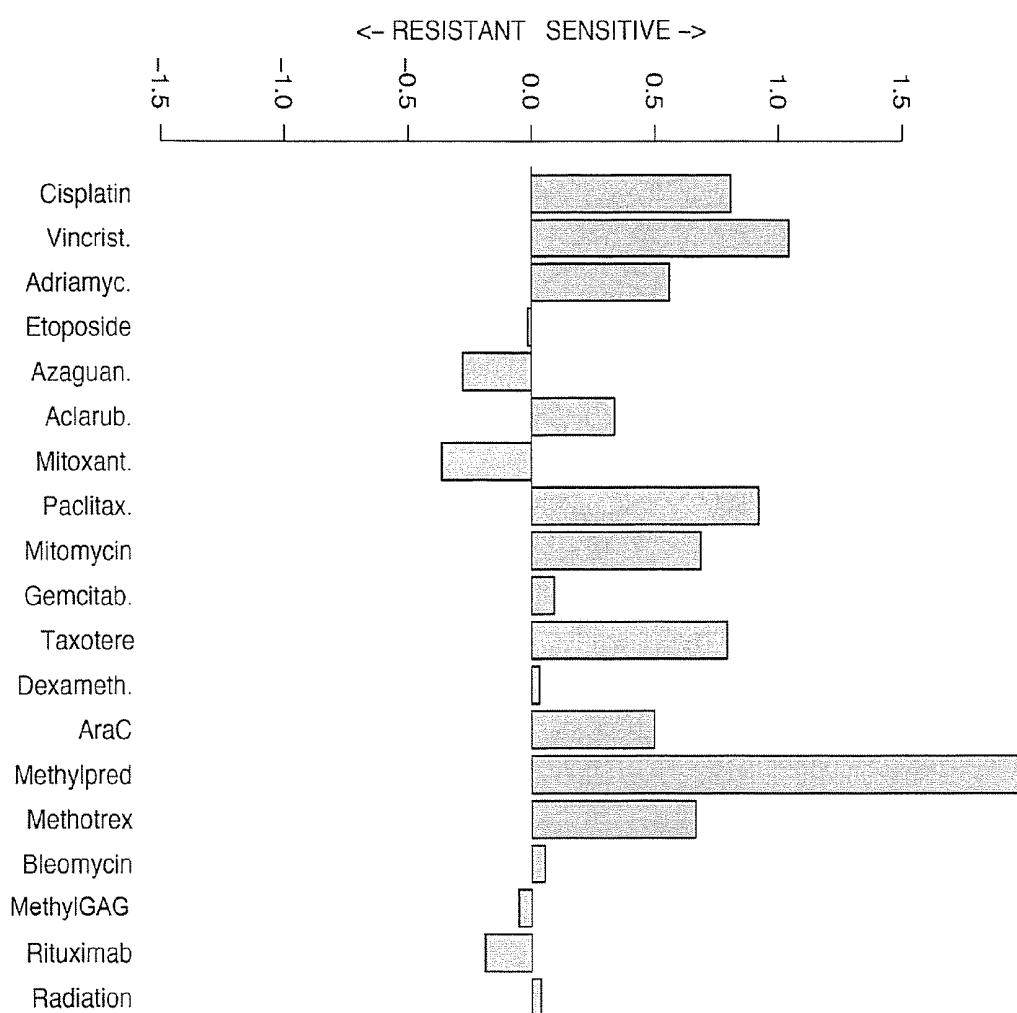


Figure 3:

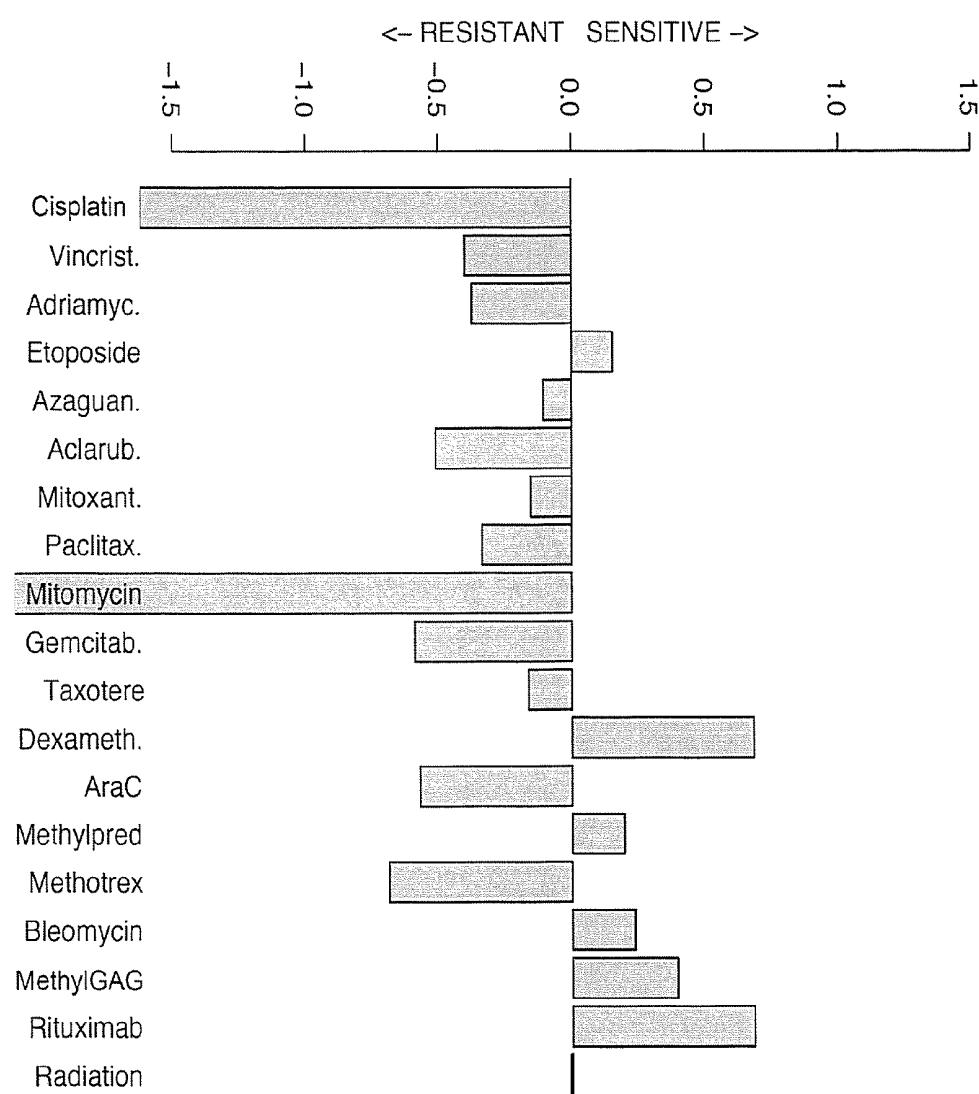


Figure 4:

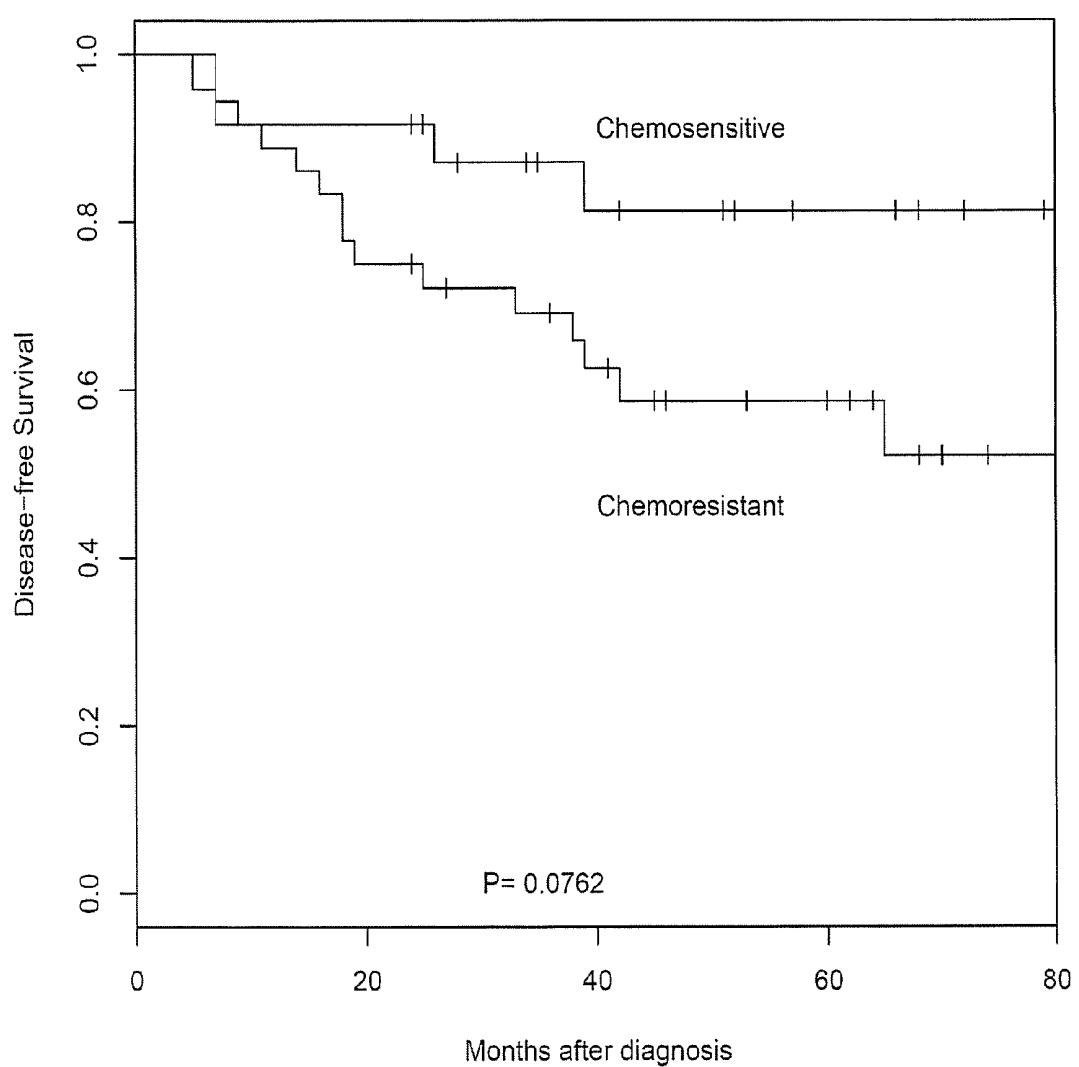


Figure 5:

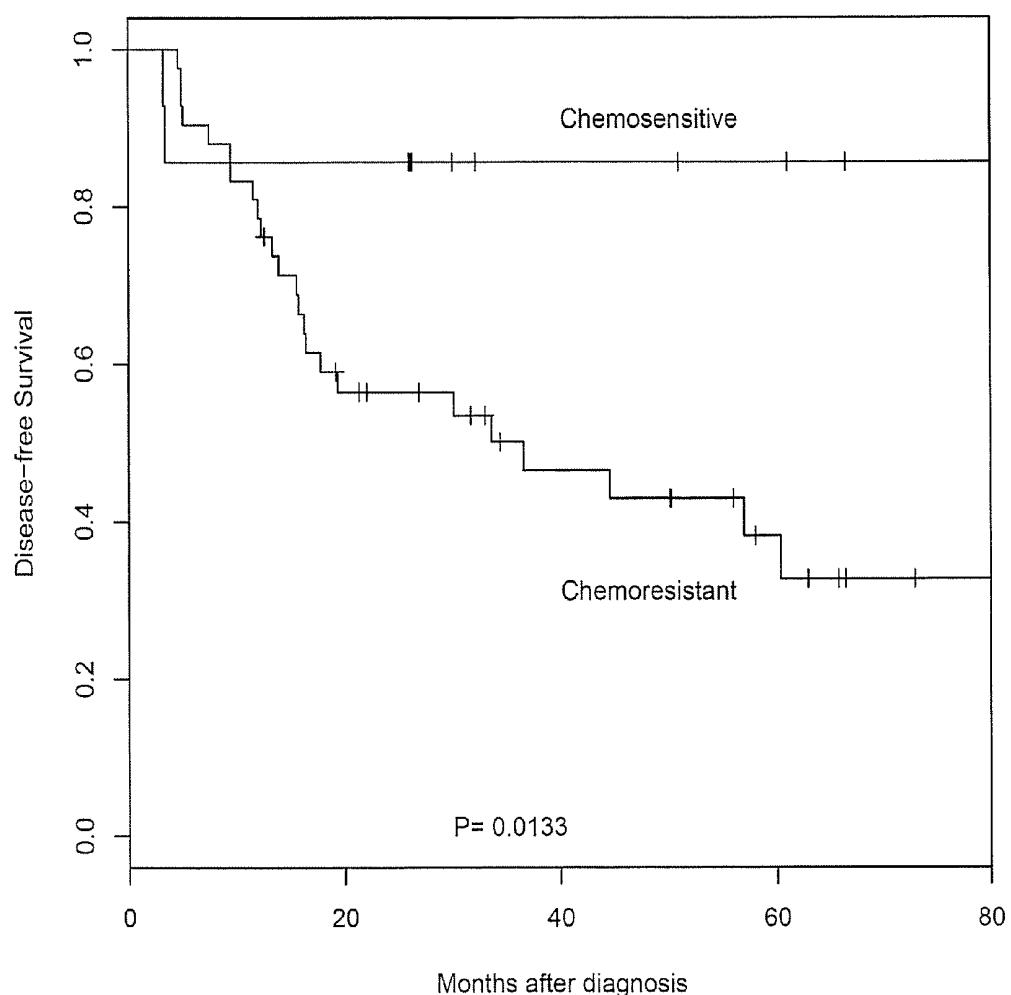


Figure 6:

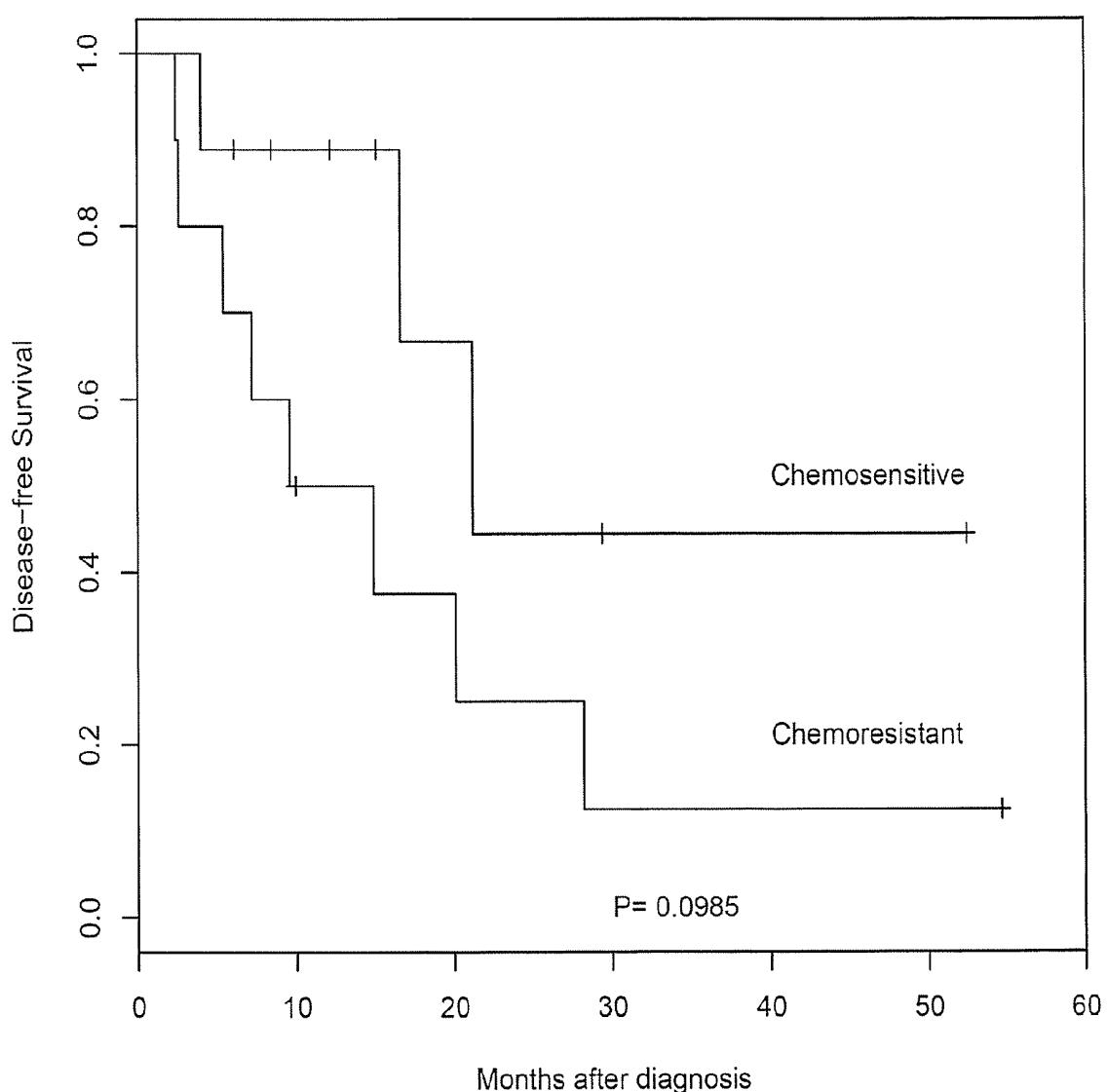


Figure 7:

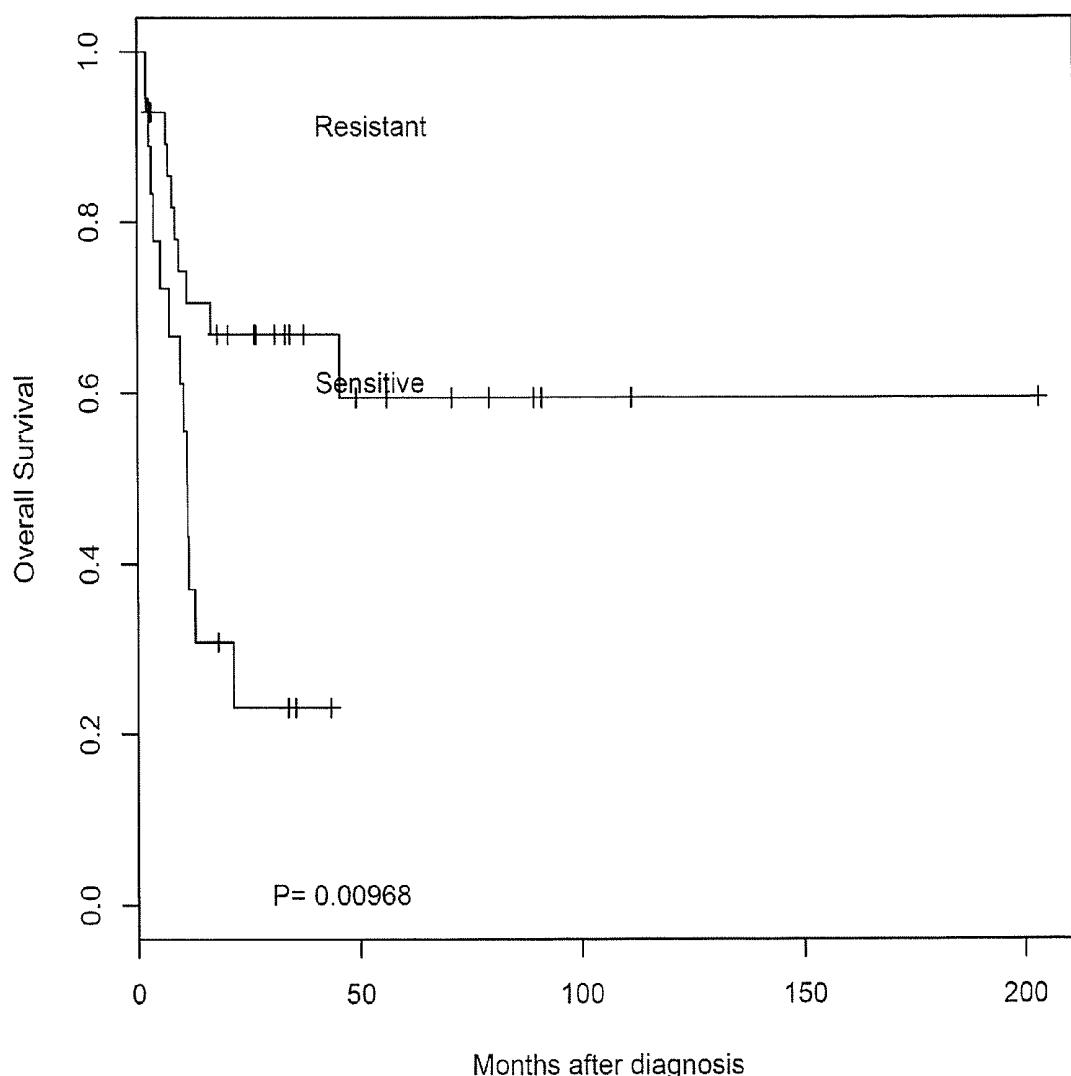


Figure 8:

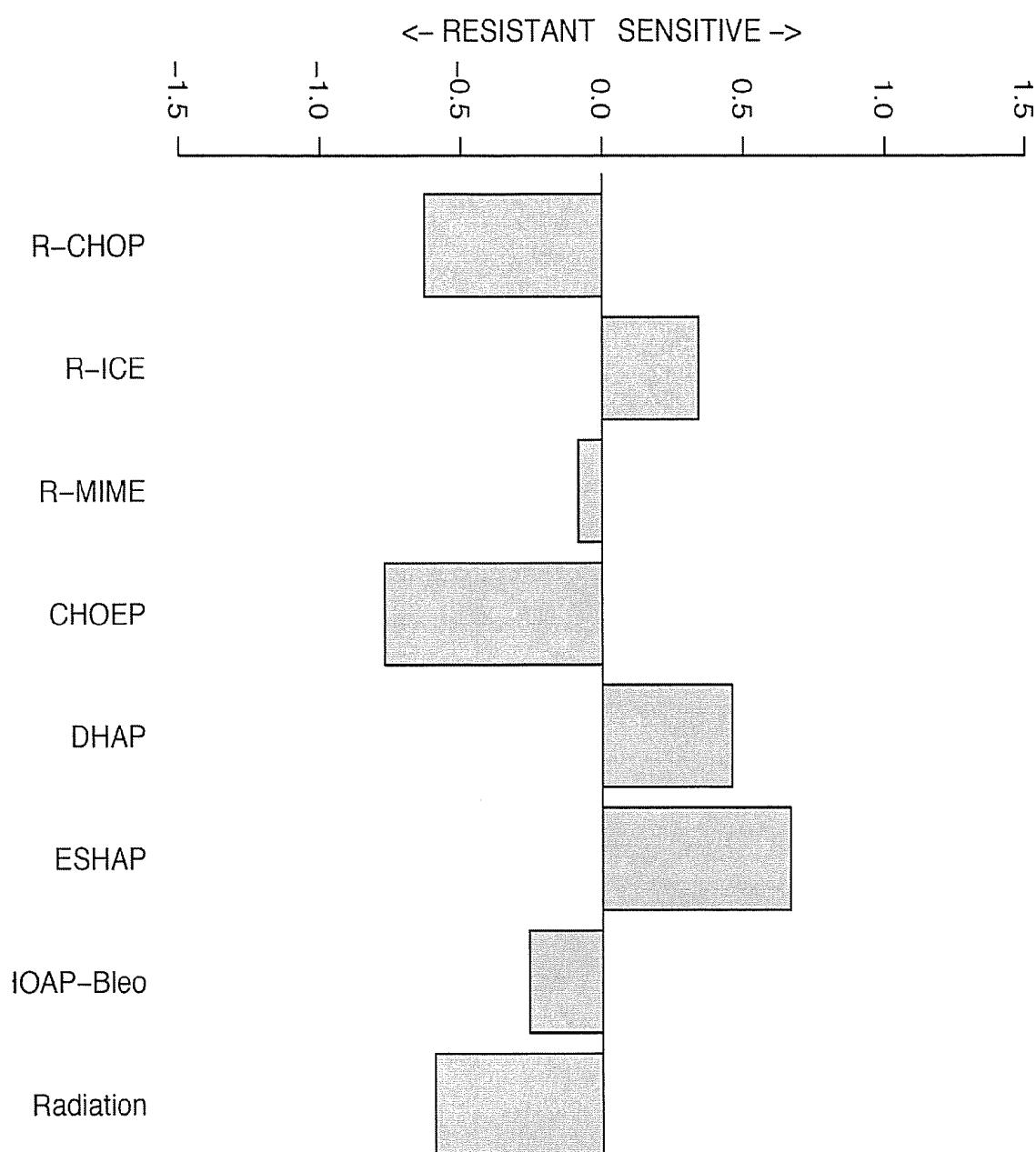


Figure 9:

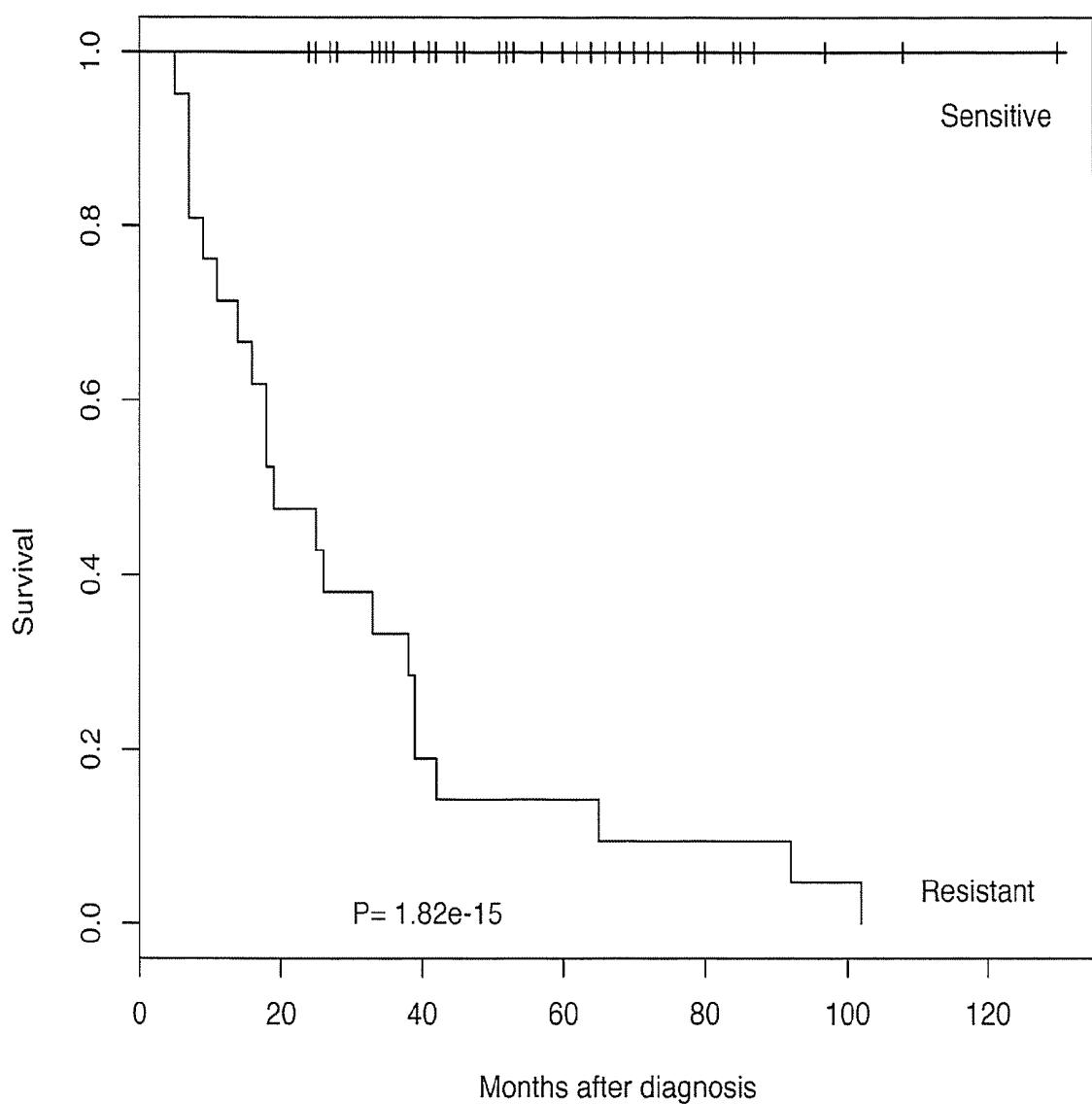


Figure 10:

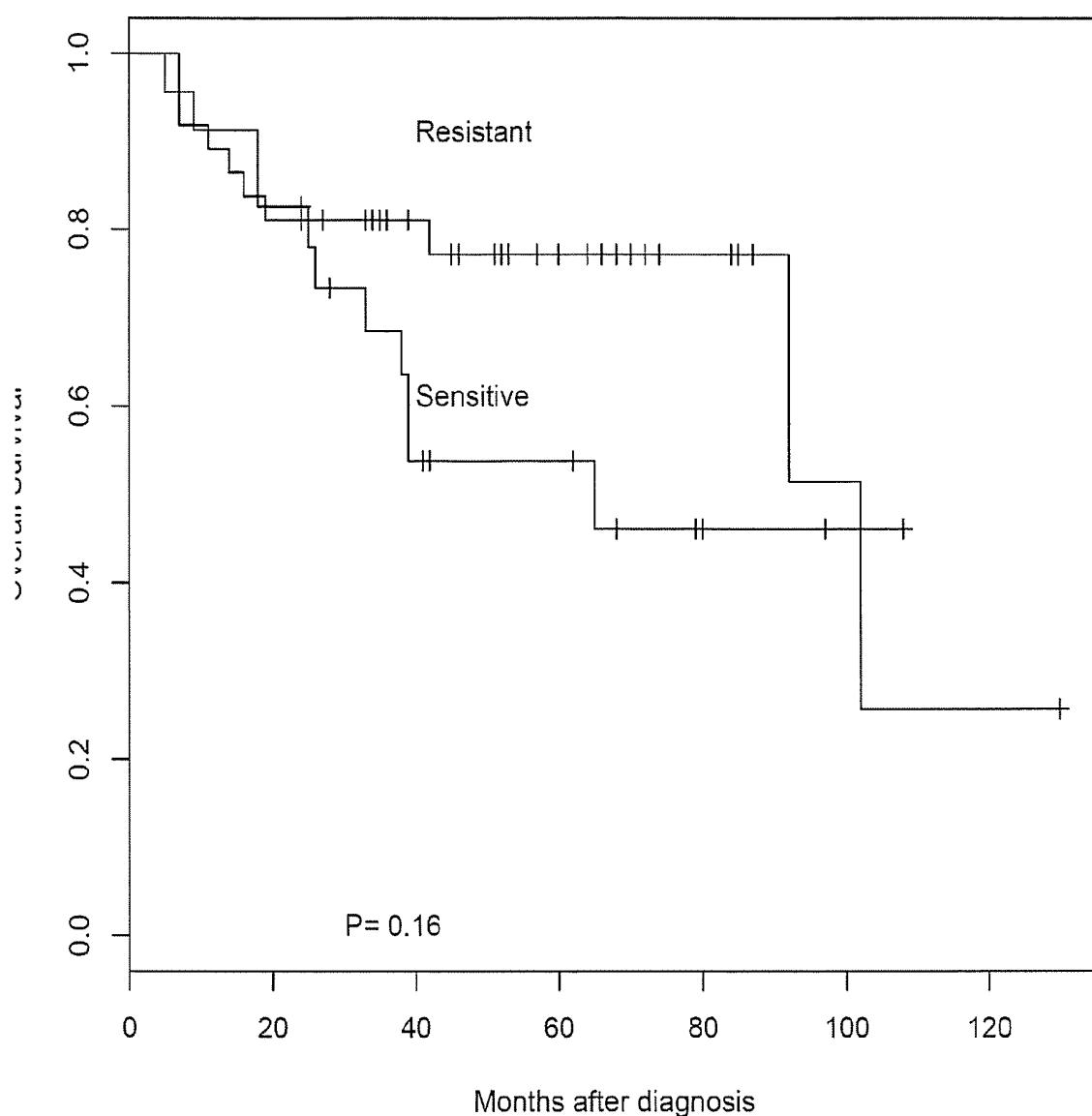
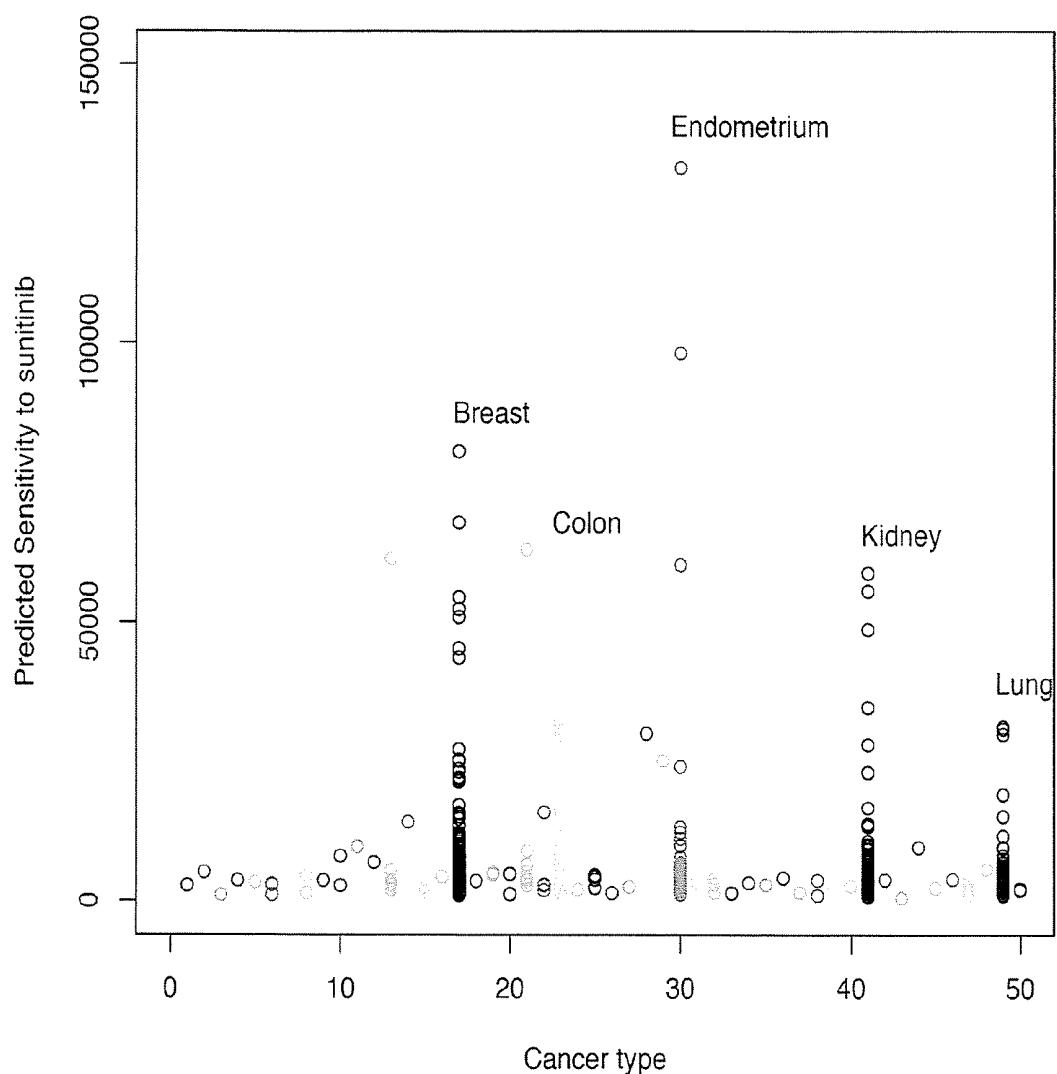


Figure 11:



**METHODS, KITS AND DEVICES FOR
IDENTIFYING BIOMARKERS OF
TREATMENT RESPONSE AND USE
THEREOF TO PREDICT TREATMENT
EFFICACY**

FIELD OF THE INVENTION

[0001] The invention features methods, kits, and devices for identifying biomarkers of patient sensitivity to medical treatments, e.g., sensitivity to chemotherapeutic agents, and predicting treatment efficacy using the biomarkers.

BACKGROUND OF THE INVENTION

[0002] DNA microarrays have been used to measure gene expression in tumor samples from patients and to facilitate diagnosis. Gene expression can reveal the presence of cancer in a patient, its type, stage, and origin, and whether genetic mutations are involved. Gene expression may even have a role in predicting the efficacy of chemotherapy. Over recent decades, the National Cancer Institute (NCI) has tested compounds, including chemotherapy agents, for their effect in limiting the growth of 60 human cancer cell lines. The NCI has also measured gene expression in these 60 cancer cell lines using DNA microarrays. Various studies have explored the relationship between gene expression and compound effect using the NCI datasets. Critical time is often lost due to a trial and error approach to finding an effective chemotherapy for patients with cancer. In addition, cancer cells often develop resistance to a previously effective therapy. In such situations, patient outcome could be greatly improved by early detection of such resistance.

[0003] There remains a need for proven methods and devices that predict the sensitivity or resistance of cancer patients to a medical treatment.

SUMMARY OF THE INVENTION

[0004] The invention features methods, kits, and devices for determining the sensitivity or resistance of a patient, e.g., a cancer patient, to a treatment, e.g., treatment with a compound, such as a chemotherapeutic agent, or radiation. In particular, the methods, kits, and devices can be used to determine the sensitivity or resistance of a cancer patient to any medical treatment, including, e.g., treatment with a compound, drug, or radiation. The methods, kits, and devices of the invention have been used to accurately determine treatment efficacy in cancer patients (e.g., patients with lung, lymphoma, and brain cancer) and can be used to determine treatment efficacy in patients diagnosed with any cancer.

[0005] Methods, kits, and devices for detecting the level of expression of biomarkers (e.g., genes and microRNAs) that indicate sensitivity or resistance to radiation therapy or the chemotherapy agents Vincristine, Cisplatin, Azaguanine, Etoposide, Adriamycin, Aclarubicin, Mitoxantrone, Mitomycin, Paclitaxel, Gemcitabine, Taxotere, Dexamethasone, Ara-C, Methylprednisolone, Methotrexate, Bleomycin, Methyl-GAG, Carboplatin, 5-FU (5-Fluorouracil), Rituximab, PXD101, (a histone deacetylase (HDAC) inhibitor), 5-Aza-2'-deoxycytidine (Decitabine), Melphalan, IL4-PE38 fusion protein, IL13-PE38QQR fusion protein (cintredekin besudotox), Valproic acid (VPA), All-trans retinoic acid (ATRA), Cytoxan, Topotecan (Hycamtin), Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza), Depsipeptide (FR901229), Bortezomib, Leukeran, Fludarabine, Vinblas-

tine, Busulfan, Dacarbazine, Oxaliplatin, Hydroxyurea, Tegafur, Daunorubicin, Bleomycin, Estramustine, Chlorambucil, Mechlorethamine, Streptozocin, Carmustine, Lomustine, Mercaptopurine, Teniposide, Dactinomycin, Tretinoin, Sunitinib, SPC2996, Ifosfamide, Tamoxifen, Floxuridine, Irinotecan, and Satraplatin are also provided. The methods, kits, and devices can be used to predict the sensitivity or resistance of a subject (e.g., a cancer patient) diagnosed with a disease condition, e.g., cancer (e.g., cancers of the breast, prostate, lung and bronchus, colon and rectum, urinary bladder, skin, kidney, pancreas, oral cavity and pharynx, ovary, thyroid, parathyroid, stomach, brain, esophagus, liver and intrahepatic bile duct, cervix larynx, heart, testis, small and large intestine, anus, anal canal and anorectum, vulva, gallbladder, pleura, bones and joints, hypopharynx, eye and orbit, nose, nasal cavity and middle ear, nasopharynx, ureter, peritoneum, omentum and mesentery, or gastrointestinal, as well as any form of cancer including, e.g., chronic myeloid leukemia, acute lymphocytic leukemia, non-Hodgkin's lymphoma, melanoma, carcinoma, basal cell carcinoma, malignant mesothelioma, neuroblastoma, multiple myeloma, leukemia, retinoblastoma, acute myeloid leukemia, chronic lymphocytic leukemia, Hodgkin's lymphoma, carcinoid tumors, acute tumor, or soft tissue sarcoma) to a treatment, e.g., treatment with a compound or drug, e.g., a chemotherapeutic agent, or radiation.

[0006] In a first aspect, the invention features a method of determining sensitivity of a cancer in a patient to a treatment for cancer by measuring the level of expression of at least one gene in a cell (e.g., a cancer cell) of the patient, in which the gene is selected from the group consisting of ACTB, ACTN4, ADA, ADAM9, ADAMTS1, ADD1, AF1Q, AIF1, AKAP1, AKAP13, AKR1C1, AKT1, ALDH2, ALDOC, ALG5, ALMS1, ALOX15B, AMIGO2, AMPD2, AMPD3, ANAPC5, ANP32A, ANP32B, ANXA1, AP1G2, APOBEC3B, APR7, ARHE, ARHGAP15, ARHGAP25, ARHGDIJ, ARHGFE6, ARL7, ASA1, ASPH, ATF3, ATIC, ATP2A2, ATP2A3, ATP5D, ATP5G2, ATP6V1B2, BC008967, BCAT1, BCHE, BCL11B, BDNF, BHLHB2, BIN2, BLMH, BMI1, BNIP3, BRDT, BRRN1, BTN3A3, C11orf2, C14orf139, C15orf25, C18orf10, C1orf24, C1orf29, C1orf38, C1QR1, C22orf18, C6orf32, CACNA1G, CACNB3, CALM1, CALML4, CALU, CAP350, CASP2, CASP6, CASP7, CAST, CBLB, CCNA2, CCNB1IP1, CCND3, CCR7, CCR9, CD1A, CD1C, CD1D, CD1E, CD2, CD28, CD3D, CD3E, CD3G, CD3Z, CD44, CD47, CD59, CD6, CD63, CD8A, CD8B1, CD99, CDC10, CDCl4B, CDH11, CDH2, CDKL5, CDKN2A, CDW52, CECR1, CENPB, CENTB1, CENTG2, CEP1, CG018, CHRNA3, CHS1, CIAPIN1, CKAP4, CKIP-1, CNP, COL4A1, COL5A2, COL6A1, CORO1C, CRABP1, CRK, CRY1, CSDA, CTBP1, CTSC, CTSL, CUGBP2, CUTC, CXCL1, CXCR4, CXorf9, CYFIP2, CYLD, CYR61, DATF1, DAZAPI, DBN1, DBT, DCTN1, DDX18, DDX5, DGKA, DIAPH1, DKC1, DKFZP434J154, DKFZP564C186, DKFZP564G2022, DKFZp564J157, DKFZP564K0822, DNAJC10, DNAJC7, DNAPTP6, DOCK10, DOCK2, DPAGT1, DPEP2, DPYSL3, DSIP1, DUSP1, DXS9879E, EEF1B2, EFNB2, EHD2, EIF5A, ELK3, ENO2, EPAS1, EPB41L4B, ERCC2, ERG, ERP70, EVER1, EVI2A, EVL, EXT1, EZH2, F2R, FABP5, FAD104, FAM46A, FAU, FCGR2A, FCGR2C, FER1L3, FHL1, FHOD1, FKBP1A, FKBP9, FLJ10350, FLJ10539, FLJ10774, FLJ12270, FLJ13373, FLJ20859, FLJ21159, FLJ22457, FLJ35036,

FLJ46603, FLNC, FLOT1, FMNL1, FNBP1, FOLH1, FOXF2, FSCN1, FTL, FYB, FYN, GOS2, G6PD, GALIG, GALNT6, GATA2, GATA3, GFPT1, GIMAP5, GIT2, GJA1, GLRB, GLTSCR2, GLUL, GMDS, GNAQ, GNB2, GNB5, GOT2, GPR65, GPRASP1, GPSM3, GRP58, GSTM2, GTF3A, GTSE1, GZMA, GZMB, H1F0, H1FX, H2AFX, H3F3A, HA-1, HEXB, HIC, HIST1H4C, HK1, HLA-A, HLA-B, HLA-DRA, HMGA1, HMGN2, HMMR, HNRPA1, HNRPD, HNRPM, HOXA9, HRMT1L1, HSA9761, HSPA5, HSU79274, HTATSF1, ICAM1, ICAM2, IER3, IFI16, IFI44, IFITM2, IFITM3, IFRG28, IGFBP2, IGSF4, IL13RA2, IL21R, IL2RG, IL4R, IL6, IL6R, IL6ST, IL8, IMPDH2, INPP5D, INSIG1, IQGAP1, IQGAP2, IRS2, ITG5, ITM2A, JARID2, JUNB, K-ALPHA-1, KHDRBS1, KIAA0355, KIAA0802, KIAA0877, KIAA0922, KIAA1078, KIAA1128, KIAA1393, KIFC1, LAIR1, LAMB1, LAMB3, LAT, LBR, LCK, LCP1, LCP2, LEF1, LEPRE1, LGALS1, LGALS9, LHFPL2, LNK, LOC54103, LOC55831, LOC81558, LOC94105, LONP, LOX, LOXL2, LPHN2, LPXN, LRMP, LRP12, LRRK5, LRRN3, LST1, LTB, LUM, LY9, LY96, MAGEB2, MAL, MAP1B, MAP1LC3B, MAP4K1, MAPK1, MARCKS, MAZ, MCAM, MCL1, MCM5, MCM7, MDH2, MDN1, MEF2C, MFNG, MGC17330, MGC21654, MGC2744, MGC4083, MGC8721, MGC8902, MGLL, MLPH, MPHOSPH6, MPP1, MPZL1, MRP63, MRPS2, MT1E, MT1K, MUF1, MVP, MYB, MYL9, MYO1B, NAP1L1, NAP1L2, NARF, NASP, NCOR2, NDN, NDUFAB1, NDUFAS6, NFKBIA, NID2, NIPA2, NME4, NME7, NNMT, NOL5A, NOL8, NOMO2, NOTCH1, NPC1, NQO1, NR1D2, NUDC, NUP210, NUP88, NVL, NXF1, OBFC1, OCRL, OGT, OXA1L, P2RX5, P4HA1, PACAP, PAF53, PAFAH1B3, PALM2-AKAP2, PAX6, PCBP2, PCCB, PFDNS5, PFN1, PFN2, PGAM1, PHEMX, PHLDA1, PIM2, PITPN1, PLAC8, PLAGL1, PLAUR, PLCB1, PLEK2, PLEKHC1, PLOD2, PLSCR1, PNAs-4, PNMA2, POLR2F, PPAP2B, PRF1, PRG1, PRIM1, PRKCH, PRKCQ, PRKD2, PRNP, PRP19, PRPF8, PRSS23, PSCDBP, PSMB9, PSMC3, PSME2, PTGER4, PTGES2, PTOV1, PTP4A3, PTPN7, PTPNS1, PTRF, PURA, PWP1, PYGL, QKI, RAB3GAP, RAB7L1, RAB9P40, RAC2, RAFTLIN, RAG2, RAP1B, RASGRP2, RBPM5, RCN1, RFC3, RFC5, RGC32, RGS3, RHOH, RIMS3, RIOK3, RIPK2, RIS1, RNASE6, RNF144, RPL10, RPL10A, RPL12, RPL13A, RPL17, RPL18, RPL36A, RPLP0, RPLP2, RPS15, RPS19, RPS2, RPS4X, RPS4Y1, RRAS, RRAS2, RRBPI, RRM2, RUNX1, RUNX3, S100A4, SART3, SATB1, SCAP1, SCARB1, SCN3A, SEC31L2, SEC61G, SELL, SELPLG, SEMA4G, SEPT10, SEPT6, SERPINA1, SERPINB1, SERPINB6, SFRS5, SFRS6, SFRS7, SH2D1A, SH3GL3, SH3TC1, SHD1, SHMT2, SIAT1, SKB1, SKP2, SLA, SLC1A4, SLC20A1, SLC25A15, SLC25A5, SLC39A14, SLC39A6, SLC43A3, SLC4A2, SLC7A11, SLC7A6, SMAD3, SMOX, SNRPA, SNRPB, SOD2, SOX4, SP140, SPANXC, SPI1, SRF, SRM, SSA2, SSBP2, SSRP1, SSSCA1, STAG3, STAT1, STAT4, STAT5A, STC1, STC2, STOML2, T3JAM, TACC1, TACC3, TAF5, TAL1, TAP1, TARP, TBKA, TCF12, TCF4, TFDP2, TFPI, TIMM17A, TIMP1, TJP1, TK2, TM4SF1, TM4SF2, TM4SF8, TM6SF1, TMEM2, TMEM22, TMSB10, TMSNB, TNFAIP3, TNFAIP8, TNFRSF10B, TNFRSF1A, TNFRSF7, TNIK, TNPO1, TOB1, TOMM20, TOX, TPK1, TPM2, TRA@, TRA1, TRAM2, TRB@, TRD@, TRIM, TRIM14, TRIM22, TRIM28, TRIP13, TRPV2, TUBGCP3, TUSC3, TXN,

TXNDC5, UBASH3A, UBE2A, UBE2L6, UBE2S, UCHL1, UCK2, UCP2, UFD1L, UGDH, ULK2, UMPS, UNG, USP34, USP4, VASP, VAV1, VLDR, VWF, WASPIP, WBSCR20A, WBSCR20C, WHSC1, WNT5A, ZAP70, ZFP36L1, ZNF32, ZNF335, ZNF593, ZNFN1A1, and ZYX; in which change in the level of expression of the gene indicates the cell is sensitive or resistant to the treatment.

[0007] In an embodiment, the method further includes determining a patient's resistance or sensitivity to radiation therapy or the chemotherapy agents Vincristine, Cisplatin, Adriamycin, Etoposide, Azaguanine, Aclarubicin, Mitoxantrone, Paclitaxel, Mitomycin, Gemcitabine, Taxotere, Dexamethasone, Methylprednisolone, Ara-C, Methotrexate, Bleomycin, Methyl-GAG, Rituximab, PXD101 (a histone deacetylase (HDAC) inhibitor), 5-Aza-2'-deoxycytidine (Decitabine), Melphalan, IL4-PE38 fusion protein, IL13-PE38QQ fusion protein (cintredekin besudotox), Valproic acid (VPA), All-trans retinoic acid (ATRA), Cytoxan, Topotecan (Hycamtin), Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza), Depsipeptide (FR901229), Bortezomib, Leukeran, Fludarabine, Vinblastine, Busulfan, Dacarbazine, Oxaliplatin, Hydroxyurea, Tegafur, Daunorubicin, Bleomycin, Estramustine, Chlorambucil, Mechlorethamine, Streptozocin, Carmustine, Lomustine, Mercaptopurine, Teniposide, Dactinomycin, Tretinoin, Sunitinib, SPC2996, Ifosfamide, Tamoxifen, Floxuridine, frinotecan, and Satraplatin by measuring the level of expression of one or more of the genes known to change (e.g., to increase or decrease) in a patient sensitive to treatment with these agents (e.g., a patient is determined to be sensitive, or likely to be sensitive, to the indicated treatment if the level of expression of one or more of the gene(s) increases or decreases relative to the level of expression of the gene(s) in a control sample (e.g., a cell or tissue) in which increased or decreased expression of the gene(s) indicates sensitivity to the treatment, and vice versa). Alternatively, a patient's resistance or sensitivity to radiation therapy or any of the chemotherapy agents listed above can be determined by measuring the level of expression of at least one microRNA in a cell (e.g., a cancer cell) known to change (e.g., the level of expression is increased or decreased) in a patient sensitive to a treatment with these agents, in which the microRNA is selected from the group consisting of ath-MIR180aNo2, Hcd102 left, Hcd111 left, Hcd115 left, Hcd120 left, Hcd142 right, Hcd145 left, Hcd148_HPR225 left, Hcd181 left, Hcd181 right, Hcd210_HPR205 right, Hcd213_HPR182 left, Hcd230 left, Hcd243 right, Hcd246 right, Hcd248 right, Hcd249 right, Hcd250 left, Hcd255 left, Hcd257 left, Hcd257 right, Hcd263 left, Hcd266 left, Hcd270 right, Hcd279 left, Hcd279 right, Hcd28_HPR39left, Hcd28_HPR39right, Hcd282PO right, Hcd289 left, Hcd294 left, Hcd318 right, Hcd323 left, Hcd330 right, Hcd338 left, Hcd340 left, Hcd350 right, Hcd355_HPR190 left, Hcd361 right, Hcd366 left, Hcd373 right, Hcd383 left, Hcd383 right, Hcd384 left, Hcd397 left, Hcd404 left, Hcd412 left, Hcd413 right, Hcd415 right, Hcd417 right, Hcd421 right, Hcd425 left, Hcd438right, Hcd434 right, Hcd438 left, Hcd440_HPR257 right, Hcd444 right, Hcd447 right, Hcd448 left, Hcd498 right, Hcd503 left, Hcd511 right, Hcd512 left, Hcd514 right, Hcd517 left, Hcd517 right, Hcd530 right, Hcd536_HPR104 right, Hcd542 left, Hcd544 left, Hcd547 left, Hcd559 right, Hcd562 right, Hcd569 right, Hcd570 right, Hcd578 right, Hcd581 right, Hcd586 left, Hcd586 right, Hcd587 right, Hcd605 left, Hcd605 left, Hcd605 right, Hcd608 right, Hcd627 left, Hcd631 left, Hcd631 right, Hcd634 left, Hcd642

right, Hcd649 right, Hcd654 left, Hcd658 right, Hcd669 right, Hcd674 left, Hcd678 right, Hcd683 left, Hcd684 right, Hcd689 right, Hcd690 right, Hcd691 right, Hcd693 right, Hcd697 right, Hcd704 left, Hcd704 left, Hcd712 right, Hcd716 right, Hcd731 left, Hcd738 left, Hcd739 right, Hcd739 right, Hcd749 right, Hcd753 left, Hcd754 left, Hcd755 left, Hcd760 left, Hcd763 right, Hcd768 left, Hcd768 right, Hcd770 left, Hcd773 left, Hcd777 left, Hcd778 right, Hcd781 left, Hcd781 right, Hcd782 left, Hcd783 left, Hcd788 left, Hcd794 right, Hcd796 left, Hcd799 left, Hcd807 right, Hcd812 left, Hcd817 left, Hcd817 right, Hcd829 right, Hcd852 right, Hcd861 right, Hcd863PO right, Hcd866 right, Hcd869 left, Hcd873 left, Hcd886 right, Hcd889 right, Hcd891 right, Hcd892 left, Hcd913 right, Hcd923 left, Hcd923 right, Hcd938 left, Hcd938 right, Hcd939 right, Hcd946 left, Hcd948 right, Hcd960 left, Hcd965 left, Hcd970 left, Hcd975 left, Hcd976 right, Hcd99 right, HPR100 right, HPR129 left, HPR154 left, HPR159 left, HPR163 left, HPR169 right, HPR172 right, HPR181 left, HPR187 left, HPR199 right, HPR206 left, HPR213 right, HPR214 right, HPR220 left, HPR220 right, HPR227 right, HPR232 right, HPR233 right, HPR244 right, HPR262 left, HPR264 right, HPR266 right, HPR271 right, HPR76 right, hsa_mir_490_Hcd20 right, HSHELA01, HSTRNL, HUMTRAB, HUMTRF, HUMTRN, HUMTRS, HUMTRV1A, let-7f-2-prec2, mir-001b-1-prec1, mir-001b-2-prec, mir-007-1-prec, mir-007-2-precNo2, mir-010a-precNo1, mir-015b-precNo2, mir-016a-chr13, mir-016b-chr3, mir-0,7-precNo1, mir-0,7-precNo2, mir-018-prec, mir-019a-prec, mir-019b-1-prec, mir-019b-2-prec, mir-020-prec, mir-022-prec, mir-023a-prec, mir-023b-prec, mir-024-2-prec, mir-025-prec, mir-027b-prec, mir-029c-prec, mir-032-precNo2, mir-033b-prec, mir-033-prec, mir-034-precNo1, mir-034-precNo2, mir-092-prec-13=092-1No2, mir-092-prec-X=092-2, mir-093-prec-7.1=093-1, mir-095-prec-4,mir-096-prec-7No1, mir-096-prec-7No2, mir-098-prec-X, mir-099b-prec-19No1, mir-100-1/2-prec, mir-100No1, mir-101-prec-9, mir-102-prec-1, mir-103-2-prec, mir-103-prec-5=103-1, mir-106aNo1, mir-106-prec-X, mir-107No1, mir-107-prec-10, mir-122a-prec, mir-123-precNo1, mir-123-precNo2, mir-124a-1-prec1, mir-124a-2-prec, mir-124a-3-prec, mir-125b-1, mir-125b-2-precNo2, mir-127-prec, mir-128b-precNo1, mir-128b-precNo2, mir-133a-1, mir-135-2-prec, mir-136-precNo2, mir-138-1-prec, mir-140No2, mir-142-prec, mir-143-prec, mir-144-precNo2, mir-145-prec, mir-146bNo1, mir-146-prec, mir-147-prec, mir-148aNo1, mir-148-prec, mir-149-prec, mir-150-prec, mir-153-1-prec1, mir-154-prec1No1, mir-155-prec, mir-15aNo1, mir-16-1No1, mir-16-2No1, mir-181a-precNo1, mir-181b-1No1, mir-181b-2No1, mir-181b-precNo1, mir-181b-precNo2, mir-181c-precNo1, mir-181dNo1, mir-188-prec, mir-18bNo2, mir-191-prec, mir-192No2, mir-193bNo2, mir-194-2No1, mir-195-prec, mir-196-2-precNo2, mir-197-prec, mir-198-prec, mir-199a-1-prec, mir-199a-2-prec, mir-199b-precNo1, mir-200a-prec, mir-200bNo1, mir-200bNo2, mir-202*, mir-202-prec, mir-204-precNo2, mir-205-prec, mir-208-prec, mir-20bNo1, mir-2,2-precNo1, mir-2,2-precNo2, mir-2,3-precNo1, mir-2,4-prec, mir-2,5-precNo2, mir-2,6-precNo1, mir-219-2No1, mir-2,9-prec, mir-223-prec, mir-29b-1No1, mir-29b-2=102prec7.1=7.2, mir-321No1, mir-321No2, mir-324No1, mir-324No2, mir-328No1, mir-342No1, mir-361No1, mir-367No1, mir-370No1, mir-371No1, mir-373*No1, mir-375, mir-376aNo1, mir-379No1, mir-380-5p, mir-382, mir-384, mir-409-3p, mir-423No1, mir-424No2, mir-429No1, mir-

429No2, mir-4323p, mir-4325p, mir-449No1, mir-450-1, mir-450-2No1, mir-483No1, mir-484, mir-487No1, mir-495No1, mir-499No2, mir-501No2, mir-503No1, mir-509No1, mir-514-1No2, mir-515-15p, mir-515-23p, mir-516-33p, mir-516-43p, mir-518e/526c, mir-519a-1/52, mir-519a-2No2, mir-519b, mir-519c/52, mir-520c/52, mir-526a-2No1, mir-526a-2No2, MPR103 right, MPR121 left, MPR121 left, MPR130 left, MPR130 right, MPR133 right, MPR141 left, MPR151 left, MPR156 left, MPR162 left, MPR174 left, MPR174 right, MPR185 right, MPR197 right, MPR203 left, MPR207 right, MPR215 left, MPR216 left, MPR224 left, MPR224 right, MPR228 left, MPR234 right, MPR237 left, MPR243 left, MPR244 right, MPR249 left, MPR254 right, MPR74 left, MPR88 right, and MPR95 left.

[0008] In an embodiment, the method includes determining the expression of two of the listed genes or microRNAs, more preferably three, four, five, six, seven, eight, nine, or ten of the listed genes, and most preferably twenty, thirty, forty, fifty, sixty, seventy, eighty, ninety, or one hundred or more of the listed genes. In another embodiment, the change in the level of gene or microRNA expression (e.g., an increase or decrease) is determined relative to the level of gene or microRNA expression in a cell or tissue known to be sensitive to the treatment, such that a similar level of gene or microRNA expression exhibited by a cell or tissue of the patient indicates the patient is sensitive to the treatment. In another embodiment, the change in the level of gene or microRNA expression (e.g., an increase or decrease) is determined relative to the level of gene or microRNA expression in a cell or tissue known to be resistant to the treatment, such that a similar level of gene or microRNA expression exhibited by a cell or tissue of the patient indicates the patient is resistant to the treatment.

[0009] In a second aspect, the invention features a method of determining sensitivity of a cancer in a patient to a treatment for cancer by measuring the level of expression of at least one microRNA in a cell (e.g., a cancer cell) of the patient, in which the microRNA is selected from the group set forth in the first aspect of the invention. In an embodiment, the method further includes determining a patient's resistance or sensitivity to radiation therapy or any of the chemotherapy agents set forth in the first aspect of the invention by measuring the level of expression of one or more of the microRNAs known to change (e.g., to increase or decrease) in a patient sensitive to treatment with these agents (e.g., a patient is determined to be sensitive, or likely to be sensitive, to the indicated treatment if the level of expression of one or more of the microRNA(s) increases or decreases relative to the level of expression of the microRNA(s) in a control sample (e.g., a cell or tissue) in which increased or decreased expression of the microRNA(s) indicates sensitivity to the treatment, and vice versa). In an embodiment, the method includes determining the expression of two of the listed genes or microRNAs, more preferably three, four, five, six, seven, eight, nine, or ten of the listed genes, and most preferably twenty, thirty, forty, fifty, sixty, seventy, eighty, ninety, or one hundred or more of the listed genes. In another embodiment, the change in the level of microRNA expression (e.g., an increase or decrease) is determined relative to the level of microRNA expression in a cell or tissue known to be sensitive to the treatment, such that a similar level of microRNA expression exhibited by a cell or tissue of the patient indicates the patient is sensitive to the treatment. In another embodiment, the change in the level of microRNA expression (e.g., an increase

or decrease) is determined relative to the level of microRNA expression in a cell or tissue known to be resistant to the treatment, such that a similar level of microRNA expression exhibited by a cell or tissue of the patient indicates the patient is resistant to the treatment.

[0010] In another embodiment, the invention features a method for determining the development of resistance by a patient (e.g., resistance of a cell, such as a cancer cell, in the patient) to a treatment to which the patient was previously sensitive. The method includes measuring the level of expression of one or more of the microRNAs set forth in the first aspect of the invention, such that the level of expression of a microRNA which is decreased in a cell or tissue known to be sensitive to the treatment indicates that the patient is resistant to or has a propensity to become resistant to the treatment. Alternatively, a decrease in the expression level of a microRNA which is increased in a cell or tissue known to be sensitive to the treatment indicates that the patient is resistant to or has a propensity to become resistant to the treatment.

[0011] In a third aspect, the invention features a kit that includes a single-stranded nucleic acid molecule (e.g., one or a plurality thereof; e.g., a deoxyribonucleic acid molecule or a ribonucleic acid molecule) that is substantially complementary to (e.g., that has at least 80%, 90%, 95% 97%, 99%, or 100% identical to the complement of) or that is substantially identical to (e.g., that has at least 80%, 90%, 95% 97%, 99%, or 100% identity to) at least 5 consecutive nucleotides (more preferably at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, or more consecutive nucleotides; the nucleic acid can also be 5-20, 25, 5-50, 50-100, or over 100 consecutive nucleotides long) of at least one of the genes (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more of the genes) set forth in the first aspect of the invention, such that the single-stranded nucleic acid molecule is sufficient for measuring the level of expression of the gene(s) by allowing specific hybridization between the single-stranded nucleic acid molecule and a nucleic acid molecule encoded by the gene, or a complement thereof. Alternatively, the kit includes one or more single-stranded nucleic acid molecules that are substantially complementary to or substantially identical to at least 5 consecutive nucleotides of at least one of the microRNAs set forth in the first aspect of the invention, such that the single-stranded nucleic acid molecule is sufficient for measuring the level of expression of the microRNA(s) by allowing specific hybridization between the single-stranded nucleic acid molecule and the microRNA, or a complement thereof. The kit further includes instructions for applying nucleic acid molecules collected from a sample from a cancer patient (e.g., from a cell of the patient), determining the level of expression of the gene(s) or microRNA(s) hybridized to the single-stranded nucleic acid, and determining the patient's sensitivity to a treatment for cancer when use of the kit indicates that the level of expression of the gene(s) or microRNA(s) changes (e.g., increases or decreases relative to a control sample (e.g., tissue or cell) known to be sensitive or resistant to the treatment, as is discussed above in connection with the first aspect of the invention). In an embodiment, the instructions further indicate that a change in the level of expression of the gene(s) or microRNA(s) relative to the expression of the gene(s) or microRNA(s) in a control sample (e.g., a cell or tissue known to be sensitive or resistant to the treatment) indicates a change in sensitivity of the patient to the treatment (e.g., a decrease in the level of expression of a gene or

microRNA known to be expressed in cells sensitive to the treatment indicates that the patient is becoming resistant to the treatment or is likely to become resistant to the treatment, and vice versa).

[0012] In another embodiment, the kit can be utilized to determine a patient's resistance or sensitivity to radiation therapy or the chemotherapy agents Vincristine, Cisplatin, Adriamycin, Etoposide, Azaguanine, Aclarubicin, Mitoxantrone, Paclitaxel, Mitomycin, Gemcitabine, Taxotere, Dexamethasone, Methylprednisolone, Ara-C, Methotrexate, Bleomycin, Methyl-GAG, Rituximab, PXD101 (a histone deacetylase (HDAC) inhibitor), 5-Aza-2'-deoxycytidine (Decitabine), Melphalan, IL4-PE38 fusion protein, IL13-PE38QQR fusion protein (cintredekin besudotox), Valproic acid (VPA), All-trans retinoic acid (ATRA), Cytoxan, Topotecan (Hycamtin), Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza), Depsipeptide (FR901229), Bortezomib, Leukeran, Fludarabine, Vinblastine, Busulfan, Dacarbazine, Oxaliplatin, Hydroxyurea, Tegafur, Daunorubicin, Bleomycin, Estramustine, Chlorambucil, Mechlorethamine, Streptozocin, Carmustine, Lomustine, Mercaptopurine, Teniposide, Dactinomycin, Tretinoin, Sunitinib, SPC2996, Ifosfamide, Tamoxifen, Floxuridine, Irinotecan, and Satraplatin by measuring the level of expression of one or more of the genes or microRNAs set forth in the first aspect of the invention and known to change (e.g., to increase or decrease) in a patient sensitive to treatment with these agents (e.g., a patient is determined to be sensitive, or likely to be sensitive, to the indicated treatment if the level of expression of one or more of the gene(s) or microRNA(s) increases or decreases relative to the level of expression of the gene(s) or microRNA(s) in a control sample (e.g., a cell or tissue) in which increased or decreased expression of the gene(s) or microRNA(s) indicates sensitivity to the treatment, and vice versa).

[0013] In another embodiment, the nucleic acid molecules are characterized by their ability to specifically identify nucleic acid molecules complementary to the genes or microRNAs in a sample collected from a cancer patient.

[0014] In a fourth aspect, the invention features a kit that includes a single-stranded nucleic acid molecule (e.g., one or a plurality thereof; e.g., a deoxyribonucleic acid molecule or a ribonucleic acid molecule) that is substantially complementary to (e.g., that has at least 80%, 90%, 95% 97%, 99%, or 100% identical to the complement of) or that is substantially identical to (e.g., that has at least 80%, 90%, 95% 97%, 99%, or 100% identity to) at least 5 consecutive nucleotides (more preferably at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, or more consecutive nucleotides; the nucleic acid can also be 5-20, 25, 5-50, 50-100, or over 100 consecutive nucleotides long) of at least one of the microRNAs (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more of the microRNAs) set forth in the first aspect of the invention, such that the single-stranded nucleic acid molecule is sufficient for measuring the level of expression of the microRNA(s) by allowing specific hybridization between the single-stranded nucleic acid molecule and a microRNA, or a complement thereof. The kit further includes instructions for applying nucleic acid molecules collected from a sample from a cancer patient (e.g., from a cell of the patient), determining the level of expression of the microRNA(s) hybridized to the single-stranded nucleic acid, and determining the patient's sensitivity to a treatment for cancer when use of the kit indicates that the level of expression of microRNA(s) changes (e.g.,

increases or decreases relative to a control sample (e.g., tissue or cell) known to be sensitive or resistant to the treatment, as is discussed above in connection with the first aspect of the invention). In an embodiment, the instructions further indicate that a change in the level of expression of microRNA(s) relative to the expression of microRNA(s) in a control sample (e.g., a cell or tissue known to be sensitive or resistant to the treatment) indicates a change in sensitivity of the patient to the treatment (e.g., a decrease in the level of expression of a microRNA known to be expressed in cells sensitive to the treatment indicates that the patient is becoming resistant to the treatment or is likely to become resistant to the treatment, and vice versa).

[0015] In another embodiment, the kit can be utilized to determine a patient's resistance or sensitivity to radiation therapy or the chemotherapy agents Vincristine, Cisplatin, Adriamycin, Etoposide, Azaguanine, Aclarubicin, Mitoxantrone, Paclitaxel, Mitomycin, Gemcitabine, Taxotere, Dexamethasone, Methylprednisolone, Ara-C, Methotrexate, Bleomycin, Methyl-GAG, Rituximab, PXD101 (a histone deacetylase (HDAC) inhibitor), 5-Aza-2'-deoxycytidine (Decitabine), Melphalan, IL4-PE38 fusion protein, IL13-PE38QQR fusion protein (cintredekin besudotox), Valproic acid (VPA), All-trans retinoic acid (ATRA), Cytoxin, Topotecan (Hycamtin), Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza), Depsiteptide (FR901229), Bortezomib, Leukeran, Fludarabine, Vinblastine, Busulfan, Dacarbazine, Oxaliplatin, Hydroxyurea, Tegafur, Daunorubicin, Bleomycin, Estramustine, Chlorambucil, Mechlorethamine, Streptozocin, Carmustine, Lomustine, Mercaptopurine, Teniposide, Dactinomycin, Tretinoin, Sunitinib, SPC2996, Ifosfamide, Tamoxifen, Floxuridine, Irinotecan, and Satraplatin by measuring the level of expression of one or more of the microRNAs set forth in the first aspect of the invention and known to change (e.g., to increase or decrease) in a patient sensitive to treatment with these agents (e.g., a patient is determined to be sensitive, or likely to be sensitive, to the indicated treatment if the level of expression of one or more of the microRNA(s) increases or decreases relative to the level of expression of the microRNA(s) in a control sample (e.g., a cell or tissue) in which increased or decreased expression of the or microRNA(s) indicates sensitivity to the treatment, and vice versa).

[0016] In another embodiment, the nucleic acid molecules are characterized by their ability to specifically identify nucleic acid molecules complementary to the microRNAs in a sample collected from a cancer patient.

[0017] In a fifth aspect, the invention features a method of identifying biomarkers (e.g., genes and microRNAs) indicative of sensitivity of a cancer patient to a treatment for cancer by obtaining pluralities of measurements of the expression level of a gene or microRNA (e.g., by detection of the expression of a gene or microRNA using a single probe or by using multiple probes directed to a single gene or microRNA) in different cell types and measurements of the growth of those cell types in the presence of a treatment for cancer relative to the growth of the cell types in the absence of the treatment for cancer; correlating each plurality of measurements of the expression level of the gene or microRNA in cells with the growth of the cells to obtain a correlation coefficient; selecting the median correlation coefficient calculated for the gene or microRNA; and identifying the gene or microRNA as a biomarker for use in determining the sensitivity of a cancer patient to said treatment for cancer if said median correlation coefficient exceeds 0.3 (preferably the gene or microRNA is

identified as a biomarker for a patient's sensitivity to a treatment if the correlation coefficient exceeds 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 0.95, or 0.99 or more). In an embodiment, the method is performed in the presence of a second treatment.

[0018] In a sixth aspect, the invention features a method of determining sensitivity of a patient (e.g., a cancer patient) to a treatment for cancer by obtaining a measurement of the level of expression of a gene or microRNA in a sample (e.g., a cell or tissue) from the patient; applying a model predictive of sensitivity to a treatment for cancer to the measurement, in which the model is developed using an algorithm selected from the group consisting of linear sums, nearest neighbor, nearest centroid, linear discriminant analysis, support vector machines, and neural networks; and determining whether or not the patient will be responsive to the treatment for cancer. In an embodiment, the measurement is obtained by measuring the level of expression of any of the genes or microRNAs set forth in the first aspect of the invention in a cell known to be sensitive or resistant to the treatment. In another embodiment, the method is performed in the presence of a second treatment. In another embodiment, the model combines the outcomes of linear sums, linear discriminant analysis, support vector machines, neural networks, k-nearest neighbors, and nearest centroids, or the model is cross-validated using a random sample of multiple measurements. In another embodiment, treatment, e.g., a compound, has previously failed to show efficacy in a patient. In several embodiments, the linear sum is compared to a sum of a reference population with known sensitivity; the sum of a reference population is the median of the sums derived from the population members' biomarker gene expression. In another embodiment, the model is derived from the components of a data set obtained by independent component analysis or is derived from the components of a data set obtained by principal component analysis. In another embodiment, the invention features a kit, apparatus, and software used to implement the method of the sixth aspect of the invention.

[0019] In several embodiments of all aspects of the invention, the level of expression of the gene(s) is determined by measuring the level of mRNA transcribed from the gene(s), by detecting the level of a protein product of the gene(s), or by detecting the level of the biological activity of a protein product of the gene(s). In further embodiments of all aspects of the invention, an increase or decrease in the expression level of the gene(s) or microRNA(s), relative to the expression level of the gene(s) or microRNA(s) in a cell or tissue sensitive to the treatment, indicates increased sensitivity of the cancer patient to the treatment. Alternatively, an increase or decrease in the expression level of the gene(s) or microRNA(s), relative to the expression level of the gene(s) or microRNA(s) in a cell or tissue resistant to the treatment, indicates increased resistance of the cancer patient to the treatment. In another embodiment of all aspects of the invention, the cell is a cancer cell. In another embodiment of all aspects of the invention, the expression level of the gene(s) is measured using a quantitative reverse transcription-polymerase chain reaction (qRT-PCR). In an embodiment of all aspects of the invention, the level of expression of two of the listed genes or microRNAs is measured, more preferably the level of expression of three, four, five, six, seven, eight, nine, or ten of the listed genes or microRNAs is measured, and most preferably twenty, thirty, forty, fifty, sixty, seventy, eighty, ninety, or one hundred or more of the listed genes or microRNAs is measured. In another embodiment of all aspects of the invention, the

expression level of the gene(s) or microRNA(s) is determined using the kit of the third or fourth aspects of the invention.

[0020] In another embodiment of all aspects of the invention, the treatment is radiation therapy or a compound, such as a chemotherapy agent selected from the group consisting of Vincristine, Cisplatin, Adriamycin, Etoposide, Azaguanine, Aclarubicin, Mitoxantrone, Paclitaxel, Mitomycin, Gemcitabine, Taxotere, Dexamethasone, Methylprednisolone, Ara-C, Methotrexate, Bleomycin, Methyl-GAG, Rituximab, PXD101 (a histone deacetylase (HDAC) inhibitor), 5-Aza-2'-deoxycytidine (Decitabine), Melphalan, IL4-PE38 fusion protein, IL13-PE38QQR fusion protein (cintredekin besudotox), Valproic acid (VPA), All-trans retinoic acid (ATRA), Cytoxan, Topotecan (Hycamtin), Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza), Depsi peptide (FR901229), Bortezomib, Leukeran, Fludarabine, Vinblastine, Busulfan, Dacarbazine, Oxaliplatin, Hydroxyurea, Tegafur, Daunorubicin, Bleomycin, Estramustine, Chlorambucil, Mechlorethamine, Streptozocin, Carmustine, Lomustine, Mercaptopurine, Teniposide, Dactinomycin, Tretinoin, Sunitinib, SPC2996, Ifosfamide, Tamoxifen, Flouxuridine, Irinotecan, and Satraplatin. In another embodiment of all aspects of the invention, the treatment has previously failed to show effect in a subject (e.g., a subject selected from a subpopulation determined to be sensitive to the treatment, a subject selected from a subpopulation predicted to die without treatment, a subject selected from a subpopulation predicted to have disease symptoms without treatment, a subject selected from a subpopulation predicted to be cured without treatment).

[0021] In another embodiment of all aspects of the invention, the treatment is, e.g., administration of a compound, a protein, an antibody, an oligonucleotide, a chemotherapeutic agent, or radiation to a patient. In an embodiment of all aspects of the invention, the treatment is, e.g., a chemotherapeutic agent, such as, e.g., Vincristine, Cisplatin, Azaguanine, Etoposide, Adriamycin, Aclarubicin, Mitoxantrone, Mitomycin, Paclitaxel, Gemcitabine, Taxotere, Dexamethasone, Ara-C, Methylprednisolone, Methotrexate, Bleomycin, Methyl-GAG, Carboplatin, 5-FU (5-Fluorouracil), a histone deacetylase (HDAC) inhibitor such as PXD101, 5-Aza-2'-deoxycytidine (Decitabine), alpha emitters such as astatine-211, bismuth-212, bismuth-213, lead-212, radium-223, actinium-225, and thorium-227, beta emitters such as tritium, strontium-90, cesium-137, carbon-11, nitrogen-13, oxygen-15, fluorine-18, iron-52, cobalt-55, cobalt-60, copper-61, copper-62, copper-64, zinc-62, zinc-63, arsenic-70, arsenic-71, arsenic-74, bromine-76, bromine-79, rubidium-82, yttrium-86, zirconium-89, indium-110, iodine-120, iodine-124, iodine-129, iodine-131, iodine-125, xenon-122, technetium-94m, technetium-94, technetium-99m, and technetium-99, gamma emitters such as cobalt-60, cesium-137, and technetium-99m, Alemtuzumab, Daclizumab, Rituximab (e.g., MABTHERATM), Trastuzumab (e.g., HERCEPTINTM), Gemtuzumab, Ibritumomab, Edrecolomab, Tositumomab, CeaVac, Epratuzumab, Mitumomab, Bevacizumab, Cetuximab, Edrecolomab, Lintuzumab, MDX-210, IGN-01, MDX-010, MAb, AME, ABX-EGF, EMD 72 000, Apolizumab, Labetuzumab, ior-t1, MDX-220, MRA, H-11 scFv, Oregovomab, huJ591 MAb, BZL, Visilizumab, TriGem, TriAb, R3, MT-201, G-250, unconjugated, ACA-125, Onyx-105, CDP-860, BrevaRex MAb, AR54, IMC-1C11, GlioMAb-H, ING-1, Anti-LCG MAbs, MT-103, KSB-303, Therex, KW-2871, Anti-HM1.24, Anti-PTHrP, 2C4 antibody,

SGN-30, TRAIL-RI MAb, CAT, Prostate cancer antibody, H22xKi-4, ABX-MA1, Imuteran, Monopharm-C, Acivicin, Aclarubicin, Acodazole Hydrochloride, Acronine, Adozelesin, Adriamycin, Aldesleukin, Altretamine, Ambomycin, A. metantrone Acetate, Aminoglutethimide, Amsacrine, Anastrozole, Anthramycin, Asparaginase, Asperlin, Azacitidine, Azetepa, Azotomycin, Batimastat, Benzodepa, Bicalutamide, Bisantrene Hydrochloride, Bisnafide Dimesylate, Bizelesin, Bleomycin Sulfate, Brequinar Sodium, Bropirimine, Busulfan, Cactinomycin, Calusterone, Camptothecin, Caracemide, Carbetimer, Carboplatin, Carmustine, Carubicin Hydrochloride, Carzelesin, Cedefingol, Chlorambucil, Cirolemycin, Cisplatin, Cladribine, Combretastatin A-4, Crisnatol Mesylate, Cyclophosphamide, Cytarabine, Dacarbazine, DACA (N-[2-(Dimethyl-amino)ethyl]acridine-4-carboxamide), Dactinomycin, Daunorubicin Hydrochloride, Daunomycin, Decitabine, Dexormaplatin, Dezaguamine, Dezaguamine Mesylate, Diaziquone, Docetaxel, Dolasatins, Doxorubicin, Doxorubicin Hydrochloride, Droloxifene, Droloxifene Citrate, Dromostanolone Propionate, Duazomycin, Edatrexate, Efudomithine Hydrochloride, Ellipticine, Elsamitracin, Enloplatin, Enpromate, Epipropidine, Epirubicin Hydrochloride, Erbulozole, Esorubicin Hydrochloride, Estramustine, Estramustine Phosphate Sodium, Etanidazole, Ethiodized Oil I 131, Etoposide, Etoposide Phosphate, Etoprine, Fadrozole Hydrochloride, Fazarabine, Fenretinide, Flouxuridine, Fludarabine Phosphate, Fluorouracil, 5-FdUMP, Fluorocitabine, Fosquidone, Fostriecin Sodium, Gemcitabine, Gemcitabine Hydrochloride, Gold Au 198, Homocamptothecin, Hydroxyurea, Idarubicin Hydrochloride, Ifosfamide, Ilmofosine, Interferon Alfa-2a, Interferon Alfa-2b, Interferon Alfa-n1, Interferon Alfa-n3, Interferon Beta-1a, Interferon Gamma-1b, Iproplatin, Irinotecan Hydrochloride, Lanreotide Acetate, Letrozole, Leuprolide Acetate, Liarozole Hydrochloride, Lometrexol Sodium, Lomustine, Losoxantrone Hydrochloride, Masoprocol, Maytansine, Mechlorethamine Hydrochloride, Megestrol Acetate, Melengestrol Acetate, Melphalan, Menogaril, Mercaptopurine, Methotrexate, Methotrexate Sodium, Metoprine, Meturedepa, Mitindomide, Mitocarcin, Mitocromin, Mitogillin, Mitomalcin, Mitomycin, Mitosper, Mitotane, Mitoxantrone Hydrochloride, Mycophenolic Acid, Nocodazole, Nogalamycin, Ormaplatin, Oxisuran, Paclitaxel, Pegaspargase, Peliomycin, Pentamustine, PeplomycinSulfate, Perfosfamide, Pipobroman, Piposulfan, Piroxantrone Hydrochloride, Plicamycin, Plomestane, Porfimer Sodium, Porfiromycin, Prednimustine, Procarbazine Hydrochloride, Puromycin, Puromycin Hydrochloride, Pyrazofurin, Rhizoxin, Rhizoxin D, Riboprine, Rogletimide, Safingol, Safingol Hydrochloride, Semustine, Simtrazene, Sparfosate Sodium, Sparsomycin, Spirogermanium Hydrochloride, Spiromustine, Spiroplatin, Streptonigrin, Streptozocin, Strontium Chloride Sr 89, Sulofenur, Talisomycin, Taxane, Taxoid, Tecogalan Sodium, Tegafur, Teloxantrone Hydrochloride, Temoporfin, Teniposide, Teroxirone, Testolactone, Thiamiprime, Thioguanine, Thiotepa, Thymitaq, Tiazofurin, Tirapazamine, Tomudex, TOP53, Topotecan Hydrochloride, Toremifene Citrate, Trestolone Acetate, Triciribine Phosphate, Trimetrexate, Trimetrexate Glucuronate, Triptorelin, Tubulozole Hydrochloride, Uracil Mustard, Uredopa, Vapreotide, Verteporfin, Vinblastine, Vinblastine Sulfate, Vincristine, Vincristine Sulfate, Vindesine, Vindesine Sulfate, Vinpipidine Sulfate, Vinglycinate Sulfate, Vinleurosine Sulfate, Vinorelbine Tartrate, Vinrosidine Sulfate, Vinzolidine Sul-

fate, Vorozole, Zeniplatin, Zinostatin, Zorubicin Hydrochloride, 2-Chlorodeoxyadenosine, 2' Deoxyformycin, 9-aminoacamptothecin, raltitrexed, N-propargyl-5,8-dideazafolic acid, 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine, anisomycin, trichostatin A, hPRL-G129R, CEP-751, linomide, sulfur mustard, nitrogen mustard (mechlор ethamine), cyclophosphamide, melphalan, chlorambucil, ifosfamide, busulfan, N-methyl-N-nitrosourea (MNU), N,N'-Bis(2-chloroethyl)-N-nitrosourea (BCNU), N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea (CCNU), N-(2-chloroethyl)-N'-(trans-4-methylcyclohexyl-N-nitrosourea (MeCCNU), N-(2-chloroethyl)-N'-(diethylethylphosphonate-N-nitrosourea (fotemustine), streptozotocin, diacarbazine (DTIC), mitozolomide, temozolamide, thiopeta, mitomycin C, AZQ, adozelesin, Cisplatin, Carboplatin, Ormaplatin, Oxaliplatin, C1-973, DWA 2114R, JM216, JM335, Bis(platinum), tomudex, azacitidine, cytarabine, gemcitabine, 6-Mercaptourine, 6-Thioguanine, Hypoxanthine, teniposide 9-amino camptothecin, Topotecan, CPT-11, Doxorubicin, Daunomycin, Epirubicin, darubicin, mitoxantrone, losoxantrone, Dactinomycin (Actinomycin D), amsacrine, pyrazoloacridine, all-trans retinol, 14-hydroxyretro-retinol, all-trans retinoic acid, N-(4-Hydroxyphenyl) retinamide, 13-cis retinoic acid, 3-Methyl TTNEB, 9-cis retinoic acid, fludarabine (2-F-ara-AMP), 2-chlorodeoxyadenosine (2-Cda), 20-pi-1,25 dihydroxyvitamin D3,5-ethynyluracil, abiraterone, aclarubicin, acylfulvene, adecyepenol, adozelesin, aldesleukin, ALL-TK antagonists, altretamine, ambamustine, amidox, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, andrographolide, angiogenesis inhibitors, antagonist D, antagonist G, antarelix, anti-dorsalizing morphogenetic protein-1, antiandrogen, prostatic carcinoma, antiestrogen, antineoplaston, antisense oligonucleotides, aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ara-CDP-DL-PTBA, argininedeaminase, asulacrine, atamestane, atrimustine, axinastatin 1, axinastatin 2, axinastatin 3, azasetron, azatoxin, azatyrosine, baccatin III derivatives, balanol, batimastat, BCR/ABL antagonists, benzochlorins, benzoylstaurosporine, beta lactam derivatives, beta-alethine, betacalamycin B, betulinic acid, bFGF inhibitor, bicalutamide, bisantrene, bisaziridinylspermine, bisnafide, bistratene A, bizelesin, breflate, bleomycin A2, bleomycin B2, bropirimine, budotitane, buthionine sulfoximine, calcipotriol, calphostin C, camptothecin derivatives (e.g., 10-hydroxy-camptothecin), canarypox IL-2, capecitabine, carboxamide-amino-triazole, carboxyamidotriazole, CaRest M3, CARN 700, cartilage derived inhibitor, carzelesin, casein kinase inhibitors (ICOS), castanospermine, cecropin B, cetrorelix, chlorins, chloroquinoxaline sulfonamide, cicaprost, cis-porphyrin, cladribine, clomifene analogues, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analogue, conagenin, crambescidin 816, cri-snatol, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentanthraquinones, cycloplatam, cypemycin, cyt-arabine ocfosfate, cytolytic factor, cytostatin, daclizimab, decitabine, dehydrodemnin B, 2'deoxycoformycin (DCF), deslorelin, dexifosfamide, dextrazoxane, dexverapamil, diaziquone, didemnin B, didox, diethylnorspermine, dihydro-5-azacytidine, 9-dihydropatol, dioxamycin, diphenyl spiro-mustine, discodermolide, docosanol, dolasetron, doxifluridine, droloxifene, dronabinol, duocarmycin SA, ebselen, ecomustine, edelfosine, edrecolomab, efomithine, elemene, emitefur, epirubicin, epithilones (A, R=H, B,

R=Me), epithilones, epristeride, estramustine analogue, estrogen agonists, estrogen antagonists, etanidazole, etoposide, etoposide 4'-phosphate (etopofos), exemestane, fadrozole, fazarabine, fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, fluasterone, fludarabine, fluorodaunorubicin hydrochloride, forfenimex, formestane, fostriecin, fotemustine, gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors, gemcitabine, glutathione inhibitors, hepsulfam, heregulin, hexamethylene bisacetamide, homoharringtonine (HHT), hypericin, ibandronic acid, idarubicin, idoxifene, idramantone, ilmofosine, ilomastat, imidazoacridones, imiquimod, immunostimulant peptides, insulin-like growth factor-1 receptor inhibitor, interferon agonists, interferons, interleukins, iobenguane, iododoxorubicin, 4-ipomeanol, irinotecan, iroplact, irsogladine, isobengazole, isohomohalicondrin B, itasetron, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, leinamycin, lenograstim, lentinan sulfate, leptolstatin, letrozole, leukemia inhibiting factor, leukocyte alpha interferon, leuprolide, estrogen, and progesterone combinations, leuprorelin, levamisole, liarozole, linear polyamine analogue, lipophilic disaccharide peptide, lipophilic platinum compounds, lisso-clinamide 7, lobaplatin, lombricine, lometrexol, lonidamine, losoxantrone, lovastatin, loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, lytic peptides, maytansine, manostatin A, marimastat, masoprolol, maspin, matrilysin inhibitors, matrix metalloproteinase inhibitors, menogaril, merbarone, meterelin, methioninase, metoclopramide, MIF inhibitor, ifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mithracin, mitoguazone, mitolactol, mitomycin analogues, mitonafide, mitotoxin fibroblast growth factor-saporin, mitoxantrone, mofarotene, molgramostim, monoclonal antibody, human chorionic gonadotrophin, monophosphoryl lipid A and mycobacterium cell wall skeleton combinations, mopidamol, multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy, mustard anticancer agent, mycaperoxide B, mycobacterial cell wall extract, myriaporone, N-acetylglucosamine, N-substituted benzamides, nafarelin, nagrestip, naloxone and pentazocine combinations, napavrin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral endopeptidase, nilutamide, nisamycin, nitric oxide modulators, nitroxide antioxidant, nitrallyn, O6-benzylguanine, octreotide, okicenone, oligonucleotides, onapristone, ondansetron, ondansetron, oracitin, oral cytokine inducer, ormaplatin, osaterone, oxaliplatin, oxaunomycin, paclitaxel analogues, paclitaxel derivatives, palauamine, palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine, pegaspargase, peldesine, pentosan polysulfate sodium, pentostatin, pentozole, perflubron, perfosfamide, perillyl alcohol, phenazinomycin, phenylacetate, phosphatase inhibitors, picibanil, pilocarpine hydrochloride, pirarubicin, pirritexim, placetin A, placetin B, plasminogen activator inhibitor, platinum complex, platinum compounds, platinum-triamine complex, podophyllotoxin, porfimer sodium, porfiromycin, propyl bis-acridone, prostaglandin J2, proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitor, protein kinase C inhibitors, microalgal, protein tyrosine phosphatase inhibitors, purine nucleoside phosphorylase inhibitors, purpurins, pyrazoloacridine, pyridoxylated hemoglobin polyoxyethylene conjugate, raf antagonists, raltitrexed, ramosetron, ras farnesyl protein transferase inhibitors, ras inhibitors, ras-GAP inhibitor, retelliptine demethylated, rhenium Re 186 etidronate, rhizoxin, ribozymes, RII retina-

mide, rogletimide, rohitukine, romurtide, roquinimex, rubigine B 1, ruboxyl, safingol, saintopin, SarCNU, sarcophytol A, sargramostim, Sdi 1 mimetics, semustine, senescence derived inhibitor 1, sense oligonucleotides, signal transduction inhibitors, signal transduction modulators, single chain antigen binding protein, sizofuran, sobuzoxane, sodium borocaptate, sodium phenylacetate, solverol, somatomedin binding protein, sonermin, sparfasic acid, spicamycin D, spiomustine, splenopentin, spongistatin 1, squalamine, stem cell inhibitor, stem-cell division inhibitors, stipiamide, stromelysin inhibitors, sulfinosine, superactive vasoactive intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic glycosaminoglycans, tallimustine, tamoxifen methiodide, tauromustine, tazarotene, tecogalan sodium, tegafur, tellurapyrylium, telomerase inhibitors, temoporfin, temozolamide, teniposide, tetrachlorodecaoxide, tetrazomine, thaliblastine, thalidomide, thiocoraline, thrombopoietin, thrombopoietin mimetic, thymalfasin, thymopoietin receptor agonist, thymotrinan, thyroid stimulating hormone, tin ethyl etiopurpurin, tirapazamine, titanocene dichloride, topotecan, topsentin, toremifene, totipotent stem cell factor, translation inhibitors, tretinoin, triacetyluridine, triciribine, trimetrexate, triptorelin, tropisetron, turosteride, tyrosine kinase inhibitors, tyrophostins, UBC inhibitors, ubenimex, urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists, vapreotide, variolin B, vector system, erythrocyte gene therapy, velaresol, veramine, verdins, verteporfin, vinorelbine, vinxaltine, vitaxin, vorozole, zanoterone, zeniplatin, zilascorb, or zinostatin stimalamer. In another embodiment of all aspects of the invention, a second treatment is utilized to determine gene expression in a sample from the patient.

[0022] In another embodiment of all aspects of the invention, the gene is selected from the group consisting of ABL1, ACTB, ACTN1, ACTN4, ACTR2, ADA, ADAM9, ADAMTS1, ADD1, ADORA2A, AF1Q, AIF1, AKAP1, AKAP13, AKR1B1, AKR1C1, AKT1, ALDH2, ALDH3A1, ALDOC, ALG5, ALMS1, ALOX15B, AMIGO2, AMPD2, AMPD3, ANAPC5, ANP32A, ANP32B, ANPEP, ANXA1, ANXA2, AP1G2, APOBEC3B, APRT, ARHE, ARHGAP15, ARHGAP25, ARHGDIB, ARHGEF6, ARL7, ASAHI, ASPH, ATF3, ATIC, ATOX1, ATP1B3, ATP2A2, ATP2A3, ATP5D, ATP5G2, ATP6V1B2, B2M, BASP1, BAX, BC008967, BCAT1, BCHE, BCL11B, BDNF, BHLHB2, BIN2, BLM, BLMH, BLVRA, BMI1, BNIP3, BRDT, BRRN1, BTN3A2, BTN3A3, C11orf2, C14orf139, C15orf25, C18orf10, C1orf24, C1orf29, C1orf38, C1QR1, C22orf18, C5orf13, C6orf32, CACNA1G, CACNB3, CALD1, CALM1, CALML4, CALU, CAP350, CAPG, CAPN2, CAPN3, CASP2, CASP6, CASP7, CAST, CBFB, CBLB, CBR1, CBX3, CCL2, CCL21, CCNA2, CCNB1IP1, CCND3, CCR7, CCR9, CCT5, CD151, CD1A, CD1B, CD1C, CD1D, CD1E, CD2, CD28, CD37, CD3D, CD3E, CD3G, CD3Z, CD44, CD47, CD53, CD59, CD6, CD63, CD81, CD8A, CD8B1, CD99, CDC10, CDCl4B, CDH11, CDH2, CDKL5, CDKN2A, CDW52, CECR1, CENPB, CENTB1, CENTG2, CEP1, CG018, CHRNA3, CHS1, CIAPIN1, CKAP4, CKIP-1, CNN3, CNP, COL1A1, COL4A1, COL4A2, COL5A2, COL6A1, COL6A2, COPA, COPEB, CORO1A, CORO1C, COX7B, CPSF1, CRABP1, CREB3L1, CRIP2, CRK, CRY1, CSDA, CSPG2, CSRPI, CST3, CTBP1, CTGF, CTNNA1, CTSB, CTSC, CTSD, CTSI, CUGBP2, CUTC, CXCL1, CXCR4, CXorf9, CYFIP2, CYLD, CYR61, DATF1, DAZAP1, DBN1, DBT, DCTN1, DDOST, DDX18, DDX5, DGKA, DIAPH1, DIPA,

DKC1, DKFZP434J154, DKFZP564C186, DKFZP564G2022, DKFZp564J157, DKFZP564K0822, DNAJC10, DNAPTP6, DOCK10, DOCK2, DPAGT1, DPEP2, DPYSL3, DSPI, DUSP1, DUSP3, DXS9879E, DYRK2, E2F4, ECE1, ECM1, EEF1A1, EEF1B2, EEF1G, EFNB2, EHD2, EIF2S2, EIF3S2, EIF4B, EIF4G3, EIF5A, ELA2B, ELK3, EMP3, ENO2, EPAS1, EPB41L4B, ERCC2, ERG, ERP70, EVER1, EVI2A, EVL, EXT1, EZH2, F2R, FABP5, FAD104, FAM46A, FARSLA, FAT, FAU, FBL, FCGR2A, FCGR2C, FER1L3, FGFR1, FHL1, FHOD1, FKBP1A, FKBP9, FLII, FLJ10350, FLJ10539, FLJ10774, FLJ12270, FLJ13373, FLJ20859, FLJ21159, FLJ22457, FLJ35036, FLJ46603, FLNC, FLOT1, FMNL1, FN1, FNBP1, FOLH1, FOXF2, FSCN1, FSTL1, FTH1, FTL, FYB, FYN, GOS2, G6PD, GALIG, GALNT6, GAPD, GAS7, GATA2, GATA3, GFPT1, GIMAP5, GIT2, GJA1, GLRB, GLTSCR2, GLUL, GMDS, GMFG, GNA15, GNAI2, GNAQ, GNB2, GNB5, GOT2, GPNMB, GPR65, GPRASPI, GPSM3, GRP58, GSTM2, GTF3A, GTSE1, GYPC, GZMA, GZMB, H1F0, H1FX, H2AFX, H3F3A, HA-1, HCLS1, HEM1, HEXB, HIC, HIST1H4C, HK1, HLA-A, HLA-B, HLA-DRA, HMGA1, HMGB2, HMGN2, HMMR, HNRPA1, HNRPD, HNRPM, HOXA9, HPRT1, HRMT1L1, HSA9761, HSPA5, HSU79274, HTATSF1, HU6800, ICAM1, ICAM2, IER3, IFI16, IFI44, IFITM2, IFITM3, IFRGP28, IGFBP2, IGFBP3, IGSF4, IL13RA2, IL21R, IL2RG, IL4R, IL6, IL6R, IL6ST, IL8, IMPDH2, INPP5D, INSIG1, IQGAP1, IQGAP2, IRS2, ITGA3, ITGA5, ITGB2, ITK, ITM2A, JAK1, JARID2, JUNB, K-ALPHA-1, KHDRBS1, KIAA0220, KIAA0355, KIAA0802, KIAA0877, KIAA0922, KIAA1078, KIAA1128, KIAA1393, KIFC1, KPNB1, LAIR1, LAMB1, LAMB3, LAMR1, LAPTMs, LAT, LBR, LCK, LCP1, LCP2, LDHB, LEF1, LEPRE1, LGALS1, LGALS9, LHFPL2, LMNB1, LNK, LOC54103, LOC55831, LOC81558, LOC94105, LONP, LOX, LOXL2, LPHN2, LPXN, LRMP, LRP12, LRRCS, LRRN3, LST1, LTB, LUM, LY9, LY96, M6PRBP1, MAD2L1BP, MAGEB2, MAL, MAN1A1, MAP1B, MAP1LC3B, MAP4K1, MAPK1, MAPRE1, MARCKS, MAZ, MCAM, MCL1, MCM5, MCM7, MDH2, MDK, MDN1, MEF2C, MFNG, MGC17330, MGC21654, MGC2744, MGC4083, MGC8721, MGC8902, MGLL, MIA, MICA, MLPH, MME, MMP2, MPHOSPH6, MPP1, MPZL1, MRP63, MRPL12, MRPS2, MSN, MT1E, MT1K, MUF1, MVP, MYB, MYC, MYL6, MYL9, MYO1B, NAP1L1, NAP1L2, NARF, NARS, NASP, NBL1, NCL, NCOR2, NDN, NDUFAB1, NDUF56, NFIL3, NFKBIA, NID2, NIPA2, NK4, NME4, NME7, NNMT, NOL5A, NOL8, NOMO2, NOTCH1, NPC1, NQO1, NR1D2, NUCB2, NUDC, NUP210, NUP88, NVL, NXF1, OBFC1, OCRL, OGT, OK/SW-c1.56, OPTN, OXAIL, P2RX5, P4HA1, PACAP, PAF53, PAFAH1B3, PALM2-AKAP2, PAX6, PBEF1, PCBP2, PCCB, PEA15, PFDN5, PFN1, PFN2, PGAMI, PGK1, PHEMX, PHLDA1, PIM2, PITPN1, PKM2, PLAC8, PLAGL1, PLAU, PLAUR, PLCB1, PLEK2, PLEKHC1, PILOD2, PLSCR1, PNAS-4, PNMA2, POLR2F, PON2, PPAP2B, PPIA, PPIF, PPP1R11, PPP2CB, PRF1, PRG1, PRIM1, PRKCA, PRKCB1, PRKCH, PRKCQ, PRKD2, PRNP, PRP19, PRPF8, PRPS1, PRSS11, PRSS23, PSCDBP, PSMB9, PSMC3, PSMC5, PSME2, PTGER4, PTGES2, PTMA, PTOV1, PTP4A3, PTPN7, PTPNS1, PTPRC, PTPRCAP, PTRF, PTS, PURA, PWI1, PYGL, QKI, RAB31, RAB3GAP, RAB7, RAB7L1, RAB9P40, RAC2, RAFTLIN, RAG2, RALY, RAP1B, RAS-

GRP2, RBMX, RBPMS, RCN1, REA, RFC3, RFC5, RGC32, RGS3, RHOC, RHOH, RIMS3, RIOK3, RIPK2, RIS1, RNASE6, RNF144, RNPS1, RPL10, RPL10A, RPL11, RPL12, RPL13, RPL13A, RPL17, RPL18, RPL18A, RPL24, RPL3, RPL32, RPL36A, RPL39, RPL7, RPL9, RPLP0, RPLP2, RPS10, RPS11, RPS15, RPS15A, RPS19, RPS2, RPS23, RPS24, RPS25, RPS27, RPS28, RPS4X, RPS4Y1, RPS6, RPS7, RPS9, RRAS, RRAS2, RRBPI, RRM2, RUNX1, RUNX3, S100A13, S100A4, SART3, SATB1, SCAP1, SCARB1, SCARB2, SCN3A, SCTR, SEC31L2, SEC61G, SELL, SELPLG, SEMA4G, SEPT6, SEPT10, SEPW1, SERPINA1, SERPINB1, SERPINB6, SFRS3, SFRS5, SFRS6, SFRS7, SH2D1A, SH3GL3, SH3TC1, SHD1, SHFM1, SHMT2, SIAT1, SKB1, SKP2, SLA, SLC1A4, SLC20A1, SLC25A15, SLC25A5, SLC39A14, SLC39A6, SLC43A3, SLC4A2, SLC7A11, SLC7A6, SMA3, SMAD3, SMARCD3, SMOX, SMS, SND1, SNRPA, SNRPB, SNRPB2, SNRPE, SNRPF, SOD2, SOX4, SP140, SPANXC, SPARC, SPI1, SRF, SRM, SRRM1, SSA2, SSBP2, SSRP1, SSSCA1, STAG3, STAT1, STAT4, STAT5A, STC1, STC2, STMN1, STOML2, SUI1, T3JAM, TACC1, TACC3, TAF5, TAGLN, TAL1, TAP1, TARP, TBCA, TCF12, TCF4, TCF7, TFDP2, TFPI, TFRC, TGFB1, TIMM17A, TIMP1, TJP1, TK2, TM4SF1, TM4SF2, TM4SF8, TM6SF1, TMEM2, TMEM22, TMSB10, TMSNB, TNFAIP3, TNFAIP8, TNFRSF10B, TNFRSF1A, TNFRSF7, TNK, TNPO1, TOB1, TOMM20, TOP2A, TOX, TPK1, TPM2, TRA@, TRA1, TRAM2, TRB@, TRD@, TRIM, TRIM14, TRIM22, TRIM28, TRIP13, TRPV2, TUBA3, TUBGCP3, TUFM, TUSC3, TXN, TXND5, UBASH3A, UBB, UBC, UBE2A, UBE2L6, UBE2S, UCHL1, UCK2, UCP2, UFD1L, UGCG, UGDH, UGT2B17, ULK2, UMPS, UNG, UROD, USP34, USP4, USP7, VASP, VAV1, VIM, VLDDL, VWF, WARS, WASPIP, WBSCR20A, WBSCR20C, WHSC1, WNT5A, XPO1, ZAP128, ZAP70, ZFP36L1, ZNF32, ZNF335, ZNF593, ZNFN1A1, or ZYX.

[0023] The nucleic acid sequence of each listed genes is publicly available through the GenBank or RefSeq database (<http://www.ncbi.nlm.nih.gov/sites/gquery>). The gene sequences are also included as part of the HG-U133A Gene-Chip from Affymetrix, Inc.

[0024] “Resistant” or “resistance” as used herein means that a cell, a tumor, a patient (e.g., a human), or a living organism is able to withstand treatment, e.g., with a compound, such as a chemotherapeutic agent, or radiation treatment, in that the treatment inhibits the growth of a cell, e.g., a cancer cell, in vitro or in a tumor, patient, or living organism by less than 10%, 20%, 30%, 40%, 50%, 60%, or 70% relative to the growth of a similar cell not exposed to the treatment. Resistance to treatment can be determined by a cell-based assay that measures the growth of treated cells as a function of the cells’ absorbance of an incident light beam as used to perform the NCI60 assays described herein. In this example, greater absorbance indicates greater cell growth, and thus, resistance to the treatment. A reduction in growth indicates more resistance to a treatment. By “chemoresistant” or “chemoresistance” is meant resistance to a compound.

[0025] “Sensitive” or “sensitivity” as used herein means that a cell, a tumor, a patient (e.g., a human), or a living organism is responsive to treatment, e.g., with a compound, such as a chemotherapeutic agent, or radiation treatment, in that the treatment inhibits the growth of a cell, e.g., a cancer cell, in vitro or in a tumor, patient, or living organism by 70%,

80%, 90%, 95%, 99%, or 100%. Sensitivity to treatment may be determined by a cell-based assay that measures the growth of treated cells as a function of the cells’ absorbance of an incident light beam as used to perform the NCI60 assays described herein. In this example, lesser absorbance indicates reduced cell growth, and thus, sensitivity to the treatment. A greater reduction in growth indicates more sensitivity to the treatment. By “chemosensitive” or “chemosensitivity” is meant sensitivity to a compound.

[0026] “Complement” of a nucleic acid sequence or a “complementary” nucleic acid sequence as used herein refers to an oligonucleotide which is in “antiparallel association” when it is aligned with the nucleic acid sequence such that the 5' end of one sequence is paired with the 3' end of the other. Nucleotides and other bases can have complements and may be present in complementary nucleic acids. Bases not commonly found in natural nucleic acids that can be included in the nucleic acids of the present invention include, for example, inosine and 7-deazaguanine. “Complementarity” may not be perfect; stable duplexes of complementary nucleic acids can contain mismatched base pairs or unmatched bases. Skilled artisans can determine duplex stability empirically considering a number of variables including, for example, the length of the oligonucleotide, percent concentration of cytosine and guanine bases in the oligonucleotide, ionic strength, and incidence of mismatched base pairs. Typically, complementarity is determined by comparing contiguous nucleic acid sequences.

[0027] When complementary nucleic acid sequences form a stable duplex, they are said to be “hybridized” or to “hybridize” to each other or it is said that “hybridization” has occurred. Nucleic acids are referred to as being “complementary” if they contain nucleotides or nucleotide homologues that can form hydrogen bonds according to Watson-Crick base-pairing rules (e.g., G with C, A with T, or A with U) or other hydrogen bonding motifs such as, for example, diaminopurine with T, 5-methyl C with G, 2-thiothymidine with A, inosine with C, and pseudouracil with G. Anti-sense RNA can be complementary to other oligonucleotides, e.g., mRNA.

[0028] “Biomarker” as used herein indicates a transcription product (e.g., RNA, such as an RNA primary transcript, mRNA, tRNA, rRNA, microRNA (miRNA), or complementary RNA or DNA (e.g., cDNA) strands thereof) or a translation product (e.g., a polypeptide or metabolite thereof) of a biomarker gene, as defined herein, whose level of expression indicates the sensitivity or resistance of a cell (e.g., a cancer cell), tissue, organism, or patient (e.g., a human) to a treatment (e.g., chemotherapy, radiation therapy, or surgery).

[0029] “Compound” as used herein means a chemical or biological substance, e.g., a drug, a protein, an antibody, or an oligonucleotide, which can be used to treat a disease or which has biological activity in vivo or in vitro. Compounds may or may not be approved by the U.S. Food and Drug Administration (FDA). Preferred compounds include, e.g., chemotherapy agents that can inhibit cancer growth. Preferred chemotherapy agents include, e.g., Vincristine, Cisplatin, Azaguanine, Etoposide, Adriamycin, Aclarubicin, Mitoxantrone, Mitomycin, Paclitaxel, Gemcitabine, Taxotere, Dexamethasone, Ara-C, Methylprednisolone, Methotrexate, Bleomycin, Methyl-GAG, Carboplatin, 5-FU (5-Fluorouracil), Rituximab (e.g., MABTHERA™), histone deacetylase (HDAC) inhibitors, and 5-Aza-2'-deoxycytidine (Decitabine). Exemplary radioactive chemotherapeutic agents

include compounds containing alpha emitters such as astatine-211, bismuth-212, bismuth-213, lead-212, radium-223, actinium-225, and thorium-227, beta emitters such as tritium, strontium-90, cesium-137, carbon-11, nitrogen-13, oxygen-15, fluorine-18, iron-52, cobalt-55, cobalt-60, copper-61, copper-62, copper-64, zinc-62, zinc-63, arsenic-70, arsenic-71, arsenic-74, bromine-76, bromine-79, rubidium-82, yttrium-86, zirconium-89, indium-110, iodine-120, iodine-124, iodine-129, iodine-131, iodine-125, xenon-122, technetium-94m, technetium-94, technetium-99m, and technetium-99, and gamma emitters such as cobalt-60, cesium-137, and technetium-99m. Exemplary chemotherapeutic agents also include antibodies such as Alemtuzumab, Daclizumab, Rituximab (e.g., MABTHERA™), Trastuzumab (e.g., HERCEPTIN™), Gemtuzumab, Ibritumomab, Edrecolomab, Tositumomab, CeaVac, Epratuzumab, Mitumomab, Bevacizumab, Cetuximab, Edrecolomab, Lintuzumab, MDX-210, IGN-101, MDX-010, MAB, AME, ABX-EGF, EMD 72 000, Apolizumab, Labetuzumab, ior-t1, MDX-220, MRA, H-11 scFv, Oregonomab, huJ591 MAb, BZL, Visilizumab, Tri-Gem, TriAb, R3, MT-201, G-250, ACA-125, Onyxav-105, CDP-860, BrevaRex MAb, AR54, IMC-1C11, GlioMAb-H, ING-1, Anti-LCG MAbs, MT-103, KSB-303, Therex, KW-2871, Anti-HM1.24, Anti-PTHRP, 2C4 antibody, SGN-30, TRAIL-R1 MAb, CAT, Prostate cancer antibody, H22xKi-4, ABX-MA1, Imuteran, and Monopharm-C. Exemplary chemotherapeutic agents also include Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adozelesin; Adriamycin; Aldesleukin; Altretamine; Ambomycin; A. metantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Camptothecin; Caracemide; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Combretastatin A-4; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; DACA (N-[2-(Dimethyl-amino) ethyl]acridine-4-carboxamide); Dactinomycin; Daunorubicin Hydrochloride; Daunomycin; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Dolasatins; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflomithine Hydrochloride; Ellipticine; Elsamitruclin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulazole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Ethiodized Oil I 131; Etoposide; Etoposide Phosphate; Etoprime; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Flouxuridine; Fludarabine Phosphate; Fluorouracil; 5-FdUMP; Fluorocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Gold Au 198; Homocamptothecin; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta-I a; Interferon Gamma-I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Meto-

prine; Meturedopa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; PeploycinSulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Rhizoxin; Rhizoxin D; Riboprime; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr 89; Sulofenur; Talisomycin; Taxane; Taxoid; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprime; Thioguanine; Thiotepa; Thymitaq; Tiazofurin; Tirapazamine; Tomudex; TOP53; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredopa; Vapreotide; Verteporfin; Vinblastine; Vinblastine Sulfate; Vincristine; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride; 2-Chlorodeoxyadenosine; 2' Deoxyformycin; 9-aminocamptothecin; raltitrexed; N-propargyl-5,8-dideazafulic acid; 2chloro-2'-arabino-fluoro-2'-deoxyadenosine; 2-chloro-2'-deoxyadenosine; anisomycin; trichostatin A; hPRL-G129R; CEP-751; linomide; sulfur mustard; nitrogen mustard (mechlор ethamine); cyclophosphamide; melphalan; chlorambucil; ifosfamide; busulfan; N-methyl-N-nitrosourea (MNU); N,N'-Bis(2-chloroethyl)-N-nitrosourea (BCNU); N-(2-chloroethyl)-N' cyclohexyl-N-nitrosourea (CCNU); N-(2-chloroethyl)-N'-trans-4-methylcyclohexyl-N-nitrosourea (MeCCNU); N-(2-chloroethyl)-N'-(diethyl)ethylphosphonate-N-nitrosourea (fotemustine); streptozotocin; diacarbazine (DTIC); mitozolomide; temozolomide; thiopeta; mitomycin C; AZQ; adozelesin; Cisplatin; Carboplatin; Ormaplatin; Oxaliplatin; C1-973; DWA 2114R; JM216; JM335; Bis(platinum); tomudex; azacitidine; cytarabine; gemcitabine; 6-Mercaptourine; 6-Thioguanine; Hypoxanthine; teniposide 9-amino camptothecin; Topotecan; CPT-11; Doxorubicin; Daunomycin; Epirubicin; darubicin; mitoxantrone; losoxantrone; Dactinomycin (Actinomycin D); amsacrine; pyrazoloacridine; all-trans retinol; 14-hydroxy-retro-retinol; all-trans retinoic acid; N-(4-Hydroxyphenyl)retinamide; 13-cis retinoic acid; 3-Methyl TTNEB; 9-cis retinoic acid; fludarabine (2-F-ara-AMP); and 2-chlorodeoxyadenosine (2-Cda).

[0030] Other chemotherapeutic agents include, but are not limited to, 20-pi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecytenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambastine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginindeaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin;

azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bleomycin A2; bleomycin B2; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives (e.g., 10-hydroxy-camptothecin); canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatan; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; daclizimab; decitabine; dehydrodideamin B; 2'deoxycoformycin (DCF); deslorelin; dexifosfamide; dexamoxane; dexverapamil; diaziquone; didemnin B; didox; diethylhydrospermine; dihydro-5-azacytidine; 9-dihydrotaxol; dioxamycin; diphenyl spiromustine; discodermolide; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebelsen; ecomustine; edelfosine; edrecolomab; efломithine; elemene; emitefur; epirubicin; epithilones (A, R=H; B, R=Me); epithilones; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide; etoposide 4'-phosphate (etoposf); exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forsenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; homoharringtonine (HHT); hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; 4-ipomeanol; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide, estrogen, and progesterone combinations; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissocliamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurotecan; lutetium texaphyrin; lysofylline; lytic peptides; maytansine; mannostatin A; marimastat; masoprolol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; ifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mithracin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A and mycobacterium cell wall skeleton combinations; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriapor-

one; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone and pentazocine combinations; napavrin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; podophyllotoxin; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rheinum Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sd±1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiomustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrophostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltinge; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

[0031] To "inhibit growth" as used herein means causing a reduction in cell growth in vivo or in vitro by, e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% or more, as evident by a reduction in the size or number of cells exposed to a treatment (e.g., exposure to a compound), relative to the size or number of cells in the absence of the treatment. Growth inhibition can be the result of a treatment that induces apoptosis in a cell, induces necrosis in a cell,

slows cell cycle progression, disrupts cellular metabolism, induces cell lysis, or induces some other mechanism that reduces the size or number of cells.

[0032] “Biomarker gene” as used herein means a gene in a cell (e.g., a cancer cell) the expression of which, as measured by, e.g., detecting the level of one or more biomarkers produced from the gene, correlates to sensitivity or resistance of the cell, tissue, organism, or patient (e.g., a human) to a treatment (e.g., chemotherapy, radiation therapy, or surgery).

[0033] “Microarray” as used herein means a device employed by any method that quantifies one or more subject oligonucleotides, e.g., DNA or RNA, or analogues thereof, at a time. One exemplary class of microarrays consists of DNA probes attached to a glass or quartz surface. Many microarrays, e.g., those made by Affymetrix, use several probes for determining the expression of a single gene. The DNA microarray can contain oligonucleotide probes that may be, e.g., full-length cDNAs complementary to an RNA or cDNA fragments that hybridize to part of an RNA. Exemplary RNAs include mRNA, miRNA, and miRNA precursors. Exemplary microarrays also include a “nucleic acid microarray” having a substrate-bound plurality of nucleic acids, hybridization to each of the plurality of bound nucleic acids being separately detectable. The substrate can be solid or porous, planar or non-planar, unitary or distributed. Exemplary nucleic acid microarrays include all of the devices so called in Schena (ed.), *DNA Microarrays: A Practical Approach* (Practical Approach Series), Oxford University Press (1999); *Nature Genet.* 21(1)(suppl.):1-60 (1999); and Schena (ed.), *Microarray Biochip: Tools and Technology*, Eaton Publishing Company/BioTechniques Books Division (2000). Additionally, exemplary nucleic acid microarrays can include a substrate-bound plurality of nucleic acids in which the plurality of nucleic acids is disposed on a plurality of beads, rather than on a unitary planar substrate, as is described, inter alia, in Brenner et al., *Proc. Natl. Acad. Sci. USA* 97(4):1665-1670 (2000). Examples of nucleic acid microarrays may be found in U.S. Pat. Nos. 6,391,623, 6,383,754, 6,383,749, 6,380,377, 6,379, 897, 6,376,191, 6,372,431, 6,351,712, 6,344,316, 6,316,193, 6,312,906, 6,309,828, 6,309,824, 6,306,643, 6,300,063, 6,287,850, 6,284,497, 6,284,465, 6,280,954, 6,262,216, 6,251,601, 6,245,518, 6,263,287, 6,251,601, 6,238,866, 6,228,575, 6,214,587, 6,203,989, 6,171,797, 6,103,474, 6,083,726, 6,054,274, 6,040,138, 6,083,726, 6,004,755, 6,001,309, 5,958,342, 5,952,180, 5,936,731, 5,843,655, 5,814,454, 5,837,196, 5,436,327, 5,412,087, and 5,405,783, herein incorporated by reference.

[0034] Exemplary microarrays can also include “peptide microarrays” or “protein microarrays” having a substrate-bound plurality of polypeptides, the binding of a oligonucleotide, a peptide, or a protein to the plurality of bound polypeptides being separately detectable. Alternatively, the peptide microarray, can have a plurality of binders, including, but not limited to, monoclonal antibodies, polyclonal antibodies, phage display binders, yeast 2 hybrid binders, aptamers, that can specifically detect the binding of specific oligonucleotides, peptides, or proteins. Examples of peptide arrays may be found in International Patent Publication Nos. WO 02/31463, WO 02/25288, WO 01/94946, WO 01/88162, WO 01/68671, WO 01/57259, WO 00/61806, WO 00/54046, WO 00/47774, WO 99/40434, WO 99/39210, and WO 97/42507, and in U.S. Pat. Nos. 6,268,210, 5,766,960, and 5,143,854, herein incorporated by reference.

[0035] “Gene expression” as used herein means the level of expression of a biomarker gene (e.g., the level of a transcription product, such as an mRNA, tRNA, or microRNA, or its complement (e.g., a cDNA complement of the transcription product), or a translation product, such as a polypeptide or metabolite thereof) in a cell, tissue, organism, or patient (e.g., a human). Gene expression can be measured by detecting the presence, quantity, or activity of a DNA, RNA, or polypeptide, or modifications thereof (e.g., splicing, phosphorylation, and acetylation) associated with a given gene.

[0036] “NCI60” as used herein means a panel of 60 cancer cell lines from lung, colon, breast, ovarian, leukemia, renal, melanoma, prostate, and brain cancers including the following cancer cell lines: NSCLC_NCIH23, NSCLC_NCIH522, NSCLC_A549ATCC, NSCLC_EKVX, NSCLC_NCIH226, NSCLC_NCIH332M, NSCLC_H460, NSCLC_HOP62, NSCLC_HOP92, COLON_HT29, COLON_HCC-2998, COLON_HCT116, COLON_SW620, COLON_COLO205, COLON_HCT15, COLON_KM12, BREAST_MCF7, BREAST_MCF7ADRr, BREAST_MDAMB231, BREAST_HS578T, BREAST_MDAMB435, BREAST_MDN, BREAST_BT549, BREAST_T47D, OVAR_OVCAR3, OVAR_OVCAR4, OVAR_OVCAR5, OVAR_OVCAR8, OVAR_IGROV1, OVAR_SKOV3, LEUK_CCRFCEM, LEUK_K562, LEUK_MOLT4, LEUK_HL60, LEUK_RPMI8266, LEUK_SR, RENAL_UO31, RENAL_SN12C, RENAL_A498, RENAL_CAKI1, RENAL_RXF393, RENAL_7860, RENAL_ACHN, RENAL_TK10, MELAN_LOXIMVI, MELAN_MALME3M, MELAN_SK-MEL2, MELAN_SKMEL5, MELAN_SKMEL28, MELAN_M14, MELAN_UACC62, MELAN_UACC257, PROSTATE_PC3, PROSTATE_DU145, CNS_SNB19, CNS_SNB75, CNS_U251, CNS_SF268, CNS_SF295, and CNS_SF539.

[0037] “Treatment” or “medical treatment” means administering to a patient (e.g., a human) or living organism or exposing to a cell or tumor a compound (e.g., a drug, a protein, an antibody, an oligonucleotide, a chemotherapeutic agent, and a radioactive agent) or some other form of medical intervention used to treat or prevent cancer or the symptoms of cancer (e.g., cryotherapy and radiation therapy). Radiation therapy includes the administration to a patient of radiation generated from sources such as particle accelerators and related medical devices that emit X-radiation, gamma radiation, or electron (Beta radiation) beams. A treatment may further include surgery, e.g., to remove a tumor from a patient or living organism.

[0038] Other features and advantages of the invention will be apparent from the following description, drawings, and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] FIG. 1 depicts an illustration of the method of identifying biomarkers and predicting patient sensitivity to a medical treatment. The method has an in vitro component where the growth inhibition of a compound or medical treatment is measured on cell lines (6 of the 60 cell lines tested are shown). The gene expression is measured on the same cell lines without compound treatment. Those genes that have a correlation above a certain cutoff (e.g., a preferred cutoff of 0.3, in which a correlation coefficient equal to or greater than the cutoff of 0.3 is deemed statistically significant by, e.g., cross-validation) to the growth inhibition are termed marker genes and the expression of those genes *in vivo*, e.g., may

predict the sensitivity or resistance of a patient's cancer to a compound or other medical treatment. The in vivo component is applied to a patient to determine whether or not the treatment will be effective in treating disease in the patient. Here, the gene expression in cells of a sample of the suspected disease tissue (e.g., a tumor) in the patient is measured before or after treatment. The activity of the marker genes in the sample is compared to a reference population of patients known to be sensitive or resistant to the treatment. The expression of marker genes in the cells of the patient known to be expressed in the cells of reference patients sensitive to the treatment indicates that the patient to be treated is sensitive to the treatment and vice versa. Based on this comparison the patient is predicted to be sensitive or resistant to treatment with the compound.

[0040] FIG. 2 depicts the treatment sensitivity predictions for a 5-year-old American boy with a brain tumor. The subject had surgery to remove the tumor and, based on the analysis of gene expression in cells from a sample of the tumor, the subject was predicted to be chemosensitive to ten chemotherapy drugs. The subject received Vincristine and Cisplatin and survived.

[0041] FIG. 3 depicts the treatment sensitivity predictions for a 7-month-old American girl with a brain tumor. The subject had surgery to remove the tumor, and based on the analysis of gene expression in cells from a sample of the tumor, the subject was predicted to be chemoresistant to twelve chemotherapy drugs. The subject received Vincristine and Cisplatin, but passed away 9 months later.

[0042] FIG. 4 depicts the survival rate of 60 brain cancer patients divided into a group predicted to be chemosensitive to Cisplatin and a group predicted to be chemoresistant to Cisplatin. All patients received Cisplatin after surgery.

[0043] FIG. 5 depicts the survival rate of 56 lymphoma patients divided into a group predicted to be chemosensitive to Vincristine and Adriamycin and a group predicted to be chemoresistant. All patients received Vincristine and Adriamycin.

[0044] FIG. 6 depicts the survival rate of 19 lung cancer patients divided into a group predicted to be chemosensitive to Cisplatin and a group predicted to be chemoresistant. All patients received Cisplatin.

[0045] FIG. 7 depicts the survival rate of 14 diffuse large-B-cell lymphoma (DLBCL) patients divided into a group predicted to be chemosensitive to the drug combination R-CHOP and a group predicted to be chemoresistant. All patients were treated with R-CHOP.

[0046] FIG. 8 depicts the predictions of sensitivity or resistance to treatment of a patient diagnosed with DLBCL. Various drug combinations and radiation therapy are considered. The drug combinations (indicated by abbreviations) are those commonly used to treat DLBCL.

[0047] FIG. 9 depicts the survival rate of 60 brain cancer patients divided into a group predicted to be sensitive to radiation treatment and a group predicted to be resistant. All patients were treated with radiation.

[0048] FIG. 10 depicts the survival rate of 60 brain cancer patients divided into a group predicted to be sensitive to radiation treatment and a group predicted to be resistant. All patients were treated with radiation. Gene biomarkers used in predicting radiation sensitivity or resistance were obtained using the correlation of the median gene expression measurement to cancer cell growth as opposed to the median of the correlations as employed in FIG. 9.

[0049] FIG. 11 depicts the predicted sensitivity of cancer patients to sunitinib. The cancer patients are grouped according to cancer type or origin and cancer types with predicted high sensitivity are labeled.

DETAILED DESCRIPTION

[0050] The invention features methods for identifying biomarkers of treatment sensitivity, e.g., chemosensitivity to compounds, or resistance, devices that include the biomarkers, kits that include the devices, and methods for predicting treatment efficacy in a patient (e.g., a human diagnosed with cancer). The kits of the invention include microarrays having oligonucleotide probes that are biomarkers of sensitivity or resistance to treatment (e.g., treatment with a therapeutic agent) that hybridize to nucleic acids derived from or obtained from a subject and instructions for using the device to predict the sensitivity or resistance of the subject to the treatment. The invention also features methods of using the microarrays to determine whether a subject, e.g., a cancer patient, will be sensitive or resistant to treatment with, e.g., a chemotherapy agent. Also featured are methods of identifying biomarkers of sensitivity or resistance to a medical treatment based on the correlation of gene or microRNA expression to treatment efficacy, e.g., the growth inhibition of cancer cells. Gene or microRNA biomarkers that identify subjects as sensitive or resistant to a treatment can also be identified within patient populations already thought to be sensitive or resistant to that treatment. Thus, the methods, devices, and kits of the invention can be used to identify patient subpopulations that are responsive to a treatment thought to be ineffective for treating disease (e.g., cancer) in the general population. More generally, cancer patient sensitivity to a compound or other medical treatment can be predicted using biomarker expression regardless of prior knowledge about patient responsiveness to treatment. The method according to the present invention can be implemented using software that is run on an apparatus (e.g., a computer) for measuring biomarker expression in connection with a microarray. The microarray (e.g., a DNA microarray), included in a kit for processing a tumor sample from a patient, and the apparatus for reading the microarray and turning the result into a chemosensitivity profile for the patient may be used to implement the methods of the invention.

Microarrays Containing Oligonucleotide Probes

[0051] The microarrays of the invention include one or more oligonucleotide probes that have nucleotide sequences that are substantially identical to or substantially complementary to, e.g., at least 5, 8, 12, 20, 30, 40, 60, 80, 100, 150, or 200 consecutive nucleotides (or nucleotide analogues) of the biomarker genes or biomarker gene products (e.g., transcription or translation gene products, such as microRNAs) listed below. The oligonucleotide probes may be, e.g., 5-20, 25, 5-50, 50-100, or over 100 nucleotides long. The oligonucleotide probes may be deoxyribonucleic acids (DNA) or ribonucleic acids (RNA). Consecutive nucleotides within the oligonucleotide probes (e.g., 5-20, 25, 5-50, 50-100, or over 100 consecutive nucleotides), which are used as biomarkers of chemosensitivity, may also appear as consecutive nucleotides in one or more of the genes described herein beginning at or near, e.g., the first, tenth, twentieth, thirtieth, fortieth, fiftieth, sixtieth, seventieth, eightieth, ninetieth, hundredth, hundred-fiftieth, two-hundredth, five-hundredth, or one-thousandth

nucleotide of the genes or microRNAs listed in Tables 1-136 below. Column List_2006 of Tables 1-21 indicates the preferred biomarker genes for the compound lists. Column List_PREFERRED of Tables 1-21 indicates the most preferred biomarker genes. Column List_2005 of Tables 1-21 indicates additional biomarkers employed in Examples 1-8. Column Correlation of Tables 1-21 indicates the correlation coefficient of the biomarker gene expression to cancer cell growth inhibition. Tables 80-136 indicate microRNA biomarkers that can be used to determine a patient's (e.g., a human's) sensitivity to a treatment. The following combinations of biomarkers have been used to detect a patient's sensitivity to the indicated treatment:

- a) One or more of the gene sequences SFRS3, CCT5, RPL39, SLC25A5, UBE2S, EEF1A1, RPLP2, RPL24, RPS23, RPL39, RPL18, NCL, RPL9, RPL10A, RPS10, E1F3S2, SHFM1, RPS28, REA, RPL36A, GAPD, HNRPA1, RPS11, HNRPA1, LDHB, RPL3, RPL11, MRPL12, RPL18A, COX7B, and RPS7, preferably gene sequences UBB, RPS4X, S100A4, NDUFS6, B2M, C14orf139, MANIA 1, SLC25A5, RPL10, RPL12, E1F5A, RPL36A, SU11, BLMH, CTBP1, TBCA, MDH2, and DXS9879E, and most preferably gene sequences RPS4X, S100A4, NDUFS6, C14orf139, SLC25A5, RPL10, RPL12, EIF5A, RPL36A, BLMH, CTBP1, TBCA, MDH2, and DXS9879E, whose expression indicates chemosensitivity to Vincristine.
- b) One or more of the gene sequences B2M, ARHGDIIB, FTL, NCL, MSN, SNRPF, XPO1, LDHB, SNRPF, GAPD, PTPN7, ARHGDIIB, RPS27, IFI16, C5orf13, and HCLS1, preferably gene sequences C1QR1, HCLS1, CD53, SLA, PTPN7, PTPRCAP, ZNFN1A1, CENTB1, PTPRC, IFI16, ARHGEF6, SEC31L2, CD3Z, GZMB, CD3D, MAP4K1, GPR65, PRF1, ARHGAP15, TM6SF1, and TCF4, and most preferably gene sequences C1QR1, SLA, PTPN7, ZNFN1A1, CENTB1, IFI16, ARHGEF6, SEC31L2, CD3Z, GZMB, CD3D, MAP4K1, GPR65, PRF1, ARHGAP15, TM6SF1, and TCF4, whose expression indicates chemosensitivity to Cisplatin.
- c) One or more of the gene sequences PRPS1, DDOST, B2M, SPARC, LGALS1, CFB, SNRPB2, MCAM, MCAM, E1F2S2, HPRT1, SRM, FKBP1A, GYPC, UROD, MSN, HNRPA1, SND1, COPA, MAPRE1, E1F3S2, ATP1B3, EMP3, ECM1, ATOX1, NARS, PGK1, OK/SW-c1.56, FN1, EEF1A1, GNAI2, PRPS1, RPL7, PSMB9, GPNMB, PPP1R11, MIA, RAB7, VIM, and SMS, preferably gene sequences MSN, SPARC, VIM, SRM, SCARB1, SIAT1, CUGBP2, GAS7, ICAM1, WASPIP, ITM2A, PALM2-AKAP2, ANPEP, PTPNS1, MPP1, LNK, FCGR2A, EMP3, RUNX3, EVI2A, BTN3A3, LCP2, BCHE, LY96, LCP1, IFI16, MCAM, MEF2C, SLC1A4, BTN3A2, FYN, FN1, C1orf38, CHS1, CAPN3, FCGR2C, TNK, AMPD2, SEPT6, RAFTLIN, SLC43A3, RAC2, LPXN, CKIP-1, FLJ10539, FLJ35036, DOCK10, TRPV2, IFRG28, LEF1, and ADAMTS1, and most preferably gene sequences SRM, SCARB1, SIAT1, CUGBP2, ICAM1, WASPIP, ITM2A, PALM2-AKAP2, PTPNS1, MPP1, LNK, FCGR2A, RUNX3, EVI2A, BTN3A3, LCP2, BCHE, LY96, LCP1, IFI16, MCAM, MEF2C, SLC1A4, FYN, C1orf38, CHS1, FCGR2C, TNK, AMPD2, SEPT6, RAFTLIN, SLC43A3, RAC2, LPXN, CKIP-1, FLJ10539, FLJ35036, DOCK10, TRPV2, IFRG28, LEF1, and ADAMTS1, whose expression indicates chemosensitivity to Azaguanine.
- d) One or more of the gene sequences B2M, MYC, CD99, RPS24, PPIF, PBEF1, and ANP32B, preferably gene sequences CD99, INSIG1, LAPT5, PRG1, MUF1, HCLS1, CD53, SLA, SSBP2, GNB5, MFNG, GMFG, PSMB9, EVI2A, PTPN7, PTGER4, CXorf9, PTPRCAP, ZNFN1A1, CENTB1, PTPRC, NAP1L1, HLA-DRA, IFI16, CORO1A, ARHGEF6, PSCDBP, SELPLG, LAT, SEC31L2, CD3Z, SH2D1A, GZMB, SCN3A, ITK, RAFTLIN, DOCK2, CD3D, RAC2, ZAP70, GPR65, PRF1, ARHGAP15, NOTCH1, and UBASH3A, and most preferably gene sequences CD99, INSIG1, PRG1, MUF1, SLA, SSBP2, GNB5, MFNG, PSMB9, EVI2A, PTPN7, PTGER4, CXorf9, ZNFN1A1, CENTB1, NAP1L1, HLA-DRA, IFI16, ARHGEF6, PSCDBP, SELPLG, LAT, SEC31L2, CD3Z, SH2D1A, GZMB, SCN3A, RAFTLIN, DOCK2, CD3D, RAC2, ZAP70, GPR65, PRF1, ARHGAP15, NOTCH1, and UBASH3A, whose expression indicates chemosensitivity to Etoposide.
- e) One or more of the gene sequences KIAA0220, B2M, TOP2A, CD99, SNRPE, RPS27, HNRPA1, CBX3, ANP32B, HNRPA1, DDX5, PPIA, SNRPF, and USP7, preferably gene sequences CD99, LAPT5, ALDOC, HCLS1, CD53, SLA, SSBP2, IL2RG, GMFG, CXorf9, RHOH, PTPRCAP, ZNFN1A1, CENTB1, TCF7, CD1C, MAP4K1, CD1B, CD3G, PTPRC, CCR9, CORO1A, CXCR4, ARHGEF6, HEM1, SELPLG, LAT, SEC31L2, CD3Z, SH2D1A, CD1A, LAIR1, ITK, TRB@, CD3D, WBSCR20C, ZAP70, IFI44, GPR65, AIF1, ARHGAP15, NARF, and PACAP, and most preferably gene sequences CD99, ALDOC, SLA, SSBP2, IL2RG, CXorf9, RHOH, ZNFN1A1, CENTB1, CD1C, MAP4K1, CD3G, CCR9, CXCR4, ARHGEF6, SELPLG, LAT, SEC31L2, CD3Z, SH2D1A, CD1A, LAIR1, TRB@, CD3D, WBSCR20C, ZAP70, IFI44, GPR65, AIF1, ARHGAP15, NARF, and PACAP, whose expression indicates chemosensitivity to Adriamycin.
- f) One or more of the gene sequences RPLP2, LAMR1, RPS25, EIF5A, TUFM, HNRPA1, RPS9, MYB, LAMR1, ANP32B, HNRPA1, HNRPA1, EIF4B, HMGB2, RPS15A, and RPS7, preferably gene sequences RPL12, RPL32, RPLP2, MYB, ZNFN1A1, SCAP1, STAT4, SP140, AMPD3, TNFAIP8, DDX18, TAF5, FBL, RPS2, PTPRC, DOCK2, GPR65, HOXA9, FLJ12270, and HNRPD, and most preferably gene sequences RPL12, RPLP2, MYB, ZNFN1A1, SCAP1, STAT4, SP140, AMPD3, TNFAIP8, DDX18, TAF5, RPS2, DOCK2, GPR65, HOXA9, FLJ12270, and HNRPD, whose expression indicates chemosensitivity to Aclarubicin.
- g) One or more of the gene sequences ARHGEF6, B2M, TOP2A, TOP2A, ELA2B, PTMA, LMNB1, TNFRSF1A, NAP1L1, B2M, HNRPA1, RPL9, C5orf13, NCOR2, ANP32B, OK/SW-c1.56, TUBA3, HMGN2, PRPS1, DDX5, PRG1, PPIA, G6PD, PSMB9, SNRPF, and MAP1B, preferably gene sequences PGAM1, DPYSL3, INSIG1, GJA1, BNIP3, PRG1, G6PD, BASP1, PLOD2, LOXL2, SSBP2, C1orf29, TOX, STC1, TNFRSF1A, NCOR2, NAP1L1, LOC94105, COL6A2, ARHGEF6, GATA3, TFPI, LAT, CD3Z, AF1Q, MAP1B, PTPRC, PRKCA, TRIM22, CD3D, BCAT1, IFI44, CCL2, RAB31, CUTC, NAP1L2, NME7, FLJ21159, and COL5A2, and most preferably gene sequences PGAM1, DPYSL3, INSIG1, GJA1, BNIP3, PRG1, G6PD, PLOD2, LOXL2, SSBP2, C1orf29, TOX, STC1, TNFRSF1A, NCOR2, NAP1L1, LOC94105, ARHGEF6, GATA3, TFPI, LAT, CD3Z, AF1Q, MAP1B, TRIM22, CD3D, BCAT1, IFI44, CUTC, NAP1L2, NME7, FLJ21159, and COL5A2, whose expression indicates chemosensitivity to Mitoxantrone.

- h) One or more of the gene sequences GAPD, GAPD, GAPD, TOP2A, SUI1, TOP2A, FTL, HNRPC, TNFRSF1A, SHC1, CCT7, P4HB, CTSI, DDX5, G6PD, and SNRPF, preferably gene sequences STC1, GPR65, DOCK10, COL5A2, FAM46A, and LOC54103, and most preferably gene sequences STC1, GPR65, DOCK10, COL5A2, FAM46A, and LOC54103, whose expression indicates chemosensitivity to Mitomycin.
- i) One or more of the gene sequences RPS23, SFRS3, KIAA0114, RPL39, SFRS3, LOC51035, RPS6, EXOSC2, RPL35, IFRD2, SMN2, EEF1A1, RPS3, RPS18, and RPS7, preferably gene sequences RPL10, RPS4X, NUDC, RALY, DKC1, DKFZP564C186, PRP19, RAB9P40, HSA9761, GMDS, CEP1, IL13RA2, MAGEB2, HMGN2, ALMS1, GPR65, FLJ10774, NOL8, DAZAP1, SLC25A15, PAF53, DXS9879E, PTPNC1, SPANXC, and KIAA1393, and most preferably RPL10, RPS4X, NUDC, DKC1, DKFZP564C186, PRP19, RAB9P40, HSA9761, GMDS, CEP1, IL13RA2, MAGEB2, HMGN2, ALMS1, GPR65, FLJ10774, NOL8, DAZAP1, SLC25A15, PAF53, DXS9879E, PTPNC1, SPANXC, and KIAA1393, whose expression indicates chemosensitivity to Paclitaxel.
- j) One or more of the gene sequences CSDA, LAMR1, and TUBA3, preferably gene sequences PFN1, PGAM1, K-ALPHA-1, CSDA, UCHL1, PWP1, PALM2-AKAP2, TNFRSF1A, ATP5G2, AF1Q, NME4, and FHOD1, and most preferably gene sequences PFN1, PGAM1, K-ALPHA-1, CSDA, UCHL1, PWP1, PALM2-AKAP2, TNFRSF1A, ATP5G2, AF1Q, NME4, and FHOD1, whose expression indicates chemosensitivity to Gemcitabine.
- k) One or more of the gene sequences RPS23, SFRS3, KIAA0114, SFRS3, RPS6, DDX39, and RPS7, preferably gene sequences ANP32B, GTF3A, RRM2, TRIM14, SKP2, TRIP13, RFC3, CASP7, TXN, MCM5, PTGES2, OBFC1, EPB41L4B, and CALML4, and most preferably gene sequences ANP32B, GTF3A, RRM2, TRIM14, SKP2, TRIP13, RFC3, CASP7, TXN, MCM5, PTGES2, OBFC1, EPB41L4B, and CALML4, whose expression indicates chemosensitivity to Taxotere.
- l) One or more of the gene sequences IL2RG, H1FX, RDBP, ZAP70, CXCR4, TM4SF2, ARHGDIb, CDA, CD3E, STMN1, GNA15, AXL, CCND3, SATB1, EIF5A, LCK, NKK2-5, LAPTMs, IQGAP2, FLII, EIF3S5, TRB, CD3D, HOXB2, GATA3, HMGB2, PSMB9, ATP5G2, CORO1A, ARHGDIb, DRAP1, PTPRCAP, RHOH, and ATP2A3, preferably gene sequences IFITM2, UBE2L6, LAPTMs, USP4, ITM2A, ITGB2, ANPEP, CD53, IL2RG, CD37, GPRASP1, PTPN7, CXorf9, RHOH, GIT2, ADORA2A, ZNFN1A1, GNA15, CEP1, TNFRSF7, MAP4K1, CCR7, CD3G, PTPRC, ATP2A3, UCP2, CORO1A, GATA3, CDKN2A, HEM1, TARP, LAIR1, SH2D1A, FLII, SEPT6, HA-1, CREB3L1, ERCC2, CD3D, LST1, AIF1, ADA, DATF1, ARHGAP15, PLAC8, CECR1, LOC81558, and EHD2, and most preferably gene sequences IFITM2, UBE2L6, USP4, ITM2A, IL2RG, GPRASP1, PTPN7, CXorf9, RHOH, GIT2, ZNFN1A1, CEP1, TNFRSF7, MAP4K1, CCR7, CD3G, ATP2A3, UCP2, GATA3, CDKN2A, TARP, LAIR1, SH2D1A, SEPT6, HA-1, ERCC2, CD3D, LST1, AIF1, ADA, DATF1, ARHGAP15, PLAC8, CECR1, LOC81558, and EHD2, whose expression indicates chemosensitivity to Dexamethasone.
- m) One or more of the gene sequences TM4SF2, ARHGDIb, ADA, H2AFZ, NAP1L1, CCND3, FABP5, LAMR1, REA, MCM5, SNRPF, and USP7, preferably gene sequences ITM2A, RHOH, PRIM1, CENTB1, GNA15, NAP1L1, ATP5G2, GATA3, PRKCQ, SH2D1A, SEPT6, NME4, RPL13, CD3D, CD1E, ADA, and FHOD1, and most preferably gene sequences ITM2A, RHOH, PRIM1, CENTB1, NAP1L1, ATP5G2, GATA3, PRKCQ, SH2D1A, SEPT6, NME4, CD3D, CD1E, ADA, and FHOD1, whose expression indicates chemosensitivity to Ara-C.
- n) One or more of the gene sequences LGALS9, CD7, IL2RG, PTPN7, ARHGEF6, CENTB1, SEPT6, SLA, LCP1, IFITM1, ZAP70, CXCR4, TM4SF2, ZNF91, ARHGDIb, TFDP2, ADA, CD99, CD3E, CD1C, STMN1, CD53, CD7, GNA15, CCND3, MAZ, SATB1, ZNF22, AES, AIF1, MYB, LCK, C5orf13, NKK2-5, ZNFN1A1, STAT5A, CHI3L2, LAPTMs, MAP4K1, DDX11, GPSM3, TRB, CD3D, CD3G, PRKCB1, CD1E, HCLS1, GATA3, TCF7, RHOG, CDW52, HMGB2, DGKA, ITGB2, PSMB9, IDH2, AES, MCM5, NUCB2, CORO1A, ARHGDIb, PTPRCAP, CD47, RHOH, LGALS9, and ATP2A3, preferably gene sequences CD99, SRRM1, ARHGDIb, LAPTMs, VWF, ITM2A, ITGB2, LGALS9, INPP5D, SATB1, CD53, TFDP2, SLA, IL2RG, MFNG, CD37, GMFG, SELL, CDW52, LRMP, ICAM2, RIMS3, PTPN7, ARHGAP25, LCK, CXorf9, RHOH, PTPRCAP, GIT2, ZNFN1A1, CENTB1, LCP2, SPI1, GNA15, GZMA, CEP1, BLM, CD8A, SCAP1, CD2, CD1C, TNFRSF7, VAV1, MAP4K1, CCR7, C6orf32, ALOX15B, BRDT, CD3G, PTPRC, LTB, ATP2A3, NVL, RASGRP2, LCP1, CORO1A, CXCR4, PRKD2, GATA3, TRA@, PRKCB1, HEM1, KIAA0922, TARP, SEC31L2, PRKCQ, SH2D1A, CHRNa3, CD1A, LST1, LAIR1, CACNA1G, TRB@, SEPT6, HA-1, DOCK2, CD3D, TRD@, T3JAM, FNBP1, CD6, AIF1, FOLH1, CD1E, LY9, UGT2B17, ADA, CDKL5, TRIM, EVL, DATF1, RGC32, PRKCH, ARHGAP15, NOTCH1, BIN2, SEMA4G, DPEP2, CECR1, BCL11B, STAG3, GALNT6, UBASH3A, PHEMX, FLJ13373, LEF1, IL21R, MGC17330, AKAP13, ZNF335, and GIMAP5, and most preferably gene sequences CD99, ARHGDIb, VWF, ITM2A, LGALS9, INPP5D, SATB1, TFDP2, SLA, IL2RG, MENG, SELL, CDW52, LRMP, ICAM2, RIMS3, PTPN7, ARHGAP25, LCK, CXorf9, RHOH, GIT2, ZNFN1A1, CENTB1, LCP2, SPI1, GZMA, CEP1, CD8A, SCAP1, CD2, CD1C, TNFRSF7, VAV1, MAP4K1, CCR7, C6orf32, ALOX15B, BRDT, CD3G, LTB, ATP2A3, NVL, RASGRP2, LCP1, CXCR4, PRKD2, GATA3, TRA@, KIAA0922, TARP, SEC31L2, PRKCQ, SH2D1A, CHRNa3, CD1A, LST1, LAIR1, CACNA1G, TRB@, SEPT6, HA-1, DOCK2, CD3D, TRD@, T3JAM, FNBP1, CD6, AIF1, FOLH1, CD1E, LY9, ADA, CDKL5, TRIM, EVL, DATF1, RGC32, PRKCH, ARHGAP15, NOTCH1, BIN2, SEMA4G, DPEP2, CECR1, BCL11B, STAG3, GALNT6, UBASH3A, PHEMX, FLJ13373, LEF1, IL21R, MGC17330, AKAP13, ZNF335, and GIMAP5, whose expression indicates chemosensitivity to Methylprednisolone.
- o) One or more of the gene sequences RPLP2, RPL4, HMGA1, RPL27, IMPDH2, LAMR1, PTMA, ATP5B, NPM1, NCL, RPS25, RPL9, TRAP1, RPL21, LAMR1, REA, HNRPA1, LDHB, RPS2, NME1, PAICS, EEF1B2, RPS15A, RPL19, RPL6, ATP5G2, SNRPF, SNRPG, and RPS7, preferably gene sequences PRPF8, RPL18, RNPS1, RPL32, EEF1G, GOT2, RPL13A, PTMA, RPS15, RPLP2, CSDA, KHDRBS1, SNRPA, IMPDH2, RPS19, NUP88, ATP5D, PCBP2, ZNF593, HSU79274, PRIM1, PFDN5, OXA1L, H3F3A, ATIC, RPL13, CIAPIN1, FBL, RPS2, PCCB, RBMX, SHMT2, RPLP0, HNRPA1, STOML2,

RPS9, SKB1, GLTSCR2, CCNB1IP1, MRPS2, FLJ20859, and FLJ12270, and most preferably gene sequences PRPF8, RPL18, GOT2, RPL13A, RPS15, RPLP2, CSDA, KHDRBS1, SNRPA, IMPDH2, RPS19, NUP88, ATP5D, PCBP2, ZNF593, HSU79274, PRIM1, PFDN5, OXA1L, H3F3A, ATIC, CIAPIN1, RPS2, PCCB, SHMT2, RPLP0, HNRPA1, STOML2, SKB1, GLTSCR2, CCNB1IP1, MRPS2, FLJ20859, and FLJ12270, whose expression indicates chemosensitivity to Methotrexate.

p) One or more of the gene sequences ACTB, COL5A1, MT1E, CSDA, COL4A2, MMP2, COL1A1, TNFRSF1A, CFHL1, TGFBI, FSCN1, NNMT, PLAUR, CSPG2, NFIL3, C5orf13, NCOR2, TUBB4, MYLK, TUBA3, PLAU, COL4A2, COL6A2, COL6A3, IFITM2, PSMB9, CSDA, and COL1A1, preferably gene sequences MSN, PFN1, HK1, ACTR2, MCL1, ZYX, RAP1B, GNB2, EPAS1, PGAM1, CKAP4, DUSP1, MYL9, K-ALPHA-1, LGALS1, CSDA, AKR1B1, IFITM2, ITGA5, VIM, DPYSL3, JUNB, ITGA3, NFKBIA, LAMB1, FHL1, INSIG1, TIMP1, GJA1, PSME2, PRG1, EXT1, DKFZP434J154, OPTN, M6PRBP1, MVP, VASP, ARL7, NNMT, TAP1, COL1A1, BASP1, PLOD2, ATF3, PALM2-AKAP2, IL8, ANPEP, LOXL2, TGFB1, IL4R, DGKA, STC2, SEC61G, NFIL3, RGS3, NK4, F2R, TPM2, PSMB9, LOX, STC1, CSPG2, PTGER4, IL6, SMAD3, PLAU, WNT5A, BDNF, TNFRSF1A, FLNC, DKFZP564K0822, FLOT1, PTRF, HLA-B, COL6A2, MGC4083, TNFRSF10B, PLAGL1, PNMA2, TFPI, LAT, GZMB, CYR61, PLAUR, FSCN1, ERP70, AF1Q, UBC, FGFR1, HIC, BAX, COL4A2, COL6A1, IFITM3, MAP1B, FLJ46603, RAFTLIN, RRAS, FTL, KIAA0877, MT1E, CDC10, DOCK2, TRIM22, RIS1, BCAT1, PRF1, DBN1, MT1K, TMSB10, RAB31, FLJ10350, C1orf24, NME7, TMEM22, TPK1, COL5A2, ELK3, CYLD, ADAMTS1, EHD2, and ACTB, and most preferably gene sequences PFN1, HK1, MCL1, ZYX, RAP1B, GNB2, EPAS1, PGAM1, CKAP4, DUSP1, MYL9, K-ALPHA-1, LGALS1, CSDA, IFITM2, ITGA5, DPYSL3, JUNB, NFKBIA, LAMB1, FHL1, INSIG1, TIMP1, GJA1, PSME2, PRG1, EXT1, DKFZP434J154, MVP, VASP, ARL7, NNMT, TAP1, PLOD2, ATF3, PALM2-AKAP2, IL8, LOXL2, IL4R, DGKA, STC2, SEC61G, RGS3, F2R, TPM2, PSMB9, LOX, STC1, PTGER4, IL6, SMAD3, WNT5A, BDNF, TNFRSF1A, FLNC, DKFZP564K0822, FLOT1, PTRF, HLA-B, MGC4083, TNFRSF10B, PLAGL1, PNMA2, TFPI, LAT, GZMB, CYR61, PLAUR, FSCN1, ERP70, AF1Q, HIC, COL6A1, IFITM3, MAP1B, FLJ46603, RAFTLIN, RRAS, FTL, KIAA0877, MT1E, CDC10, DOCK2, TRIM22, RIS1, BCAT1, PRF1, DBN1, MT1K, TMSB10, FLJ10350, C1orf24, NME7, TMEM22, TPK1, COL5A2, ELK3, CYLD, ADAMTS1, EHD2, and ACTB, whose expression indicates chemosensitivity to Bleomycin.

q) One or more of the gene sequences NOS2A, MUC1, TFF3, GPIBB, IGLL1, BATF, MYB, PTPRS, NEFL, AIP, CEL, DGKA, RUNX1, ACTR1A, and CLCNKA, preferably gene sequences PTMA, SSRP1, NUDC, CTSC, AP1G2, PSME2, LBR, EFNB2, SERPINA1, SSSCA1, EZH2, MYB, PRIM1, H2AFX, HMGA1, HMMR, TK2, WHSC1, DIAPH1, LAMB3, DPAGT1, UCK2, SERPINB1, MDN1, BRRN1, GOS2, RAC2, MGC21654, GTSE1, TACC3, PLEK2, PLAC8, HNRPD, and PNAS-4, and most preferably gene sequences SSRP1, NUDC, CTSC, AP1G2, PSME2, LBR, EFNB2, SERPINA1, SSSCA1, EZH2, MYB, PRIM1, H2AFX, HMGA1, HMMR, TK2, WHSC1, DIAPH1, LAMB3, DPAGT1, UCK2, SERPINB1, MDN1, BRRN1,

GOS2, RAC2, MGC21654, GTSE1, TACC3, PLEK2, PLAC8, HNRPD, and PNAS-4, whose expression indicates chemosensitivity to Methyl-GAG.

r) One or more of the gene sequences MSN, ITGA5, VIM, TNFAIP3, CSPG2, WNT5A, FOXF2, LOC94105, IFI16, LRRN3, FGFR1, DOCK10, LEPRE1, COL5A2, and ADAMTS1, and most preferably gene sequences ITGA5, TNFAIP3, WNT5A, FOXF2, LOC94105, IFI16, LRRN3, DOCK10, LEPRE1, COL5A2, and ADAMTS1, whose expression indicates chemosensitivity to carboplatin.

s) One or more of the gene sequences RPL18, RPL10A, RNPS1, ANAPC5, EEF1B2, RPL13A, RPS15, AKAP1, NDUFAB1, APRT, ZNF593, MRP63, IL6R, RPL13, SART3, RPS6, UCK2, RPL3, RPL17, RPS2, PCCB, TOMM20, SHMT2, RPLP0, GTF3A, STOML2, DKFZp564J157, MRPS2, ALG5, and CALML4, and most preferably gene sequences RPL18, RPL10A, ANAPC5, EEF1B2, RPL13A, RPS15, AKAP1, NDUFAB1, APRT, ZNF593, MRP63, IL6R, SART3, UCK2, RPL17, RPS2, PCCB, TOMM20, SHMT2, RPLP0, GTF3A, STOML2, DKFZp564J157, MRPS2, ALG5, and CALML4, whose expression indicates chemosensitivity to 5-FU(5-Fluorouracil).

t) One or more of the gene sequences ITK, KIFC1, VLDDL, RUNX1, PAFAH1B3, H1FX, RNF144, TMSNB, CRY1, MAZ, SLA, SRF, UMPS, CD3Z, PRKCQ, HNRPM, ZAP70, ADD1, RFC5, TM4SF2, PFN2, BMI1, TUBGCP3, ATP6V1B2, RALY, PSMC5, CD1D, ADA, CD99, CD2, CNP, ERG, MYL6, CD3E, CD1A, CD1B, STMN1, PSMC3, RPS4Y1, AKT1, TAL1, GNA15, UBE2A, TCF12, UBE2S, CCND3, PAX6, MDK, CAPG, RAG2, ACTN1, GSTM2, SATB1, NASP, IGFBP2, CDH2, CRABP1, DBN1, CTNNA1, AKR1C1, CACNB3, FARSLA, CASP2, CASP2, E2F4, LCP2, CASP6, MYB, SFRS6, GLRB, NDN, CPSF1, GNAQ, TUSC3, GNAQ, JARID2, OCRL, FHL1, EZH2, SMOX, SLC4A2, UFD1L, SEPW1, ZNF32, HTATSF1, SHD1, PTOV1, NXF1, FYB, TRIM28, BC008967, TRB@, TFRC, H1F0, CD3D, CD3G, CENPB, ALDH2, ANXA1, H2AFX, CD1E, DDX5, ABL1, CCNA2, ENO2, SNRPB, GATA3, RRM2, GLUL, TCF7, FGFR1, SOX4, MAL, NUCB2, SMA3, FAT, UNG, ARHGDI, RUNX1, MPHOSPH6, DCTN1, SH3GL3, VIM, PLEKHC1, CD47, POLR2F, RHOH, ADD1, and ATP2A3, preferably gene sequences ITK, KIFC1, VLDDL, RUNX1, PAFAH1B3, H1FX, RNF144, TMSNB, CRY1, MAZ, SLA, SRF, UMPS, CD3Z, PRKCQ, HNRPM, ZAP70, ADD1, RFC5, TM4SF2, PFN2, BMI1, TUBGCP3, ATP6V1B2, RALY, PSMC5, CD1D, ADA, CD99, CD2, CNP, ERG, MYL6, CD3E, CD1A, CD1B, STMN1, PSMC3, RPS4Y1, AKT1, TAL1, GNA15, UBE2A, TCF12, UBE2S, CCND3, PAX6, MDK, CAPG, RAG2, ACTN1, GSTM2, SATB1, NASP, IGFBP2, CDH2, CRABP1, DBN1, CTNNA1, AKR1C1, CACNB3, FARSLA, CASP2, CASP2, E2F4, LCP2, CASP6, MYB, SFRS6, GLRB, NDN, CPSF1, GNAQ, TUSC3, GNAQ, JARID2, OCRL, FHL1, EZH2, SMOX, SLC4A2, UFD1L, SEPW1, ZNF32, HTATSF1, SHD1, PTOV1, NXF1, FYB, TRIM28, BC008967, TRB@, TFRC, H1F0, CD3D, CD3G, CENPB, ALDH2, ANXA1, H2AFX, CD1E, DDX5, ABL1, CCNA2, ENO2, SNRPB, GATA3, RRM2, GLUL, TCF7, FGFR1, SOX4, MAL, NUCB2, SMA3, FAT, UNG, ARHGDI, RUNX1, MPHOSPH6, DCTN1, SH3GL3, VIM, PLEKHC1, CD47, POLR2F, RHOH, ADD1, and ATP2A3, and most preferably gene sequences KIFC1, VLDDL, RUNX1, PAFAH1B3, H1FX, RNF144, TMSNB, CRY1, MAZ, SLA, SRF, UMPS, CD3Z, PRKCQ, HNRPM,

ZAP70, ADD1, RFC5, TM4SF2, PFN2, BMI1, TUBGCP3, ATP6V1B2, CD1D, ADA, CD99, CD2, CNP, ERG, CD3E, CD1A, PSMC3, RPS4Y1, AKT1, TAL1, UBE2A, TCF12, UBE2S, CCND3, PAX6, RAG2, GSTM2, SATB1, NASP, IGFBP2, CDH2, CRABP1, DBN1, AKR1C1, CACNB3, CASP2, CASP2, LCP2, CASP6, MYB, SFRS6, GLRB, NDN, GNAQ, TUSC3, GNAQ, JARID2, OCRL, FHL1, EZH2, SMOX, SLC4A2, UFD1L, ZNF32, HTATSF1, SHD1, PTOV1, NXF1, FYB, TRIM28, BC008967, TRB@, H1F0, CD3D, CD3G, CENPB, ALDH2, ANXA1, H2AFX, CD1E, DDX5, CCNA2, ENO2, SNRBP, GATA3, RRM2, GLUL, SOX4, MAL, UNG, ARHGDI, RUNX1, MPHOSPH6, DCTN1, SH3GL3, PLEKHC1, CD47, POLR2F, RHOH, and ADD1, whose expression indicates chemosensitivity to Rituximab (e.g., MABTHERA™).

u) One or more of the gene sequences CCL21, ANXA2, SCARB2, MAD2L1BP, CAST, PTS, NBL1, ANXA2, CD151, TRAM2, HLA-A, CRIP2, UGCG, PRSS11, MME, CBR1, LGALS1, DUSP3, PFN2, MICA, FTH1, RHOC, ZAP128, PON2, COL5A2, CST3, MCAM, IGFBP3, MMP2, GALIG, CTSD, ALDH3A1, CSRP1, S100A4, CALD1, CTGF, CAPG, HLA-A, ACTN1, TAGLN, FSTL1, SCTR, BLVRA, COPEB, DIPA, SMARCD3, FN1, CTSL, CD63, DUSP1, CKAP4, MVP, PEA15, S100A13, and ECE1, preferably gene sequences TRA1, ACTN4, WARS, CALM1, CD63, CD81, FKBP1A, CALU, IQGAP1, CTSB, MGC8721, STAT1, TACC1, TM4SF8, CD59, CKAP4, DUSP1, RCN1, MGC8902, LGALS1, BHLHB2, RRBP1, PKM2, PRNP, PPP2CB, CNN3, ANXA2, ER3, JAK1, MARCKS, LUM, FER1L3, SLC20A1, EIF4G3, HEXB, EXT1, TJP1, CTSL, SLC39A6, RIOK3, CRK, NNMT, COL1A1, TRAM2, ADAM9, DNAJC7, PLSCR1, PRSS23, PLOD2, NPC1, TOB1, GFPT1, IL8, DYRK2, PYGL, LOXL2, KIAA0355, UGDH, NFL3, PURA, ULK2, CENTG2, NID2, CAP350, CXCL1, BTN3A3, IL6, WNT5A, FOXF2, LPHN2, CDH11, P4HA1, GRP58, ACTN1, CAPN2, DSPI, MAP1LC3B, GALIG, IGSF4, IRS2, ATP2A2, OGT, TNFRSF10B, KIAA1128, TM4SF1, RBPM, RIPK2, CBLB, NR1D2, BTN3A2, SLC7A11, MPZL1, IGFBP3, SSA2, FN1, NQO1, ASPH, ASA1, MGLL, SERPINB6, HSPA5, ZFP36L1, COL4A2, COL4A1, CD44, SLC39A14, NIPA2, FKBP9, IL6ST, DKFZP564G2022, PPAP2B, MAP1B, MAPK1, MYO1B, CAST, RRAS2, QKI, LHFPL2, 38970, ARHE, KIAA1078, FTL, KIAA0877, PLCB1, KIAA0802, KPNB1, RAB3GAP, SERPINB1, TIMM17A, SOD2, HLA-A, NOMO2, LOC55831, PHLDA1, TMEM2, MLPH, FAD104, LRRC5, RAB7L1, FLJ35036, DOCK10, LRP12, TXNDC5, CDC14B, HRMT1L1, CORO1C, DNAJC10, TNPO1, LONP, AMIGO2, DNAPTP6, and ADAMTS1, and most preferably gene sequences TRA1, ACTN4, CALM1, CD63, FKBP1A, CALU, IQGAP1, MGC8721, STAT1, TACC1, TM4SF8, CD59, CKAP4, DUSP1, RCN1, MGC8902, LGALS1, BHLHB2, RRBP1, PRNP, IER3, MARCKS, LUM, FER1L3, SLC20 μl, HEXB, EXT1, TJP1, CTSL, SLC39A6, RIOK3, CRK, NNMT, TRAM2, ADAM9, DNAJC7, PLSCR1, PRSS23, PLOD2, NPC1, TOB1, GFPT1, IL8, PYGL, LOXL2, KIAA0355, UGDH, PURA, ULK2, CENTG2, NID2, CAP350, CXCL1, BTN3A3, IL6, WNT5A, FOXF2, LPHN2, CDH11, P4HA1, GRP58, DSPI, MAP1LC3B, GALIG, IGSF4, IRS2, ATP2A2, OGT, TNFRSF10B, KIAA1128, TM4SF1, RBPM, RIPK2, CBLB, NR1D2, SLC7A11, MPZL1, SSA2, NQO1, ASPH, ASA1, MGLL, SERPINB6, HSPA5, ZFP36L1, COL4A1, CD44,

SLC39A14, NIPA2, FKBP9, IL6ST, DKFZP564G2022, PPAP2B, MAP1B, MAPK1, MYO1B, CAST, RRAS2, QKI, LHFPL2, 38970, ARHE, KIAA1078, FTL, KIAA0877, PLCB1, KIAA0802, RAB3GAP, SERPINB1, TIMM17A, SOD2, HLA-A, NOMO2, LOC55831, PHLDA1, TMEM2, MLPH, FAD104, LRRC5, RAB7L1, FLJ35036, DOCK10, LRP12, TXNDC5, CDC14B, HRMT1L1, CORO1C, DNAJC10, TNPO1, LONP, AMIGO2, DNAPTP6, and ADAMTS1, whose expression indicates sensitivity to radiation therapy.

v) One or more of the gene sequences FAU, NOL5A, ANP32A, ARHGDI, LBR, FABP5, ITM2A, SFRS5, IQGAP2, SLC7A6, SLA, IL2RG, MFNG, GPSM3, PIM2, EVER1, LRMP, ICAM2, RIMS3, FMNL1, MYB, PTPN7, LCK, CXorf9, RHOH, ZNFN1A1, CENTB1, LCP2, DBT, CEP1, IL6R, VAV1, MAP4K1, CD28, PTP4A3, CD3G, LTB, USP34, NVL, CD8B1, SFRS6, LCP1, CXCR4, PSCDBP, SELPLG, CD3Z, PRKCQ, CD1A, GATA2, P2RX5, LAIR1, C1orf38, SH2D1A, TRB@, SEPT6, HA-1, DOCK2, WBSCR20C, CD3D, RNASE6, SFRS7, WBSCR20A, NUP210, CD6, HNRPA1, AIF1, CYFIP2, GLTSCR2, C11orf2, ARHGAP15, BIN2, SH3TC1, STAG3, TM6SF1, C15orf25, FLJ22457, PACAP, and MGC2744, whose expression indicates sensitivity to an HDAC inhibitor.

w) One or more of the gene sequences CD99, SNRPA, CUGBP2, STAT5A, SLA, IL2RG, GTSE1, MYB, PTPN7, CXorf9, RHOH, ZNFN1A1, CENTB1, LCP2, HIST1H4C, CCR7, APOBEC3B, MCM7, LCP1, SELPLG, CD3Z, PRKCQ, GZMB, SCN3A, LAIR1, SH2D1A, SEPT6, CG018, CD3D, C18orf10, PRF1, AIF1, MCM5, LPXN, C22orf18, ARHGAP15, and LEF1, whose expression indicates sensitivity to 5-Aza-2'-deoxycytidine (Decitabine).

[0052] Probes that may be employed on microarrays of the invention include oligonucleotide probes having sequences complementary to any of the biomarker gene or microRNA sequences described above. Additionally, probes employed on microarrays of the invention may also include proteins, peptides, or antibodies that selectively bind any of the oligonucleotide probe sequences or their complementary sequences. Exemplary probes are listed in Tables 22-44, wherein for each treatment listed, the biomarkers indicative of treatment sensitivity, the correlation of biomarker expression to growth inhibition, and the sequence of an exemplary probe (Tables 22-44) to detect biomarker (Tables 1-21) expression are shown.

Identification of Biomarker Genes

[0053] The gene expression measurements of the NCI60 cancer cell lines were obtained from the National Cancer Institute and the Massachusetts Institute of Technology (MIT). Each dataset was normalized so that sample expression measured by different chips could be compared. The preferred method of normalization is the logit transformation, which is performed for each gene y on each chip:

$$\text{logit}(y) = \log[(y - \text{background}) / (\text{saturation} - y)],$$

where background is calculated as the minimum intensity measured on the chip minus 0.1% of the signal intensity range: $\text{min} - 0.001 * (\text{max} - \text{min})$, and saturation is calculated as the maximum intensity measured on the chip plus 0.1% of the signal intensity range: $\text{max} + 0.001 * (\text{max} - \text{min})$. The resulting logit transformed data is then z-transformed to mean zero and standard deviation 1.

[0054] Next, gene expression is correlated to cancer cell growth inhibition. Growth inhibition data (GI50) of the NCI60 cell lines in the presence of any one of thousands of tested compounds was obtained from the NCI. The correlation between the logit-transformed expression level of each gene in each cell line and the logarithm of GI50 (the concentration of a given compound that results in a 50% inhibition of growth) can be calculated, e.g., using the Pearson correlation coefficient or the Spearman Rank-Order correlation coefficient. Instead of using GI50s, any other measure of patient sensitivity to a given compound may be correlated to the patient's gene expression. Since a plurality of measurements may be available for a single gene, the most accurate determination of correlation coefficient was found to be the median of the correlation coefficients calculated for all probes measuring expression of the same gene.

[0055] The median correlation coefficient of gene expression measured on a probe to growth inhibition or patient sensitivity is calculated for all genes, and genes that have a median correlation above 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 0.95, or 0.99 are retained as biomarker genes. Preferably, the correlation coefficient of biomarker genes will exceed 0.3. This is repeated for all the compounds to be tested. The result is a list of marker genes that correlates to sensitivity for each compound tested.

Predicting Patient Sensitivity or Resistance to Medical Treatment

[0056] For a given compound, the biomarker whose expression has been shown to correlate to chemosensitivity can be used to classify a patient, e.g., a cancer patient, as sensitive to a medical treatment, e.g., administration of a chemotherapeutic agent or radiation. Using a tumor sample or a blood sample (e.g., in case of leukemia or lymphoma) from a patient, expression of the biomarker in the cells of the patient in the presence of the treatment agent is determined (using, for example, an RNA extraction kit, a DNA microarray and a DNA microarray scanner). The biomarker expression measurements are then logit transformed as described above. The sum of the expression measurements of the biomarkers is then compared to the median of the sums derived from a training set population of patients having the same tumor. If the sum of biomarker expression in the patient is closest to the median of the sums of expression in the surviving members of the training set, the patient is predicted to be sensitive to the compound or other medical treatment. If the sum of expression in the patient is closest to the median of the sums of expression in the non-surviving members of the training set, the patient is predicted to be resistant to the compound.

[0057] Machine learning techniques such as Neural Networks, Support Vector Machines, K Nearest Neighbor, and Nearest Centroids may also be employed to develop models that discriminate patients sensitive to treatment from those resistant to treatment using biomarker expression as model variables which assign each patient a classification as resistant or sensitive. Machine learning techniques used to classify patients using various measurements are described in U.S. Pat. No. 5,822,715; U.S. Patent Application Publication Nos. 2003/0073083, 2005/0227266, 2005/0208512, 2005/0123945, 2003/0129629, and 2002/0006613; and in Vapnik V N. Statistical Learning Theory, John Wiley & Sons, New York, 1998; Hastie et al., 2001, The Elements of Statistical Learning: Data Mining, Inference, and Prediction, Springer, N.Y.; Agresti, 1996, An Introduction to Categorical Data

Analysis, John Wiley & Sons, New York; and V. Tresp et al., "Neural Network Modeling of Physiological Processes", in Hanson S. J. et al. (Eds.), Computational Learning Theory and Natural Learning Systems 2, MIT Press, 1994, hereby incorporated by reference.

[0058] Other variables can be used to determine relative biomarker expression between a patient (e.g., a cancer patient) and a normal subject (e.g., a control subject), including but not limited to, measurement of biomarker DNA copy number and the identification of biomarker genetic mutations.

[0059] A more compact microarray can be designed using only the oligonucleotide probes having measurements yielding the median correlation coefficients with cancer cell growth inhibition. Thus, in this embodiment, only one probe needs to be used to measure expression of each biomarker. Biomarkers include polypeptides and metabolites thereof. A skilled artisan can use employ assays that measure changes in polypeptide biomarker expression (e.g., Western blot, immunofluorescent staining, and flow cytometry) to determine a patient's sensitivity to a treatment (e.g., chemotherapy, radiation therapy, or surgery).

Identifying a Subpopulation of Patients Sensitive to a Treatment for Cancer

[0060] The invention can also be used to identify a subpopulation of patients, e.g., cancer patients, that are sensitive to a compound or other medical treatment previously thought to be ineffective for the treatment of cancer. To this end, genes or microRNAs whose expression correlates to sensitivity to a compound or other treatment can be identified so that patients sensitive to a compound or other treatment may be identified. To identify such biomarkers, gene or microRNA expression within cell lines can be correlated to the growth of those cell lines in the presence of the same compound or other treatment. Preferably, genes or microRNAs whose expression correlates to cell growth with a correlation coefficient exceeding 0.3 may be considered possible biomarkers.

[0061] Alternatively, genes or microRNAs can be identified as biomarkers according to their ability to discriminate patients known to be sensitive to a treatment from those known to be resistant. The significance of the differences in gene or microRNA expression between the sensitive and resistant patients may be measured using, e.g., t-tests. Alternatively, naïve Bayesian classifiers may be used to identify gene biomarkers that discriminate sensitive and resistant patient subpopulations given the gene expressions of the sensitive and resistant subpopulations within a treated patient population.

[0062] The patient subpopulations considered can be further divided into patients predicted to survive without treatment, patients predicted to die without treatment, and patients predicted to have symptoms without treatment. The above methodology may be similarly applied to any of these further defined patient subpopulations to identify biomarkers able to predict a subject's sensitivity to compounds or other treatments for the treatment of cancer.

[0063] Patients with elevated expression of biomarkers correlated to sensitivity to a compound or other medical treatment would be predicted to be sensitive to that compound or other medical treatment.

[0064] The invention is particularly useful for recovering compounds or other treatments that failed in clinical trials by identifying sensitive patient subpopulations using the gene or

microRNA expression methodology disclosed herein to identify biomarkers that can be used to predict clinical outcome.

Kit, Apparatus, and Software for Clinical Use

[0065] This invention can also be used to predict patients who are resistant or sensitive to a particular treatment by using a kit that includes a kit for RNA extraction from tumors (e.g., Trizol from Invitrogen Inc.), a kit for RNA amplification (e.g., MessageAmp from Ambion Inc.), a microarray for measuring biomarker expression (e.g., HG-U133A GeneChip from Affymetrix Inc.), a microarray hybridization station and scanner (e.g., GeneChip System 3000Dx from Affymetrix Inc.), and software for analyzing the expression of marker genes as described in herein (e.g., implemented in R from R-Project or S-Plus from Insightful Corp.).

Methodology of the In Vitro Cancer Growth Inhibition Screen

[0066] The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells are inoculated into 96 well microtiter plates in 100 μ L at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37° C., 5% CO₂, 95% air, and 100% relative humidity for 24 hrs prior to addition of experimental compounds.

[0067] After 24 hrs, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of compound addition (Tz). Experimental compounds are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of compound addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μ g/mL Gentamicin. Additional four, 10-fold or V2 log serial dilutions are made to provide a total of five compound concentrations plus control. Aliquots of 100 μ L of these different compound dilutions are added to the appropriate microtiter wells already containing 100 μ L of medium, resulting in the required final compound concentrations.

[0068] Following compound addition, the plates are incubated for an additional 48 hrs at 37° C., 5% CO₂, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μ L of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4° C. The supernatant is discarded, and the plates are washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 μ L) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air-dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μ L of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of compound at the five concentration levels (Ti)], the percentage growth is

calculated at each of the compound concentrations levels. Percentage growth inhibition is calculated as:

$$\frac{[(T_i - T_z)/(C - T_z)] \times 100}{T_i >= T_z}$$

$$\frac{[(T_i - T_z)/T_z] \times 100}{T_i < T_z}$$

[0069] Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI50) is calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is the compound concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the compound incubation. The compound concentration resulting in total growth inhibition (TGI) is calculated from $T_i = T_z$. The LC50 (concentration of compound resulting in a 50% reduction in the measured protein at the end of the compound treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

RNA Extraction and Gene Expression Measurement

[0070] Cell/tissue samples are snap frozen in liquid nitrogen until processing. RNA is extracted using e.g., Trizol Reagent (Invitrogen) following manufacturers instructions. RNA is amplified using e.g., MessageAmp kit (Ambion) following manufacturers instructions. Amplified RNA is quantified using e.g., HG-U133A GeneChip (Affymetrix) and compatible apparatus e.g., GCS3000Dx (Affymetrix), using manufacturers instructions.

[0071] The resulting gene expression measurements are further processed as described in this document. The procedures described can be implemented using R software available from R-Project and supplemented with packages available from Bioconductor.

[0072] For many drugs 10-30 biomarkers are sufficient to give an adequate response, thus, given the relatively small number of biomarkers required, procedures, such as quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), can be performed to measure, with greater precision, the amount of biomarker genes expressed in a sample. This will provide an alternative to or a complement to microarrays so that a single companion test, typically more quantitative than microarrays alone, employing biomarkers of the invention can be used to predict sensitivity to a new drug. qRT-PCR can be performed alone or in combination with a microarray described herein. Procedures for performing qRT-PCR are described in, e.g., U.S. Pat. No. 7,101,663 and U.S. Patent Application Nos. 2006/0177837 and 2006/008856. The methods of the invention are readily applicable to newly discovered drugs as well as drugs described herein.

[0073] The following examples are provided so that those of ordinary skill in the art can see how to use the methods and kits of the invention. The examples are not intended to limit the scope of what the inventor regards as their invention.

EXAMPLES

Example 13

Identification of Gene Biomarkers for Chemosensitivity to Common Chemotherapy Drugs

[0074] DNA chip measurements of the 60 cancer cell lines of the NCI60 data set were downloaded from the Broad

Institute (Cambridge, Mass.) and logit normalized. Growth inhibition data of thousands of compounds against the same cell lines were downloaded from the National Cancer Institute. Compounds where the difference concentration to achieve 50% in growth inhibition (GI50) was less than 1 log were deemed uninformative and rejected. Each gene's expression in each cell line was correlated to its growth (-log (GI50)) in those cell lines in the presence of a given compound. The median Pearson correlation coefficient was used when multiple expression measurements were available for a given gene, and genes having a median correlation coefficient greater than 0.3 were identified as biomarkers for a given compound.

Example 2

Prediction of Treatment Sensitivity for Brain Cancer Patients

[0075] DNA chip measurements of gene expression in tumors from 60 brain cancer patients were downloaded from the Broad Institute. All data files were logit normalized. For each of the common chemotherapy drugs Cisplatin, Vincristine, Adriamycin, Etoposide, Aclarubicine, Mitoxantrone and Azaguanine, the gene expression for the marker genes was summed. The sum was normalized by dividing by the standard deviation of all patients and compared to the median of the sums of patients who survived and the median of the sums of patients who died:

$$\text{NormalizedSum}(\text{compound}) = \frac{\text{sum(marker genes for compound)}}{\text{sd(sums of all patients)}}$$

Sensitivity(compound) =

$$\left[\frac{\text{NormalizedSum}(\text{compound}) - \text{median}(\text{NormalizedSum}_{\text{deadpatients}}(\text{compound}))}{\text{median}(\text{NormalizedSum}_{\text{deadpatients}}(\text{compound}))} \right]^2 - \left[\frac{\text{NormalizedSum}(\text{compound}) - \text{median}(\text{NormalizedSum}_{\text{survivingpatients}}(\text{compound}))}{\text{median}(\text{NormalizedSum}_{\text{survivingpatients}}(\text{compound}))} \right]^2$$

[0076] FIGS. 2 and 3 show the resulting treatment sensitivity predictions for two of the 60 patients. All patients received Cisplatin and the prediction of survival amongst the 60 patients based on their Cisplatin chemosensitivity yielded the Kaplan-Meier survival curve shown in FIG. 4. The expression of the 16 Cisplatin biomarker genes was first reduced to 5 components (dimensions) using Independent Component Analysis (fastICA). Five different classification methods were trained on the five components from the 60 patients: K Nearest Neighbor with K=1, K Nearest Neighbor with K=3, Nearest Centroid, Support Vector Machine, and Neural Network. Chemosensitivity or sensitivity to radiation treatment was predicted by combining the classifications of the five methods wherein each classification method was assigned a single vote: unanimous chemosensitive/treatment sensitive prediction resulted in a prediction of chemosensitive/treatment sensitive. All other predictions resulted in a prediction of chemoresistant/treatment resistant. The performance of the combined classifier was validated using leave-one-out cross validation and the survival of the two predicted groups shown in FIG. 4. The survival rate of the patients predicted to be chemosensitive was higher than the patients predicted to be chemoresistant.

Example 3

Prediction of Chemosensitivity for Lymphoma (DLBCL) Patients

[0077] DNA chip measurements of gene expression in the tumors from 56 DLBCL (diffuse large B-cell lymphoma) patients were downloaded from the Broad Institute. All data files were logit normalized. All patients received Vincristine and Adriamycin and the prediction of survival amongst the 56 patients based on their Vincristine and Adriamycin chemosensitivity yielded the Kaplan-Meier survival curve shown in FIG. 5. The expression of the 33 Vincristine genes and 16 Adriamycin genes was first reduced to 3 components (dimensions) using Independent Component Analysis (fastICA). Five different classification methods were trained on the independent components from the 56 patients: K Nearest Neighbor with K=1, K Nearest Neighbor with K=3, Nearest Centroid, Support Vector Machine, and Neural Network. Chemosensitivity was predicted by combining the classifications of the five methods wherein each classification method was assigned a single vote: unanimous chemosensitive prediction resulted in a prediction of chemosensitive. All other predictions resulted in a prediction of chemoresistant. The performance of the combined classifier was validated using leave-one-out cross validation and the survival of the two predicted groups is shown in FIG. 5. The survival rate of the patients predicted to be chemosensitive was higher than the patients predicted to be chemoresistant.

Example 4

Prediction of Chemosensitivity for Lung Cancer Patients

[0078] DNA chip measurements of gene expression in the tumors from 86 lung cancer (adenocarcinoma) patients was downloaded from the University of Michigan, Ann Arbor. Of the 86 patients, 19 had Stage III of the disease and received adjuvant chemotherapy. Raw data was logit normalized. Instead of the combined classifier described for the brain cancer and lymphoma examples above, the sum of biomarker gene expression was calculated for each patient and used to discriminate chemosensitive and chemoresistant patients. For each patient, the gene expression of the 16 marker genes for Cisplatin sensitivity (all Stage III patients received Cisplatin after surgery) was summed. If the sum was closer to the median of the sums of the surviving patients, the patient was predicted to be sensitive to Cisplatin. If the sum was closest to the median of the sums of the non-surviving patients, the patient was predicted to be resistant to Cisplatin. The survival rates of the two predicted groups are shown in FIG. 6. The survival rate of the patients predicted to be chemosensitive was higher than the patients predicted to be chemoresistant.

Example 5

Prediction of Rituximab Sensitivity for Lymphoma (DLBCL) Patients

[0079] The method is not limited to cytotoxic chemicals. It is also applicable to predicting the efficacy of protein therapeutics, such as monoclonal antibodies, approved for treating cancer. For example, the monoclonal antibody Rituximab (e.g., MABTHERA™ and RITUXAN™) was examined. Data for cytotoxicity of Rituximab in cell lines in vitro were obtained from published reports (Ghetie et al., *Blood* 97(5):

1392-1398, 2001). This cytotoxicity in each cell line was correlated to the expression of genes in these cell lines (downloaded from the NCBI Gene Expression Omnibus database using accession numbers GSE2350, GSE1880, GDS181). The identified marker genes were used to predict the sensitivity of DLBCL to Rituximab in a small set of 14 patients treated with Rituximab and CHOP(R—CHOP) (downloaded from NCBI Gene Expression Omnibus under accession number GSE4475). Conversion between different chip types was performed using matching tables available through Affymetrix.

[0080] The survival of patients predicted to be sensitive to be R-CHOP is compared to the survival of patients predicted to be resistant to R-CHOP in FIG. 7. The survival rate of the patients predicted to be chemosensitive was higher than the patients predicted to be chemoresistant.

[0081] To predict the sensitivity toward combination therapies, such as those used to treat Diffuse Large B-cell Lymphoma (DLBCL), patient sensitivity to a particular combination therapy is predicted by combining the marker genes for the individual compounds used in the combination. An example of this is shown in FIG. 8, where the predicted sensitivities of one patient towards a number of combination therapies used against DLBCL (identified by their acronyms) are shown: R-CHOP contains Rituximab (e.g., MABTHERATM), Vincristine, Doxorubicin (Adriamycin), Cyclophosphamide, and Prednisolone; R-ICE contains Rituximab, Ifosfamide, Carboplatin, and Etoposide; R-MIME contains Rituximab, Mitoguazone, Ifosfamide, Methotrexate, and Etoposide; CHOEP contains Cyclophosphamide, Doxorubicin, Etoposide, Vincristine and Prednisone; DHAP contains Dexamethasone, Cytarabine (Ara C), and Cisplatin; ESHAP contains Etoposide, Methylprednisolone (Solumedrol), Cytarabine (Ara-C) and Cisplatin; and HOAP-Bleo contains Doxorubicin, Vincristine, Ara C, Prednisone, and Bleomycin.

Example 6

Prediction of Radiosensitivity for Brain Tumor (Medulloblastoma) Patients

[0082] The method of identifying biomarkers can also be applied to other forms of treatment such as radiation therapy. For example, sensitivity to radiation therapy was predicted for brain tumor patients. Radiation therapy in the form of craniospinal irradiation yielding 2,400-3,600 centigray (cGy) with a tumor dose of 5,300-7,200 cGy was administered to the brain tumor patients using a medical device that emits beams of radiation. Sensitivity of the 60 cancer cell lines used in the NCI60 dataset to radiation treatment was obtained from published reports. This sensitivity was correlated to the expression of genes in the cell lines as described above to identify marker genes. DNA microarray measurements of gene expression in brain tumors obtained from patients subsequently treated with radiation therapy were obtained from the Broad Institute. The identified gene biomarkers were used to classify the patients as sensitive or resistant to radiation therapy. The survival of the patients in the two predicted categories is shown in FIG. 9. The survival rate of the patients predicted to be sensitive to radiation therapy was higher than the patients predicted to be resistant to radiation therapy.

Example 7

Drug Rescue

[0083] Every member of a population may not be equally responsive to a particular treatment. For example, new com-

pounds often fail in late clinical trials because of lack of efficacy in the population tested. While such compounds may not be effective in the overall population, there may be subpopulations sensitive to those failed compounds due to various reasons, including inherent differences in gene expression. The method as described herein can be used to rescue failed compounds by identifying a patient subpopulation sensitive to a compound using their gene expression as an indicator. Subsequent clinical trials restricted to a sensitive patient subpopulation may demonstrate efficacy of a previously failed compound within that particular patient subpopulation, advancing the compound towards approval for use in that subpopulation.

[0084] To this end, in vitro measurements of the inhibitory effects of a compound on various cancer cell lines are compared to the gene expression of cells. The growth of the cancer cell samples can be correlated to gene expression measurements as described above. This will identify marker genes that can be used to predict patient sensitivity to the failed compound. Once biomarkers are identified, the expression of biomarker genes in cells obtained from patients can be measured according to the procedure detailed above. The patients are predicted to be responsive or non-responsive to compound treatment according to their gene biomarker expression profile. Clinical effect must then be demonstrated in the group of patients that are predicted to be sensitive to the failed compound.

[0085] The method may be further refined if patients responsive to the compound treatment are further subdivided into those predicted to survive without the compound and those predicted to die or suffer a relapse without the compound. Clinical efficacy in the subpopulation that is predicted to die or suffer relapse can be further demonstrated. Briefly, the gene expression at the time of diagnosis of patients who later die from their disease is compared to gene expression at the time of diagnosis of patients who are still alive after a period of time (e.g., 5 years). Genes differentially expressed between the two groups are identified as prospective biomarkers and a model is built using those gene biomarkers to predict treatment efficacy.

[0086] Examples of compounds that have failed in clinical trials include Gefinitib (e.g., Iressa, AstraZeneca) in refractory, advanced non-small-cell lung cancer (NSCLC), Bevacizumab (e.g., Avastin, Genentech) in first-line treatment for advanced pancreatic cancer, Bevacizumab (e.g., Avastin, Genentech) in relapsed metastatic breast cancer patients, and Erlotinib (e.g., Tarceva, Genentech) in metastatic non-small cell lung cancer (NSCLC). The method of the invention may be applied to these compounds, among others, so that sensitive patient subpopulations responsive to those compounds may be identified.

Example 8

Median of the Correlations Versus Correlation of the Median

[0087] The median of the correlations of the individual probe measurements to cancer cell growth as employed by the invention was compared to the correlation of the median probe measurements: this will determine at which step of the method a median calculation should be performed. In the former, several correlations are calculated for each gene since multiple probes measure a given gene's expression, but only the median of the correlation coefficients is finally retained to

identify biomarkers. In the latter, only one correlation is calculated for each gene because only the median gene expression measurement is considered for each gene. FIG. 10 shows the results of using the correlation of the median expression measurements to identify biomarker genes of radiation sensitivity predicting the survival of 60 brain cancer patients. The difference in survival between the group predicted to be radiation sensitive and the group predicted to be radiation resistant in FIG. 10 is much smaller than the difference depicted in FIG. 9 which employed a median correlation coefficient suggesting that the invention's median of the correlations employed in FIG. 9 outperforms the correlation of the median depicted in FIG. 10.

[0088] If we look at individual marker genes like OMD, the median of the correlation to measured radiosensitivity of cell lines in vitro is 0.32. The correlation of the median, however, is 0.39. Adjusting the cutoff from 0.3 to 0.4 to compensate for the difference does not improve on FIG. 10, however.

[0089] We have also compared median correlation to weighted voting as proposed by Staunton et al., *PNAS* 98(19): 10787-10792, 2001). Weighted voting produced a poor result similar to that of FIG. 10, with a P-value of 0.11.

Example 9

Other Methods of Identifying Biomarkers

[0090] The examples shown above all rely on the availability of measurements of inhibition by a compound or treatment of the growth of cell lines in vitro. Such measurements may not always be available or practical. In that case an alternative method of identifying biomarkers can be employed. If the target(s) of the compound is/are known, it is possible to build a model based on the gene expression of the known target(s). One example is the drug sunitinib (SU11248), for which eight targets are known. Sunitinib inhibits at least eight receptor protein-tyrosine kinases including vascular endothelial growth factor receptors 1-3 (VEGFR1-VEGFR3), platelet-derived growth factor receptors (PDGFRA and PDGFRB), stem cell factor receptor (Kit), Flt-3, and colony-stimulating factor-1 receptor (CSF-1R). U.S. Patent Application Publication 2006/0040292 mentions prediction of response measuring just two targets, PDGFRA and KIT. Using the sum of the gene expression of four targets it is possible to predict with more reliability the response to sunitinib. As an example, the predicted sunitinib sensitivity of cell lines HT29, U118, 786, and H226 is 0.24, 2.3, 0.14 and 0.60, respectively, based on the sum of the four targets PDGFRB, KDR, KIT and FLT3. This correlates well with the measured response in mouse xenografts of these cells (correlation coefficient 0.86) as well as with the measured anti-angiogenic effect measured in mouse xenografts (Potapova et al. Contribution of individual targets to the antitumor efficacy of the multitargeted receptor tyrosine kinase inhibitor SU11248 (*Mol. Cancer Ther.* 5(5): 1280-9, 2006)). This is better than a model based only on two targets PDGFRA and KIT (correlation coefficient 0.56).

[0091] This four-gene predictor of sunitinib response can be applied to a large number of tumor samples from patients with different tumors from which gene expression analysis has been performed in order to get an idea of the range of sensitivities within each cancer type as well as which cancer types are most susceptible to treatment with sunitinib. FIG. 11 shows just a small fraction of the cancer samples available from www.intgen.org/expo.html. The comparison is based on normalizing the samples in such a way (e.g., logit normaliza-

tion) that different cancer types become comparable. Sunitinib is currently approved by the FDA for renal cancer and gastrointestinal cancer. Both kidney and colon show a good response in this plot.

[0092] Any other drug response predictor based on gene expression can be tested in the same manner as shown in FIG. 11.

[0093] The approach of identifying biomarkers based on known targets can also be applied to RNA antagonists such as SPC2996 targeted against Bcl-2. A response predictor can be built based on measuring the gene expression of Bcl-2 in samples from cancer patients. The same approach can be used for the targets of all mRNA antagonists or inhibitors.

Example 10

Identifying Candidate Drugs for a Known Target

[0094] The methods of the invention described herein can also be used for identifying candidate drugs to a known target. Basically, the method of identifying biomarkers is run backwards in order to identify candidate drugs. If one starts with a known target, the expression of its corresponding gene is determined in the NCI 60 cell lines and correlated to the measured growth inhibition of all the thousands of drugs tested in the NCI 60 cell lines. This provides a list, ranked by correlation coefficient, of candidate drugs for the target. It is even possible to test new drugs and compare their correlation coefficient to the target gene expression to the correlation coefficients of the already tested drugs.

Example 11

Using MicroRNAs as Biomarkers of Drug Response

[0095] In recent years it has become clear that microRNAs (miRNA) play an important role in regulating the translation of mRNAs. As such, microRNAs may contain important information relevant for the prediction of drug sensitivity. This information may be complementary to the information contained in mRNA expression. Shown below is the correlation between predicted and measured chemosensitivity of the NCI 60 cell lines. The prediction is based either on mRNA measurements with DNA microarrays as described herein or predictions based on measurements of microRNA concentration (ArrayExpress accession number E-MEXP-1029) using a microRNA specific microarray (ArrayExpress accession number A-MEXP-620). Whenever more than one probe is used to determine the concentration of a given microRNA, the median correlation procedure is used for calculating correlation between microRNA concentration and $-\log(GI50)$.

	miRNA	mRNA	Combined
cisplatin	0.16	0.02	0.21
PXD101	0.44	0.31	0.50
vincristine	0.06	0.11	0.26
etoposide	0.32	0.41	0.44
adriamycin	0.24	0.22	0.28

As the above table shows, the correlation (determined using leave-one-out cross-validation) is highest when using a combination (linear sum) of microRNA and mRNA predictions. These results suggest that a more accurate drug response predictor can be built using a combination of microRNA and mRNA. It is possible to measure both in the same experiment,

as long as one takes into consideration that microRNAs in general do not have a polyA tail as mRNA does. Only slight modifications to the amplification and labeling methods used for mRNA may be needed to incorporate microRNAs into the analysis. Commercial kits for microRNA extraction, amplification, and labeling are available from suppliers (e.g., Ambion Inc.).

[0096] Tables 22A-76A list the microRNA probes that are useful for detection of sensitivity to individual drugs, as determined by their median correlation to $-\log(GI50)$ for the indicated drug.

OTHER EMBODIMENTS

[0097] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

Legend:

[0098] List_2006: biomarkers identified in 2006 using the new U133A chip measurements

List_2005: biomarkers listed in 2005 patent filing

HU6800: biomarkers obtained with old HU6800 chip measurements

List_Prior: matching biomarkers in prior art

List_Preferr: Preferred list of biomarkers

Correlation: The correlation of the biomarker to sensitivity to the compound

TABLE 1

<u>Vincristine biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[1.] UBB	UBB			0.39
[2.] RPS4X		RPS4X		0.34
[3.] S100A4		S100A4		0.32
[4.] NDUFS6		NDUFS6		0.31
[5.] B2M	B2M			0.35
[6.] C14orf139		C14orf139		0.3
[7.] MAN1A1		MAN1A1		0.33
[8.] SLC25A5	SLC25A5	SLC25A5		0.32
[9.] RPL10		RPL10		0.38
[10.] RPL12		RPL12		0.31
[11.] EIF5A		EIF5A		0.31
[12.] RPL36A	RPL36A	RPL36A		0.3
[13.] SUI1	SUI1			0.33
[14.] BLMH		BLMH		0.32
[15.] CTBP1		CTBP1		0.32
[16.] TBCA		TBCA		0.3
[17.] MDH2		MDH2		0.34
[18.] DXS9879E		DXS9879E		0.35

TABLE 1-continued

<u>Vincristine biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[19.]	SFRS3			
[20.]	CCTS			
[21.]	RPL39			
[22.]	UBE2S			
[23.]	EEF1A1			
[24.]	COX7B			
[25.]	RPLP2			
[26.]	RPL24			
[27.]	RPS23			
[28.]	RPL18			
[29.]	NCL			
[30.]	RPL9			
[31.]	RPL10A			
[32.]	RPS10			
[33.]	EIF3S2			
[34.]	SHFM1			
[35.]	RPS28			
[36.]	REA			
[37.]	GAPD			
[38.]	HNRPAl			
[39.]	RPS11			
[40.]	LDHB			
[41.]	RPL3			
[42.]	RPL11			
[43.]	MRPL12			
[44.]	RPL18A			
[45.]	RPS7			

TABLE 2

<u>Cisplatin biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[1.] C1QR1			C1QR1	0.3
[2.] HCLS1	HCLS1	HCLS1		0.33
[3.] CD53		CD53		0.35
[4.] SLA			SLA	0.37
[5.] PTPN7	PTPN7		PTPN7	0.31
[6.] PTPRCAP		PTPRCAP		0.32
[7.] ZNFN1A1			ZNFN1A1	0.33
[8.] CENTB1			CENTB1	0.37
[9.] PTPRC		PTPRC		0.36
[10.] IFI16	IFI16		IFI16	0.31
[11.] ARHGEF6			ARHGEF6	0.35
[12.] SEC31L2			SEC31L2	0.32
[13.] CD3Z			CD3Z	0.32
[14.] GZMB			GZMB	0.3
[15.] CD3D			CD3D	0.34
[16.] MAP4K1			MAP4K1	0.32
[17.] GPR65			GPR65	0.39
[18.] PRF1			PRF1	0.31
[19.] ARHGAP15			ARHGAP15	0.35
[20.] TM6SF1			TM6SF1	0.41
[21.] TCF4			TCF4	0.4
[22.]		GAPD		
[23.]		ARHGDB		
[24.]		RPS27		
[25.]		C5orf13		
[26.]		LDHB		
[27.]		SNRPF		
[28.]		B2M		
[29.]		FTL		
[30.]		NCL		
[31.]		MSN		
[32.]		XPO1		

TABLE 3

<u>Azaguanine biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[1.] MSN	MSN	MSN		0.36
[2.] SPARC	SPARC	SPARC		0.48
[3.] VIM	VIM	VIM		0.47
[4.] SRM	SRM		SRM	0.32
[5.] SCARB1			SCARB1	0.4
[6.] SIAT1			SIAT1	0.31
[7.] CUGBP2			CUGBP2	0.37
[8.] GAS7		GAS7		0.34
[9.] ICAM1			ICAM1	0.43
[10.] WASPIP			WASPIP	0.44
[11.] ITM2A			ITM2A	0.31
[12.] PALM2-AKAP2			PALM2-AKAP2	0.31
[13.] ANPEP		ANPEP		0.33
[14.] PTPNS1			PTPNS1	0.39
[15.] MPP1			MPP1	0.32
[16.] LNK			LNK	0.43
[17.] FCGR2A			FCGR2A	0.3
[18.] EMP3	EMP3	EMP3		0.33
[19.] RUNX3			RUNX3	0.43
[20.] EVI2A			EVI2A	0.4
[21.] BTN3A3			BTN3A3	0.4
[22.] LCP2			LCP2	0.34
[23.] BCHE			BCHE	0.35
[24.] LY96			LY96	0.47
[25.] LCP1			LCP1	0.42
[26.] IFI16			IFI16	0.33
[27.] MCAM	MCAM		MCAM	0.37
[28.] MEF2C			MEF2C	0.41
[29.] SLC1A4			SLC1A4	0.49
[30.] BTN3A2		BTN3A2		0.43
[31.] FYN			FYN	0.31
[32.] FN1	FN1	FN1		0.33
[33.] C1orf38			C1orf38	0.37
[34.] CHS1			CHS1	0.33
[35.] CAPN3		CAPN3		0.5
[36.] FCGR2C			FCGR2C	0.34
[37.] TNK			TNIK	0.35
[38.] AMPD2			AMPD2	0.3
[39.] SEPT6			SEPT6	0.41
[40.] RAFTLIN			RAFTLIN	0.39
[41.] SLC43A3			SLC43A3	0.52
[42.] RAC2			RAC2	0.33
[43.] LPXN			LPXN	0.54
[44.] CKIP-1			CKIP-1	0.33
[45.] FLJ10539			FLJ10539	0.33
[46.] FLJ35036			FLJ35036	0.36
[47.] DOCK10			DOCK10	0.3
[48.] TRPV2			TRPV2	0.31
[49.] IFRG28			IFRG28	0.3
[50.] LEF1			LEF1	0.31
[51.] ADAMTS1			ADAMTS1	0.36
[52.] PRPS1				
[53.] DDOST				
[54.] B2M				
[55.] LGALS1				
[56.] CBFB				
[57.] SNRPB2				
[58.] EIF2S2				
[59.] HPRT1				
[60.] FKBP1A				
[61.] GYPC				
[62.] UROD				
[63.] HNRPA1				
[64.] SND1				
[65.] COPA				
[66.] MAPRE1				
[67.] EIF3S2				
[68.] ATP1B3				
[69.] ECM1				
[70.] ATOX1				
[71.] NARS				
[72.] PGK1				

TABLE 3-continued

<u>Azaguanine biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[73.]	OK/SW-cl.56			
[74.]	EEF1A1			
[75.]	GNAI2			
[76.]	RPL7			
[77.]	PSMB9			
[78.]	GPNMB			
[79.]	PPP1R11			
[80.]	MIA			
[81.]	RAB7			
[82.]	SMS			

TABLE 4

<u>Etoposide biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[1.] CD99	CD99	CD99	0.3	
[2.] INSIG1		INSIG1	0.35	
[3.] LAPTMS5		LAPTMS5	0.32	
[4.] PRG1		PRG1	0.34	
[5.] MUF1		MUF1	0.35	
[6.] HCLS1		HCLS1	0.33	
[7.] CD53		CD53	0.32	
[8.] SLA		SLA	0.37	
[9.] SSBP2		SSBP2	0.37	
[10.] GNB5		GNB5	0.35	
[11.] MFNG		MFNG	0.33	
[12.] GMFG		GMFG	0.32	
[13.] PSMB9		PSMB9	0.31	
[14.] EVI2A		EVI2A	0.41	
[15.] PTPN7		PTPN7	0.3	
[16.] PTGER4		PTGER4	0.3	
[17.] CXorf9		CXorf9	0.3	
[18.] PTPRCAP		PTPRCAP	0.3	
[19.] ZNFN1A1		ZNFN1A1	0.35	
[20.] CENTB1		CENTB1	0.3	
[21.] PTPRC		PTPRC	0.31	
[22.] NAPI1L1		NAPI1L1	0.31	
[23.] HLA-DRA		HLA-DRA	0.34	
[24.] IFI16		IFI16	0.38	
[25.] CORO1A		CORO1A	0.3	
[26.] ARHGEF6		ARHGEF6	0.33	
[27.] PSCDBP		PSCDBP	0.4	
[28.] SELPLG		SELPLG	0.35	
[29.] LAT		LAT	0.3	
[30.] SEC31L2		SEC31L2	0.42	
[31.] CD3Z		CD3Z	0.36	
[32.] SH2D1A		SH2D1A	0.33	
[33.] GZMB		GZMB	0.34	
[34.] SCN3A		SCN3A	0.3	
[35.] ITK		ITK	0.35	
[36.] RAFTLIN		RAFTLIN	0.39	
[37.] DOCK2		DOCK2	0.33	
[38.] CD3D		CD3D	0.31	
[39.] RAC2		RAC2	0.34	
[40.] ZAP70		ZAP70	0.35	
[41.] GPR65		GPR65	0.35	
[42.] PRF1		PRF1	0.32	
[43.] ARHGAP15		ARHGAP15	0.32	
[44.] NOTCH1		NOTCH1	0.31	
[45.] UBASH3A		UBASH3A	0.32	
[46.] B2M				
[47.] MYC				
[48.] RPS24				
[49.] PPIF				

TABLE 4-continued

<u>Etoposide biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[50.]		PBEF1		
[51.]		ANP32B		

TABLE 5

<u>Adriamycin biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[1.] CD99	CD99	CD99	CD99	0.41
[2.] LAPTMS5		LAPTMS5	ALDOC	0.39
[3.] ALDOC			ALDOC	0.31
[4.] HCLS1		HCLS1	HCLS1	0.32
[5.] CD53		CD53	CD53	0.31
[6.] SLA			SLA	0.35
[7.] SSBP2			SSBP2	0.34
[8.] IL2RG			IL2RG	0.38
[9.] GMFG		GMFG	GMFG	0.32
[10.] CXorf9		CXorf9	CXorf9	0.32
[11.] RHOH		RHOH	RHOH	0.31
[12.] PTPRCAP		PTPRCAP	PTPRCAP	0.32
[13.] ZNFN1A1		ZNFN1A1	ZNFN1A1	0.43
[14.] CENTB1		CENTB1	CENTB1	0.36
[15.] TCF7		TCF7	TCF7	0.32
[16.] CD1C		CD1C	CD1C	0.3
[17.] MAP4K1		MAP4K1	MAP4K1	0.35
[18.] CD1B		CD1B	CD1B	0.39
[19.] CD3G		CD3G	CD3G	0.31
[20.] PTPRC		PTPRC	PTPRC	0.38
[21.] CCR9		CCR9	CCR9	0.34
[22.] CORO1A		CORO1A	CORO1A	0.38
[23.] CXCR4		CXCR4	CXCR4	0.3
[24.] ARHGEF6		ARHGEF6	ARHGEF6	0.31
[25.] HEM1		HEM1	HEM1	0.32
[26.] SELPLG		SELPLG	SELPLG	0.31
[27.] LAT		LAT	LAT	0.31
[28.] SEC31L2		SEC31L2	SEC31L2	0.33
[29.] CD3Z		CD3Z	CD3Z	0.37
[30.] SH2D1A		SH2D1A	SH2D1A	0.37
[31.] CD1A		CD1A	CD1A	0.4
[32.] LAIR1		LAIR1	LAIR1	0.39
[33.] ITK		ITK	ITK	0.3
[34.] TRB@		TRB@	TRB@	0.34
[35.] CD3D		CD3D	CD3D	0.33
[36.] WBSCR20C		WBSCR20C	WBSCR20C	0.34
[37.] ZAP70		ZAP70	ZAP70	0.33
[38.] IFI44		IFI44	IFI44	0.32
[39.] GPR65		GPR65	GPR65	0.31

TABLE 5-continued

<u>Adriamycin biomarkers</u>					
	List_2006	List_2005	List_Prior	List_Preferr	Correlation
[40,]	AIF1		AIF1		0.3
[41,]	ARHGAP15		ARHGAP15		0.37
[42,]	NARF		NARF		0.3
[43,]	PACAP		PACAP		0.32
[44,]	KIAA0220				
[45,]	B2M				
[46,]	TOP2A				
[47,]	SNRPE				
[48,]	RPS27				
[49,]	HNRP1A1				
[50,]	CBX3				
[51,]	ANP32B				
[52,]	DDX5				
[53,]	PPIA				
[54,]	SNRPF				
[55,]	USP7				

TABLE 6

<u>Aclarubicin biomarkers</u>					
	List_2006	List_2005	List_Prior	List_Preferr	Correlation
[1,]	RPL12		RPL12		0.3
[2,]	RPL32		RPL32		0.37
[3,]	RPLP2	RPLP2	RPLP2		0.37
[4,]	MYB	MYB	MYB		0.31
[5,]	ZNFN1A1		ZNFN1A1		0.34
[6,]	SCAP1		SCAP1		0.33
[7,]	STAT4		STAT4		0.31
[8,]	SP140		SP140		0.4
[9,]	AMPD3		AMPD3		0.3
[10,]	TNFAIP8		TNFAIP8		0.4
[11,]	DDX18		DDX18		0.31
[12,]	TAF5		TAF5		0.3
[13,]	FBL	FBL			0.41
[14,]	RPS2		RPS2		0.34
[15,]	PTPRC	PTPRC			0.37
[16,]	DOCK2		DOCK2		0.32
[17,]	GPR65		GPR65		0.35
[18,]	HOXA9		HOXA9		0.33
[19,]	FLJ12270		FLJ12270		0.31
[20,]	HNRPD		HNRPD		0.4
[21,]	LAMR1				
[22,]	RPS25				
[23,]	EIF5A				
[24,]	TUFM				
[25,]	HNRPA1				
[26,]	RPS9				
[27,]	ANP32B				
[28,]	EIF4B				
[29,]	HMGB2				
[30,]	RPS15A				
[31,]	RPS7				

TABLE 7

<u>Mitoxantrone biomarkers</u>					
	List_2006	List_2005	List_Prior	List_Preferr	Correlation
[1,]	PGAM1		PGAM1		0.32
[2,]	DPYSL3		DPYSL3		0.36
[3,]	INSIG1		INSIG1		0.32
[4,]	GJA1		GJA1		0.31

TABLE 7-continued

<u>Mitoxantrone biomarkers</u>						
	List_2006	List_2005	List_Prior	List_Preferr	Correlation	
[5,]	BNIP3				BNIP3	0.31
[6,]	PRG1	PRG1			PRG1	0.39
[7,]	G6PD	G6PD			G6PD	0.34
[8,]	BASPI		BASPI		BASPI	0.31
[9,]	PLOD2				PLOD2	0.34
[10,]	LOXL2				LOXL2	0.31
[11,]	SSBP2				SSBP2	0.36
[12,]	C1orf29				C1orf29	0.35
[13,]	TOX				TOX	0.35
[14,]	STC1				STC1	0.39
[15,]	TNFRSF1A	TNFRSF1A			TNFRSF1A	0.34
[16,]	NCOR2	NCOR2			NCOR2	0.3
[17,]	NAPI1L1	NAPI1L1			NAPI1L1	0.32
[18,]	LOC94105				LOC94105	0.34
[19,]	COL6A2		COL6A2		COL6A2	0.3
[20,]	ARHGEF6	ARHGEF6			ARHGEF6	0.34
[21,]	GATA3				GATA3	0.35
[22,]	TFPI				TFPI	0.31
[23,]	LAT				LAT	0.31
[24,]	CD3Z				CD3Z	0.37
[25,]	AF1Q				AF1Q	0.33
[26,]	MAP1B	MAP1B			MAP1B	0.34
[27,]	PTPRC		PTPRC		PTPRC	0.31
[28,]	PRKCA		PRKCA		PRKCA	0.35
[29,]	TRIM22				TRIM22	0.3
[30,]	CD3D				CD3D	0.31
[31,]	BCAT1				BCAT1	0.32
[32,]	IFI44				IFI44	0.33
[33,]	CCL2		CCL2		CCL2	0.37
[34,]	RAB31		RAB31		RAB31	0.31
[35,]	CUTC				CUTC	0.33
[36,]	NAPI1L2				NAPI1L2	0.33
[37,]	NME7				NME7	0.35
[38,]	FLJ21159				FLJ21159	0.33
[39,]	COL5A2				COL5A2	0.38
[40,]	B2M					
[41,]	OK/SW-cl.56					
[42,]	TOP2A					
[43,]	ELA2B					
[44,]	PTMA					
[45,]	LMNB1					
[46,]	HNRPA1					
[47,]	RPL9					
[48,]	C5orf13					
[49,]	ANP32B					
[50,]	TUBA3					
[51,]	HMGN2					
[52,]	PRPS1					
[53,]	DDX5					
[54,]	PPIA					
[55,]	PSMB9					
[56,]	SNRPF					

TABLE 8

<u>Mitomycin biomarkers</u>						
	List_2006	HU6800	List_Prior	List_Preferr	Correlation	
[1,]	STC1				STC1	0.34
[2,]	GPR65				GPR65	0.32
[3,]	DOCK10				DOCK10	0.35
[4,]	COL5A2				COL5A2	0.33
[5,]	FAM46A				FAM46A	0.36
[6,]	LOC54103				LOC54103	0.39

TABLE 9

<u>Paclitaxel (Taxol) biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] RPL10		RPL10		0.31
[2,] RPS4X		RPS4X		0.31
[3,] NUDC		NUDC		0.3
[4,] RALY	RALY			0.31
[5,] DCK1		DCK1		0.3
[6,] DKFZP564C186		DKFZP564C186		0.32
[7,] PRP19		PRP19		0.31
[8,] RAB9P40		RAB9P40		0.33
[9,] HSA9761		HSA9761		0.37
[10,] GMDS		GMDS		0.3
[11,] CEP1		CEP1		0.3
[12,] IL13RA2		IL13RA2		0.34
[13,] MAGEB2		MAGEB2		0.41
[14,] HMGN2		HMGN2		0.35
[15,] ALMS1		ALMS1		0.3
[16,] GPR65		GPR65		0.31
[17,] FLJ10774		FLJ10774		0.31
[18,] NOL8		NOL8		0.31
[19,] DAZAP1		DAZAP1		0.32
[20,] SLC25A15		SLC25A15		0.31
[21,] PAF53		PAF53		0.36
[22,] DXS9879E		DXS9879E		0.31
[23,] PITPNC1		PITPNC1		0.33
[24,] SPANXC		SPANXC		0.3
[25,] KIAA1393		KIAA1393		0.33

TABLE 10

<u>Gemcitabine (Gemzar) biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] PFN1		PFN1		0.37
[2,] PGAMI		PGAMI		0.35
[3,] K-ALPHA-1		K-ALPHA-1		0.34
[4,] CSDA		CSDA		0.31
[5,] UCHL1		UCHL1		0.36
[6,] PWP1		PWP1		0.37
[7,] PALM2-		PALM2-		0.31
AKAP2		AKAP2		
[8,] TNFRSF1A		TNFRSF1A		0.31
[9,] ATP5G2		ATP5G2		0.36
[10,] AF1Q		AF1Q		0.31
[11,] NME4		NME4		0.31
[12,] FHOD1		FHOD1		0.32

TABLE 11

<u>Taxotere (docetaxel) biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[1,] ANP32B		ANP32B		0.45
[2,] GTF3A		GTF3A		0.31
[3,] RRM2		RRM2		0.31
[4,] TRIM14		TRIM14		0.31
[5,] SKP2		SKP2		0.33
[6,] TRIP13		TRIP13		0.36
[7,] RFC3		RFC3		0.45
[8,] CASP7		CASP7		0.32
[9,] TXN		TXN		0.36
[10,] MCM5		MCM5		0.34
[11,] PTGES2		PTGES2		0.39
[12,] OBFC1		OBFC1		0.37

TABLE 11-continued

<u>Taxotere (docetaxel) biomarkers</u>				
List_2006	List_2005	List_Prior	List_Preferr	Correlation
[13,] EPB41L4B			EPB41L4B	0.32
[14,] CALML4			CALML4	0.31

TABLE 12

<u>Dexamethasone biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] IFITM2			IFITM2	0.38
[2,] UBE2L6			UBE2L6	0.32
[3,] LAPT M5	LAPT M5	LAPT M5		0.36
[4,] USP4			USP4	0.33
[5,] ITM2A			ITM2A	0.38
[6,] ITGB2		ITGB2		0.42
[7,] ANPEP		ANPEP		0.31
[8,] CD53		CD53		0.34
[9,] IL2RG	IL2RG		IL2RG	0.36
[10,] CD37		CD37		0.34
[11,] GPRASP1			GPRASP1	0.36
[12,] PTPN7			PTPN7	0.31
[13,] CXorf9			CXorf9	0.36
[14,] RHOH	RHOH		RHOH	0.33
[15,] GIT2			GIT2	0.31
[16,] ADORA2A		ADORA2A		0.31
[17,] ZNFN1A1			ZNFN1A1	0.35
[18,] GNA15	GNA15	GNA15		0.33
[19,] CEP1			CEP1	0.31
[20,] TNFRSF7			TNFRSF7	0.46
[21,] MAP4K1			MAP4K1	0.3
[22,] CCR7			CCR7	0.33
[23,] CD3G			CD3G	0.35
[24,] PTPRC			PTPRC	0.41

TABLE 12-continued

<u>Dexamethasone biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[25,] ATP2A3	ATP2A3		ATP2A3	0.4
[26,] UCP2		UCP2	UCP2	0.3
[27,] CORO1A	CORO1A	CORO1A		0.39
[28,] GATA3	GATA3		GATA3	0.37
[29,] CDKN2A			CDKN2A	0.32
[30,] HEM1		HEM1		0.3
[31,] TARP			TARP	0.3
[32,] LAIR1			LAIR1	0.34
[33,] SH2D1A			SH2D1A	0.34
[34,] FLII	FLII	FLII		0.33
[35,] SEPT6			SEPT6	0.34
[36,] HA-1			HA-1	0.34
[37,] CREB3L1		CREB3L1		0.31
[38,] ERCC2			ERCC2	0.65
[39,] CD3D	CD3D		CD3D	0.32
[40,] LST1			LST1	0.39
[41,] AIF1			AIF1	0.35
[42,] ADA			ADA	0.33
[43,] DATF1			DATF1	0.41
[44,] ARHGAP15			ARHGAP15	0.3
[45,] PLAC8			PLAC8	0.31
[46,] CECR1			CECR1	0.31
[47,] LOC81558			LOC81558	0.33
[48,] EHD2			EHD2	0.37

TABLE 13

<u>Ara-C (Cytarabine hydrochloride) biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] ITM2A			ITM2A	0.32
[2,] RHOH			RHOH	0.31
[3,] PRIM1			PRIM1	0.3
[4,] CENTB1			CENTB1	0.31
[5,] GNA15		GNA15		0.32
[6,] NAP1L1	NAP1L1		NAP1L1	0.31
[7,] ATP5G2			ATP5G2	0.31
[8,] GATA3			GATA3	0.33
[9,] PRKCQ			PRKCQ	0.32
[10,] SH2D1A			SH2D1A	0.3
[11,] SEPT6			SEPT6	0.42
[12,] PTPRC	PTPRC			0.35
[13,] NME4			NME4	0.33
[14,] RPL13		RPL13		0.3
[15,] CD3D			CD3D	0.31
[16,] CD1E			CD1E	0.32
[17,] ADA	ADA		ADA	0.34
[18,] FHOD1			FHOD1	0.31

TABLE 14

<u>Methylprednisolone biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] CD99	CD99		CD99	0.31
[2,] SRRM1		SRRM1		0.31
[3,] ARHGDI	ARHGDI		ARHGDI	0.31
[4,] LAPTMS5	LAPTMS5	LAPTMS5		0.37
[5,] VWF			VWF	0.45
[6,] ITM2A			ITM2A	0.35
[7,] ITGB2	ITGB2	ITGB2		0.43
[8,] LGALS9	LGALS9		LGALS9	0.43
[9,] INPP5D			INPP5D	0.34

TABLE 14-continued

<u>Methylprednisolone biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[10,] SATB1	SATB1		SATB1	0.32
[11,] CD53	CD53		CD53	0.33
[12,] TFDP2	TFDP2		TFDP2	0.4
[13,] SLA	SLA		SLA	0.31
[14,] IL2RG	IL2RG		IL2RG	0.3
[15,] MFNG			MFNG	0.3
[16,] CD37		CD37		0.37
[17,] GMFG		GMFG		0.4
[18,] SELL			SELL	0.33
[19,] CDW52	CDW52		CDW52	0.33
[20,] LRMP			LRMP	0.32
[21,] ICAM2			ICAM2	0.38
[22,] RIMS3			RIMS3	0.36
[23,] PTPN7	PTPN7		PTPN7	0.39
[24,] ARHGAP25			ARHGAP25	0.37
[25,] LCK	LCK		LCK	0.3
[26,] CXorf9			CXorf9	0.3
[27,] RHOH	RHOH		RHOH	0.51
[28,] PTPRCAP	PTPRCAP	PTPRCAP	PTPRCAP	0.5
[29,] GIT2			GIT2	0.33
[30,] ZNFN1A1	ZNFN1A1		ZNFN1A1	0.53
[31,] CENTB1			CENTB1	0.36
[32,] LCP2			LCP2	0.34
[33,] SPI1			SPI1	0.3
[34,] GNA15	GNA15	GNA15	GNA15	0.39
[35,] GZMA			GZMA	0.31
[36,] CEP1			CEP1	0.37
[37,] BLM		BLM		0.33
[38,] CD8A			CD8A	0.38
[39,] SCAP1			SCAP1	0.32
[40,] CD2			CD2	0.48
[41,] CD1C	CD1C		CD1C	0.37
[42,] TNFRSF7			TNFRSF7	0.31
[43,] VAV1			VAV1	0.41
[44,] MAP4K1	MAP4K1		MAP4K1	0.36
[45,] CCR7			CCR7	0.37
[46,] C6orf32			C6orf32	0.38
[47,] ALOX15B			ALOX15B	0.43
[48,] BRDT			BRDT	0.33
[49,] CD3G	CD3G		CD3G	0.51
[50,] PTPRC			PTPRC	0.37
[51,] LTB			LTB	0.32
[52,] ATP2A3	ATP2A3		ATP2A3	0.3
[53,] NVL			NVL	0.31
[54,] RASGRP2			RASGRP2	0.35
[55,] LCP1	LCP1		LCP1	0.34
[56,] CORO1A	CORO1A	CORO1A	CORO1A	0.41
[57,] CXCR4			CXCR4	0.3
[58,] PRKD2			PRKD2	0.33
[59,] GATA3	GATA3		GATA3	0.39
[60,] TRA@			TRA@	0.4
[61,] PRKCB1	PRKCB1	PRKCB1	PRKCB1	0.35
[62,] HEM1		HEM1		0.32
[63,] KIAA0922			KIAA0922	0.36
[64,] TARP			TARP	0.49
[65,] SEC31L2			SEC31L2	0.32
[66,] PRKCQ			PRKCQ	0.37
[67,] SH2D1A			SH2D1A	0.33
[68,] CHRNA3			CHRNA3	0.5
[69,] CD1A			CD1A	0.44
[70,] LST1			LST1	0.36
[71,] LAIR1			LAIR1	0.47
[72,] CACNA1G			CACNA1G	0.33
[73,] TRB@	TRB@	TRB@		0.31
[74,] SEPT6		SEPT6		0.33
[75,] HA-1			HA-1	0.42
[76,] DOCK2			DOCK2	0.32
[77,] CD3D	CD3D		CD3D	0.41
[78,] TRD@			TRD@	0.38
[79,] T3JAM			T3JAM	0.37
[80,] FNBP1			FNBP1	0.37

TABLE 14-continued

<u>Methylprednisolone biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[81,] CD6		CD6	0.4	
[82,] AIF1	AIF1	AIF1	0.31	
[83,] FOLH1		FOLH1	0.45	
[84,] CD1E	CD1E	CD1E	0.58	
[85,] LY9		LY9	0.39	
[86,] UGT2B17		UGT2B17	0.47	
[87,] ADA	ADA	ADA	0.39	
[88,] CDKL5		CDKL5	0.44	
[89,] TRIM		TRIM	0.38	
[90,] EVL		EVL	0.39	
[91,] DATF1		DATF1	0.31	
[92,] RGC32		RGC32	0.51	
[93,] PRKCH		PRKCH	0.3	
[94,] ARHGAP15		ARHGAP15	0.34	
[95,] NOTCH1		NOTCH1	0.36	
[96,] BIN2		BIN2	0.31	
[97,] SEMA4G		SEMA4G	0.35	
[98,] DPEP2		DPEP2	0.33	
[99,] CECR1		CECR1	0.36	
[100,] BCL11B		BCL11B	0.33	
[101,] STAG3		STAG3	0.41	
[102,] GALNT6		GALNT6	0.32	
[103,] UBASH3A		UBASH3A	0.3	
[104,] PHEMX		PHEMX	0.38	
[105,] FLJ13373		FLJ13373	0.34	
[106,] LEF1		LEF1	0.49	
[107,] IL21R		IL21R	0.42	
[108,] MGC17330		MGC17330	0.33	
[109,] AKAP13		AKAP13	0.53	
[110,] ZNF335		ZNF335	0.3	
[111,] GIMAP5		GIMAP5	0.34	

TABLE 15

<u>Methotrexate biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] PRPF8		PRPF8	0.34	
[2,] RPL18		RPL18	0.34	

TABLE 15-continued

<u>Methotrexate biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[3,] RNPS1		RNPS1	0.36	
[4,] RPL32		RPL32	0.39	
[5,] EEF1G		EEF1G	0.34	
[6,] GOT2			GOT2	0.31
[7,] RPL13A			RPL13A	0.31
[8,] PTMA	PTMA	PTMA	0.41	
[9,] RPS15			RPS15	0.39
[10,] RPLP2	RPLP2	RPLP2	0.32	
[11,] CSDA			CSDA	0.39
[12,] KHDRBS1			KHDRBS1	0.32
[13,] SNRPA			SNRPA	0.31
[14,] IMPDH2	IMPDH2	IMPDH2	0.39	
[15,] RPS19			RPS19	0.47
[16,] NUP88			NUP88	0.36
[17,] ATP5D			ATP5D	0.33
[18,] PCBP2			PCBP2	0.32
[19,] ZNF593			ZNF593	0.4
[20,] HSU79274			HSU79274	0.32
[21,] PRIM1			PRIM1	0.3
[22,] PFDN5			PFDN5	0.33
[23,] OXA1L			OXA1L	0.37
[24,] H3F3A			H3F3A	0.42
[25,] ATIC			ATIC	0.31
[26,] RPL13	RPL13	RPL13	0.36	
[27,] CIAPIN1			CIAPIN1	0.34
[28,] FBL	FBL	FBL	0.33	
[29,] RPS2	RPS2	RPS2	0.32	
[30,] PCCB			PCCB	0.36
[31,] RBMX	RBMX	RBMX	0.33	
[32,] SHMT2			SHMT2	0.34
[33,] RPLP0			RPLP0	0.35
[34,] HNRPA1	HNRPA1	HNRPA1	0.35	
[35,] STOML2			STOML2	0.32
[36,] RPS9	RPS9	RPS9	0.36	
[37,] SKB1			SKB1	0.33
[38,] GLTSCR2			GLTSCR2	0.37
[39,] CCNB1IP1			CCNB1IP1	0.3
[40,] MRPS2			MRPS2	0.33
[41,] FLJ20859			FLJ20859	0.34
[42,] FLJ12270			FLJ12270	0.3

TABLE 16

<u>Bleomycin biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] MSN		MSN	0.3	
[2,] PFN1		PFN1	0.45	
[3,] HK1		HK1	0.33	
[4,] ACTR2		ACTR2	0.31	
[5,] MCL1		MCL1	0.31	
[6,] ZYX		ZYX	0.32	
[7,] RAP1B		RAP1B	0.34	
[8,] GNB2		GNB2	0.32	
[9,] EPAS1		EPAS1	0.31	
[10,] PGAM1		PGAM1	0.42	
[11,] CKAP4		CKAP4	0.31	
[12,] DUSP1		DUSP1	0.4	
[13,] MYL9		MYL9	0.4	
[14,] K-ALPHA-1		K-ALPHA-1	0.37	
[15,] LGALS1		LGALS1	0.38	
[16,] CSDA	CSDA	CSDA	0.3	
[17,] AKR1B1		AKR1B1	0.32	
[18,] IFITM2	IFITM2	IFITM2	0.36	
[19,] ITGA5		ITGA5	0.43	
[20,] VIM	VIM	VIM	0.39	

TABLE 16-continued

<u>Bleomycin biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[21,] DPYSL3			DPYSL3	0.44
[22,] JUNB			JUNB	0.32
[23,] ITGA3		ITGA3		0.38
[24,] NFKBIA			NFKBIA	0.32
[25,] LAMB1			LAMB1	0.37
[26,] FHL1			FHL1	0.31
[27,] INSIG1			INSIG1	0.31
[28,] TIMP1			TIMP1	0.48
[29,] GJA1			GJA1	0.54
[30,] PSME2			PSME2	0.34
[31,] PRG1			PRG1	0.46
[32,] EXT1			EXT1	0.35
[33,] DKFZP434J154			DKFZP434J154	0.31
[34,] OPTN		OPTN		0.31
[35,] M6PRBP1		M6PRBP1		0.52
[36,] MVP			MVP	0.34
[37,] VASP			VASP	0.31
[38,] ARL7			ARL7	0.39
[39,] NNMT	NNMT		NNMT	0.34
[40,] TAP1			TAP1	0.3
[41,] COL1A1	COL1A1	COL1A1		0.33
[42,] BASP1		BASP1		0.35
[43,] PLOD2			PLOD2	0.37
[44,] ATF3			ATF3	0.42
[45,] PALM2-AKAP2			PALM2-AKAP2	0.33
[46,] IL8			IL8	0.34
[47,] ANPEP		ANPEP		0.35
[48,] LOXL2			LOXL2	0.32
[49,] TGFBI		TGFBI		0.31
[50,] IL4R			IL4R	0.31
[51,] DGKA			DGKA	0.32
[52,] STC2			STC2	0.31
[53,] SEC61G			SEC61G	0.41
[54,] NFL3	NFL3	NFL3		0.47
[55,] RGS3			RGS3	0.37
[56,] NK4		NK4		0.34
[57,] F2R			F2R	0.34
[58,] TPM2			TPM2	0.35
[59,] PSMB9	PSMB9		PSMB9	0.34
[60,] LOX			LOX	0.37
[61,] STC1			STC1	0.35
[62,] CSPG2	CSPG2	CSPG2		0.35
[63,] PTGER4			PTGER4	0.31
[64,] IL6			IL6	0.34
[65,] SMAD3			SMAD3	0.38
[66,] PLAU	PLAU	PLAU		0.35
[67,] WNT5A			WNT5A	0.44
[68,] BDNF			BDNF	0.34
[69,] TNFRSF1A	TNFRSF1A		TNFRSF1A	0.46
[70,] FLNC			FLNC	0.34
[71,] DKFZP564K0822			DKFZP564K0822	0.34
[72,] FLOT1			FLOT1	0.38
[73,] PTRF			PTRF	0.39
[74,] HLA-B			HLA-B	0.36
[75,] COL6A2	COL6A2	COL6A2		0.32
[76,] MGC4083			MGC4083	0.32
[77,] TNFRSF10B			TNFRSF10B	0.34
[78,] PLAGL1			PLAGL1	0.31
[79,] PNMA2			PNMA2	0.38
[80,] TFPI			TFPI	0.38
[81,] LAT			LAT	0.46
[82,] GZMB			GZMB	0.51
[83,] CYR61			CYR61	0.37
[84,] PLAUR	PLAUR		PLAUR	0.35
[85,] FSCN1	FSCN1		FSCN1	0.32
[86,] ERP70			ERP70	0.32
[87,] AF1Q			AF1Q	0.3
[88,] UBC		UBC		0.37
[89,] FGFR1		FGFR1		0.33
[90,] HIC			HIC	0.33
[91,] BAX		BAX		0.35
[92,] COL4A2	COL4A2	COL4A2		0.32

TABLE 16-continued

<u>Bleomycin biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[93,] COL6A1		COL6A1		0.32
[94,] IFITM3		IFITM3		0.3
[95,] MAP1B		MAP1B		0.38
[96,] FLJ46603		FLJ46603		0.37
[97,] RAFTLIN		RAFTLIN		0.34
[98,] RRAS		RRAS		0.31
[99,] FTL		FTL		0.3
[100,] KIAA0877		KIAA0877		0.31
[101,] MT1E	MT1E	MT1E		0.31
[102,] CDC10		CDC10		0.51
[103,] DOCK2		DOCK2		0.32
[104,] TRIM22		TRIM22		0.36
[105,] RIS1		RIS1		0.37
[106,] BCAT1		BCAT1		0.42
[107,] PRF1		PRF1		0.34
[108,] DBN1		DBN1		0.36
[109,] MT1K		MT1K		0.3
[110,] TMSB10		TMSB10		0.42
[111,] RAB31	RAB31			0.45
[112,] FLJ10350		FLJ10350		0.4
[113,] C1orf24		C1orf24		0.34
[114,] NME7		NME7		0.46
[115,] TMEM22		TMEM22		0.3
[116,] TPK1		TPK1		0.37
[117,] COL5A2		COL5A2		0.34
[118,] ELK3		ELK3		0.38
[119,] CYLD		CYLD		0.4
[120,] ADAMTS1		ADAMTS1		0.31
[121,] EHD2		EHD2		0.41
[122,] ACTB	ACTB	ACTB		0.33

TABLE 17

<u>Methyl-GAG (Methyl glyoxal bis(amidinohydrazone) dihydrochloride)</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] PTMA	PTMA			0.32
[2,] SSRP1		SSRP1		0.37
[3,] NUDC		NUDC		0.35
[4,] CTSC		CTSC		0.35
[5,] AP1G2		AP1G2		0.33
[6,] PSME2		PSME2		0.3
[7,] LBR		LBR		0.38
[8,] EFNB2		EFNB2		0.31
[9,] SERPINA1		SERPINA1		0.34
[10,] SSSCA1		SSSCA1		0.32
[11,] EZH2		EZH2		0.36
[12,] MYB	MYB	MYB		0.33
[13,] PRIM1		PRIM1		0.39
[14,] H2AFX		H2AFX		0.33
[15,] HMGA1		HMGA1		0.35
[16,] HMMR		HMMR		0.33
[17,] TK2		TK2		0.42
[18,] WHSC1		WHSC1		0.35
[19,] DIAPH1		DIAPH1		0.34
[20,] LAMB3		LAMB3		0.31
[21,] DPAGT1		DPAGT1		0.42
[22,] UCK2		UCK2		0.31
[23,] SERPINB1		SERPINB1		0.31
[24,] MDN1		MDN1		0.35
[25,] BRRN1		BRRN1		0.33
[26,] GOS2		GOS2		0.43
[27,] RAC2		RAC2		0.35
[28,] MGC21654		MGC21654		0.36
[29,] GTSE1		GTSE1		0.35
[30,] TACC3		TACC3		0.31

TABLE 17-continued

<u>Methyl-GAG (Methyl glyoxal bis(amidinohydrazone) dihydrochloride)</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[31,] PLEK2			PLEK2	0.32
[32,] PLAC8			PLAC8	0.31
[33,] HNRPD			HNRPD	0.35
[34,] PNAS-4			PNAS-4	0.3

TABLE 18

<u>Carboplatin biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] MSN		MSN		0.31
[2,] ITGA5			ITGA5	0.43
[3,] VIM		VIM		0.34
[4,] TNFAIP3			TNFAIP3	0.4
[5,] CSPG2		CSPG2		0.35
[6,] WNT5A			WNT5A	0.34
[7,] FOXF2			FOXF2	0.36
[8,] LOC94105			LOC94105	0.32
[9,] IFI16		IFI16		0.38
[10,] LRRN3			LRRN3	0.33
[11,] FGFR1			FGFR1	0.37
[12,] DOCK10			DOCK10	0.4
[13,] LEPRE1			LEPRE1	0.32
[14,] COL5A2			COL5A2	0.3
[15,] ADAMTS1			ADAMTS1	0.34

TABLE 19

5-FU (5-Fluorouracil) biomarkers				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1,] RPL18		RPL18		0.39
[2,] RPL10A		RPL10A		0.36
[3,] RNPS1	RNPS1			0.3
[4,] ANAPC5		ANAPC5		0.5
[5,] EEF1B2		EEF1B2		0.4
[6,] RPL13A		RPL13A		0.38
[7,] RPS15		RPS15		0.34
[8,] AKAP1		AKAP1		0.37
[9,] NDUFAB1		NDUFAB1		0.3
[10,] APRT		APRT		0.32
[11,] ZNF593		ZNF593		0.37
[12,] MRP63		MRP63		0.31
[13,] IL6R		IL6R		0.31
[14,] RPL13	RPL13			0.31
[15,] SART3		SART3		0.35
[16,] RPS6	RPS6			0.49
[17,] UCK2		UCK2		0.38
[18,] RPL3	RPL3			0.32
[19,] RPL17		RPL17		0.34
[20,] RPS2		RPS2		0.32
[21,] PCCB		PCCB		0.31
[22,] TOMM20		TOMM20		0.39
[23,] SHMT2		SHMT2		0.36
[24,] RPLP0		RPLP0		0.3
[25,] GTF3A		GTF3A		0.5
[26,] STOML2		STOML2		0.4
[27,] DKFZp564J157		DKFZp564J157		0.38
[28,] MRPS2		MRPS2		0.34
[29,] ALG5		ALG5		0.37
[30,] CALML4		CALML4		0.3

TABLE 20

Rituximab (e.g., Mabthera) biomarkers			
List_2006	List_Prior	List_Preferr	Correlation
[1,] ITK	ITK		0.36
[2,] KIFC1		KIFC1	0.36
[3,] VLDLR		VLDLR	0.39
[4,] RUNX1		RUNX1	0.32
[5,] PAFAH1B3		PAFAH1B3	0.32
[6,] H1FX		H1FX	0.43
[7,] RNF144		RNF144	0.38
[8,] TMSNB		TMSNB	0.47
[9,] CRY1		CRY1	0.37
[10,] MAZ		MAZ	0.33
[11,] SLA		SLA	0.35
[12,] SRF		SRF	0.37
[13,] UMPS		UMPS	0.41
[14,] CD3Z		CD3Z	0.33
[15,] PRKCQ		PRKCQ	0.31
[16,] HNRPM		HNRPM	0.45
[17,] ZAP70		ZAP70	0.38
[18,] ADD1		ADD1	0.31
[19,] RFC5		RFC5	0.35
[20,] TM4SF2		TM4SF2	0.33
[21,] PFN2		PFN2	0.3
[22,] BMI1		BMI1	0.31
[23,] TUBGCP3		TUBGCP3	0.33
[24,] ATP6V1B2	RALY	ATP6V1B2	0.42
[25,] RALY			0.31
[26,] PSMC5	PSMC5		0.36
[27,] CD1D		CD1D	0.32
[28,] ADA	ADA		0.34
[29,] CD99		CD99	0.33
[30,] CD2	CD2		0.43
[31,] CNP		CNP	0.48

TABLE 20-continued

Rituximab (e.g., Mabthera) biomarkers			
List_2006	List_Prior	List_Preferr	Correlation
[32,] ERG		ERG	0.47
[33,] MYL6	MYL6		0.41
[34,] CD3E		CD3E	0.36
[35,] CD1A		CD1A	0.46
[36,] CD1B	CD1B		0.47
[37,] STMN1		STMN1	0.32
[38,] PSMC3		PSMC3	0.38
[39,] RPS4Y1		RPS4Y1	0.36
[40,] AKT1		AKT1	0.38
[41,] TAL1		TAL1	0.37
[42,] GNA15	GNA15		0.37
[43,] UBE2A		UBE2A	0.35
[44,] TCF12		TCF12	0.35
[45,] UBE2S		UBE2S	0.52
[46,] CCND3		CCND3	0.38
[47,] PAX6		PAX6	0.35
[48,] MDK	MDK		0.3
[49,] CAPG		CAPG	0.36
[50,] RAG2		RAG2	0.39
[51,] ACTN1	ACTN1		0.37
[52,] GSTM2		GSTM2	0.47
[53,] SATB1		SATB1	0.36
[54,] NASP		NASP	0.3
[55,] IGFBP2		IGFBP2	0.46
[56,] CDH2		CDH2	0.49
[57,] CRABP1		CRABP1	0.36
[58,] DBN1		DBN1	0.49
[59,] CTNNA1	CTNNA1		0.53
[60,] AKR1C1		AKR1C1	0.32
[61,] CACNB3	CACNB3		0.37
[62,] FARSLA		FARSLA	0.35

TABLE 20-continued

Rituximab (e.g., Mabthera) biomarkers				
	List_2006	List_Prior	List_Preferr	Correlation
[63.]	CASP2	CASP2	0.42	E2F4
[64.]	CASP2	CASP2	0.31	
[65.]	E2F4		0.36	
[66.]	LCP2	LCP2	0.35	
[67.]	CASP6	CASP6	0.32	
[68.]	MYB	MYB	0.3	
[69.]	SFRS6	SFRS6	0.44	
[70.]	GLRB	GLRB	0.34	
[71.]	NDN	NDN	0.39	
[72.]	CPSF1	CPSF1	0.33	
[73.]	GNAQ	GNAQ	0.44	CPSF1
[74.]	TUSC3	TUSC3	0.41	
[75.]	GNAQ	GNAQ	0.54	
[76.]	JARID2	JARID2	0.44	
[77.]	OCRL	OCRL	0.5	
[78.]	FHL1	FHL1	0.36	
[79.]	EZH2	EZH2	0.4	
[80.]	SMOX	SMOX	0.35	
[81.]	SLC4A2	SLC4A2	0.35	
[82.]	UFD1L	UFD1L	0.3	
[83.]	SEPW1	SEPW1	0.31	SEPW1
[84.]	ZNF32	ZNF32	0.35	
[85.]	HTATSF1	HTATSF1	0.35	
[86.]	SHD1	SHD1	0.43	
[87.]	PTOV1	PTOV1	0.42	
[88.]	NXF1	NXF1	0.46	
[89.]	FYB	FYB	0.47	
[90.]	TRIM28	TRIM28	0.38	
[91.]	BC008967	BC008967	0.4	
[92.]	TRB@	TRB@	0.3	
[93.]	TFRC	TFRC	0.31	TFRC
[94.]	H1F0	H1F0	0.36	
[95.]	CD3D	CD3D	0.32	
[96.]	CD3G	CD3G	0.4	

TABLE 20-continued

Rituximab (e.g., Mabthera) biomarkers				
	List_2006	List_Prior	List_Preferr	Correlation
[97.]	CENPB	CENPB	0.36	ABL1
[98.]	ALDH2	ALDH2	0.33	
[99.]	ANXA1	ANXA1	0.35	
[100.]	H2AFX	H2AFX	0.51	
[101.]	CD1E	CD1E	0.33	
[102.]	DDX5	DDX5	0.39	
[103.]	ABL1	ABL1	0.3	
[104.]	CCNA2	CCNA2	0.3	
[105.]	ENO2	ENO2	0.35	
[106.]	SNRPB	SNRPB	0.38	
[107.]	GATA3	GATA3	0.36	FGFR1
[108.]	RRM2	RRM2	0.48	
[109.]	GLUL	GLUL	0.4	
[110.]	TCF7	TCF7	0.39	
[111.]	FGFR1	FGFR1	0.33	
[112.]	SOX4	SOX4	0.3	
[113.]	MAL	MAL	0.3	
[114.]	NUCB2	NUCB2	0.38	
[115.]	SMA3	SMA3	0.31	
[116.]	FAT	FAT	0.52	
[117.]	UNG	UNG	0.31	VIM
[118.]	ARHGDI	ARHGDI	0.36	
[119.]	RUNX1	RUNX1	0.38	
[120.]	MPHOSPH6	MPHOSPH6	0.5	
[121.]	DCTN1	DCTN1	0.34	
[122.]	SH3GL3	SH3GL3	0.38	
[123.]	VIM	VIM	0.41	
[124.]	PLEKHC1	PLEKHC1	0.3	
[125.]	CD47	CD47	0.32	
[126.]	POLR2F	POLR2F	0.37	
[127.]	RHOH	RHOH	0.43	ATP2A3
[128.]	ADD1	ADD1	0.46	
[129.]	ATP2A3	ATP2A3	0.38	

TABLE 21

Radiation sensitivity biomarkers					
	List_2006	HU6800	List_Prior	List_Preferr	Correlation
[1.]	TRA1		TRA1	0.36	WARS
[2.]	ACTN4		ACTN4	0.36	
[3.]	WARS			0.39	
[4.]	CALM1		CALM1	0.32	
[5.]	CD63	CD63	CD63	0.32	
[6.]	CD81	CD81		0.43	
[7.]	FKBP1A		FKBP1A	0.38	
[8.]	CALU		CALU	0.47	
[9.]	IQGAP1		IQGAP1	0.37	
[10.]	CTSB	CTSB		0.33	
[11.]	MGC8721		MGC8721	0.35	CD81
[12.]	STAT1		STAT1	0.37	
[13.]	TACC1		TACC1	0.41	
[14.]	TM4SF8		TM4SF8	0.33	
[15.]	CD59		CD59	0.31	
[16.]	CKAP4	CKAP4	CKAP4	0.45	
[17.]	DUSP1	DUSP1	DUSP1	0.38	
[18.]	RCN1		RCN1	0.31	
[19.]	MGC8902		MGC8902	0.35	
[20.]	LGALS1	LGALS1	LGALS1	0.33	
[21.]	BHLHB2		BHLHB2	0.3	ATP2A3
[22.]	RRBP1		RRBP1	0.31	
[23.]	PKM2	PKM2		0.33	
[24.]	PRNP		PRNP	0.42	
[25.]	PPP2CB	PPP2CB		0.31	
[26.]	CNN3		CNN3	0.36	
[27.]	ANXA2	ANXA2	ANXA2	0.32	
[28.]	IER3		IER3	0.34	

TABLE 21-continued

Radiation sensitivity biomarkers				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[29,] JAK1		JAK1		0.33
[30,] MARCKS		MARCKS		0.43
[31,] LUM		LUM		0.48
[32,] FER1L3		FER1L3		0.47
[33,] SLC20A1		SLC20A1		0.41
[34,] EIF4G3		EIF4G3		0.36
[35,] HEXB		HEXB		0.46
[36,] EXT1		EXT1		0.47
[37,] TJP1		TJP1		0.32
[38,] CTSL	CTSL	CTSL		0.38
[39,] SLC39A6		SLC39A6		0.36
[40,] RIOK3		RIOK3		0.38
[41,] CRK		CRK		0.37
[42,] NNMT		NNMT		0.37
[43,] COL1A1		COL1A1		0.35
[44,] TRAM2	TRAM2	TRAM2		0.35
[45,] ADAM9		ADAM9		0.52
[46,] DNAJC7		DNAJC7		0.38
[47,] PLSCR1		PLSCR1		0.35
[48,] PRSS23		PRSS23		0.3
[49,] PLOD2		PLOD2		0.36
[50,] NPC1		NPC1		0.39
[51,] TOB1		TOB1		0.37
[52,] GFPT1		GFPT1		0.47
[53,] IL8		IL8		0.36
[54,] DYRK2		DYRK2		0.3
[55,] PYGL		PYGL		0.46
[56,] LOXL2		LOXL2		0.49
[57,] KIAA0355		KIAA0355		0.36
[58,] UGDH		UGDH		0.49
[59,] NFIL3		NFIL3		0.53
[60,] PURA		PURA		0.32
[61,] ULK2		ULK2		0.37
[62,] CENTG2		CENTG2		0.35
[63,] NID2		NID2		0.42
[64,] CAP350		CAP350		0.31
[65,] CXCL1		CXCL1		0.36
[66,] BTN3A3		BTN3A3		0.35
[67,] IL6		IL6		0.32
[68,] WNT5A		WNT5A		0.3
[69,] FOXF2		FOXF2		0.44
[70,] LPHN2		LPHN2		0.34
[71,] CDH11		CDH11		0.39
[72,] P4HA1		P4HA1		0.33
[73,] GRP58		GRP58		0.44
[74,] ACTN1	ACTN1	ACTN1		0.41
[75,] CAPN2		CAPN2		0.54
[76,] DSPI		DSPI		0.44
[77,] MAP1LC3B		MAP1LC3B		0.5
[78,] GALIG	GALIG	GALIG		0.36
[79,] IGSF4		IGSF4		0.4
[80,] IRS2		IRS2		0.35
[81,] ATP2A2		ATP2A2		0.35
[82,] OGT		OGT		0.3
[83,] TNFRSF10B		TNFRSF10B		0.31
[84,] KIAA1128		KIAA1128		0.35
[85,] TM4SF1		TM4SF1		0.35
[86,] RBPMs		RBPMs		0.43
[87,] RIPK2		RIPK2		0.42
[88,] CBLB		CBLB		0.46
[89,] NR1D2		NR1D2		0.47
[90,] BTN3A2		BTN3A2		0.38
[91,] SLC7A11		SLC7A11		0.4
[92,] MPZL1		MPZL1		0.3
[93,] IGFBP3	IGFBP3	IGFBP3		0.31
[94,] SSA2		SSA2		0.36
[95,] FN1	FN1	FN1		0.32
[96,] NQO1		NQO1		0.4
[97,] ASPH		ASPH		0.36
[98,] ASAHI		ASAHI		0.33
[99,] MGLL		MGLL		0.35
[100,] SERPINB6		SERPINB6		0.51

TABLE 21-continued

<u>Radiation sensitivity biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[101,]	HSPA5		HSPA5	0.33
[102,]	ZFP36L1		ZFP36L1	0.39
[103,]	COL4A2		COL4A2	0.3
[104,]	COL4A1		COL4A1	0.3
[105,]	CD44		CD44	0.35
[106,]	SLC39A14		SLC39A14	0.38
[107,]	NIPA2		NIPA2	0.36
[108,]	FKBP9		FKBP9	0.48
[109,]	IL6ST		IL6ST	0.4
[110,]	DKFZP564G2022		DKFZP564G2022	0.39
[111,]	PPAP2B		PPAP2B	0.33
[112,]	MAP1B		MAP1B	0.3
[113,]	MAPK1		MAPK1	0.3
[114,]	MYO1B		MYO1B	0.38
[115,]	CAST	CAST	CAST	0.31
[116,]	RRAS2		RRAS2	0.52
[117,]	QKI		QKI	0.31
[118,]	LHFPL2		LHFPL2	0.36
[119,]	SEPT10		SEPT10	0.38
[120,]	ARHE		ARHE	0.5
[121,]	KIAA1078		KIAA1078	0.34
[122,]	FTL		FTL	0.38
[123,]	KIAA0877		KIAA0877	0.41
[124,]	PLCB1		PLCB1	0.3
[125,]	KIAA0802		KIAA0802	0.32
[126,]	KPNB1	KPNB1		0.37
[127,]	RAB3GAP		RAB3GAP	0.43
[128,]	SERPINB1		SERPINB1	0.46
[129,]	TIMM17A		TIMM17A	0.38
[130,]	SOD2		SOD2	0.35
[131,]	HLA-A	HLA-A	HLA-A	0.33
[132,]	NOMO2		NOMO2	0.43
[133,]	LOC55831		LOC55831	0.32
[134,]	PHLDA1		PHLDA1	0.32
[135,]	TMEM2		TMEM2	0.47
[136,]	MLPH		MLPH	0.35
[137,]	FAD104		FAD104	0.34
[138,]	LRRC5		LRRC5	0.42
[139,]	RAB7L1		RAB7L1	0.41
[140,]	FLJ35036		FLJ35036	0.36
[141,]	DOCK10		DOCK10	0.41
[142,]	LRP12		LRP12	0.36
[143,]	TXNDC5		TXNDC5	0.4
[144,]	CDC14B		CDC14B	0.39
[145,]	HRMT1L1		HRMT1L1	0.38
[146,]	CORO1C		CORO1C	0.38
[147,]	DNAJC10		DNAJC10	0.31
[148,]	TNPO1		TNPO1	0.33
[149,]	LONP		LONP	0.32
[150,]	AMIGO2		AMIGO2	0.38
[151,]	DNAPTP6		DNAPTP6	0.31
[152,]	ADAMTS1		ADAMTS1	0.37
[153,]	CCL21			
[154,]	SCARB2			
[155,]	MAD2L1BP			
[156,]	PTS			
[157,]	NBL1			
[158,]	CD151			
[159,]	CRIP2			
[160,]	UGCG			
[161,]	PRSS11			
[162,]	MME			
[163,]	CBR1			
[164,]	DUSP3			
[165,]	PFN2			
[166,]	MICA			
[167,]	FTH1			
[168,]	RHOC			
[169,]	ZAP128			
[170,]	PON2			
[171,]	COL5A2			
[172,]	CST3			

TABLE 21-continued

<u>Radiation sensitivity biomarkers</u>				
List_2006	HU6800	List_Prior	List_Preferr	Correlation
[173,]	MCAM			
[174,]	MMP2			
[175,]	CTSD			
[176,]	ALDH3A1			
[177,]	CSRP1			
[178,]	S100A4			
[179,]	CALD1			
[180,]	CTGF			
[181,]	CAPG			
[182,]	TAGLN			
[183,]	FSTL1			
[184,]	SCTR			
[185,]	BLVRA			
[186,]	COPEB			
[187,]	DIPA			
[188,]	SMARCD3			
[189,]	MVP			
[190,]	PEA15			
[191,]	S100A13			
[192,]	ECE1			

TABLE 22

<u>Vincristine biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Medianprobe	
1	SLC25A5	0.32	TCCTGTACTTGTCCCTCAGCTGGGC	
2	RPL10	0.38	GCCCCACTGGACAACACTGATT CCT	
3	RPL12	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG	
4	RPS4X	0.39	AAATGTTCCCTTGCCCTGCTCCTG	
5	EIF5A	0.31	TCCTGTACTTGTCCCTCAGCTGGGC	
6	BLMH	0.32	AAGCCTATACTGTTCTGTGGAGTAA	
7	TBCA	0.3	ACTTGTCCCTCAGCTTGGCTTCTC	
8	MDH2	0.34	TCCTGTACTTGTCCCTCAGCTGGGC	
9	S100A4	0.32	TGGACCCCCTGGCTGAGAACCTGG	
10	C14orf139	0.3	TTGGACATCTCTAGTGTAGCTGCCA	

TABLE 23

Cisplatin biomarkers.

SEQ ID NO	Gene	Corre- lation	Medianprobe
11	C1QR1	0.3	CACCCAGCTGGCCTGTGGATGGGA
12	SLA	0.37	TGCCTGCTCCTGTACTTGTCCCTCAG
13	PTPN7	0.31	ACTTGTCCCTCAGCTTGGGCTTCTC
14	ZNFN1A1	0.33	CACCCAGCTGGCCTGTGGATGGGA
15	CENTB1	0.37	TTGGACATCTCTAGTGTAGCTGCCA

TABLE 23-continued

<u>Cisplatin biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Medianprobe	
16	IFI16	0.31	TCCTCCATCACCTGAAACACTGGAC	
17	ARHGEF6	0.35	TGCCTGCTCCTGTACTTGTCCCTCAG	
18	SEC31L2	0.32	AAGCCTATACTGTTCTGTGGAGTAA	
19	CD3Z	0.32	TTGGACATCTCTAGTGTAGCTGCCA	
20	GZMB	0.3	TCCTCCATCACCTGAAACACTGGAC	
21	CD3D	0.34	TCCTCCATCACCTGAAACACTGGAC	
22	MAP4K1	0.32	CACCCAGCTGGCCTGTGGATGGGA	
23	GPR65	0.39	CACCCAGCTGGCCTGTGGATGGGA	
24	PRF1	0.31	TCCTTGTGCCTGCTCCTGTACTTGT	
25	ARHGAP15	0.35	CACCCAGCTGGCCTGTGGATGGGA	
26	TM6SF1	0.41	TGCCTGCTCCTGTACTTGTCCCTCAG	
27	TCF4	0.4	AAATGTTCCCTGTGCCTGCTCCTG	

TABLE 24

<u>Etoposide biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Medianprobe	
28	CD99	0.3	AAGCCTATACTGTTCTGTGGAGTAA	
29	INSIG1	0.35	TCCTTGTGCCTGCTCCTGTACTTGT	

TABLE 24-continued

<u>Etoposide biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
30	PRG1	0.34	GCCCCACTGGACAAACACTGATTCCCT
31	MUF1	0.35	AAGCCTATACTACGTTCTGTGGAGTAA
32	SLA	0.37	CACCCAGCTGGCCTGTGGATGGGA
33	SSBP2	0.37	TGGACCCCCTGGCTGAGAACATCTGG
34	GNB5	0.35	TCCTTGTCCTGCTCCTGTACTTGT
35	MFNG	0.33	GCCCCACTGGACAAACACTGATTCCCT
36	PSMB9	0.31	AAGCCTATACTACGTTCTGTGGAGTAA
37	EVI2A	0.41	TCCTCCATCACCTGAAACACTGGAC
38	PTPN7	0.3	AAGCCTATACTACGTTCTGTGGAGTAA
39	PTGER4	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
40	CXorf9	0.3	GCCCCACTGGACAAACACTGATTCCCT
41	ZNFN1A1	0.35	ACTTGTCCCTCAGCTGGGCTCTTC
42	CENTB1	0.3	TGGACCCCCTGGCTGAGAACATCTGG
43	NAP1L1	0.31	TCCTCCATCACCTGAAACACTGGAC
44	HLA-DRA	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
45	IFI16	0.38	CACCCAGCTGGCCTGTGGATGGGA
46	ARHGEF6	0.33	TGGACCCCCTGGCTGAGAACATCTGG
47	PSCDBP	0.4	AAGCCTATACTACGTTCTGTGGAGTAA
48	SELPLG	0.35	TTGGACATCTCTAGTGTAGCTGCCA
49	SEC31L2	0.42	AAATGTTCCCTGTGCCTGCTCCTG
50	CD3Z	0.36	TGCCTGCTCCTGTACTTGTCCCTCAG
51	SH2D1A	0.33	CACCCAGCTGGCCTGTGGATGGGA
52	GZMB	0.34	TGGACCCCCTGGCTGAGAACATCTGG
53	SCN3A	0.3	GCCCCACTGGACAAACACTGATTCCCT
54	RAFTLIN	0.39	TCCTCCATCACCTGAAACACTGGAC
55	DOCK2	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
56	CD3D	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
57	ZAP70	0.35	TCCTCCATCACCTGAAACACTGGAC
58	GPR65	0.35	TGGACCCCCTGGCTGAGAACATCTGG
59	PRF1	0.32	TGGACCCCCTGGCTGAGAACATCTGG
60	ARHGAP15	0.32	ACTTGTCCCTCAGCTGGGCTCTTC
61	NOTCH1	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
62	UBASH3A	0.32	ACTTGTCCCTCAGCTGGGCTCTTC

TABLE 25

<u>Azaquanine biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
63	SRM	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
64	SCARB1	0.4	TTGGACATCTCTAGTGTAGCTGCCA
65	SIAT1	0.31	AAATGTTCCCTGTGCCTGCTCCTG
66	CUGBP2	0.37	TGGACCCCCTGGCTGAGAACATCTGG
67	WASPIP	0.44	TCCTGTACTTGTCCCTCAGCTTGGGC
68	ITM2A	0.31	AAGCCTATACTGTTCTGTGGAGTAA
69	PALM2-AKAP2	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
70	LNK	0.43	TTGGACATCTCTAGTGTAGCTGCCA
71	FCGR2A	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
72	RUNX3	0.43	TCCTGTACTTGTCCCTCAGCTTGGGC
73	EVI2A	0.4	AAATGTTCCCTGTGCCTGCTCCTG
74	BTN3A3	0.4	ACTTGTCCCTCAGCTGGGCTCTTC
75	LCP2	0.34	TCCTTGCCCTGCTCCTGTACTTGT
76	BCHE	0.35	TCCTCCATCACCTGAAACACTGGAC
77	LY96	0.47	TGCCTGCTCCTGTACTTGTCCCTCAG
78	LCP1	0.42	ACTTGTCCCTCAGCTGGGCTCTTC
79	IFI16	0.33	CACCCAGCTGGCCTGTGGATGGGA
80	MCAM	0.37	TTGGACATCTCTAGTGTAGCTGCCA
81	MEF2C	0.41	CACCCAGCTGGCCTGTGGATGGGA
82	FYN	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
83	Clorf38	0.37	AAGCCTATACTGTTCTGTGGAGTAA
84	FCGR2C	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
85	TNIK	0.35	AAGCCTATACTGTTCTGTGGAGTAA
86	AMPD2	0.3	TCCTGTACTTGTCCCTCAGCTTGGGC
87	SEPT6	0.41	AAATGTTCCCTGTGCCTGCTCCTG
88	RAFTLIN	0.39	TCCTTGCCCTGCTCCTGTACTTGT
89	SLC43A3	0.52	CACCCAGCTGGCCTGTGGATGGGA
90	LPXN	0.54	AAGCCTATACTGTTCTGTGGAGTAA
91	CKIP-1	0.33	TCCTGTACTTGTCCCTCAGCTTGGGC
92	FLJ10539	0.33	TCCTTGCCCTGCTCCTGTACTTGT
93	FLJ35036	0.36	AAGCCTATACTGTTCTGTGGAGTAA
94	DOCK10	0.3	GCCCCACTGGACAAACACTGATTCCCT
95	TRPV2	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
96	IFRG28	0.3	TCCTTGCCCTGCTCCTGTACTTGT

TABLE 25-continued

<u>Azaquanine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
97	LEF1	0.31	ACTTGTCCCTCAGCTTGGGCTTC
98	ADAMTS1	0.36	TGGACCCCCTGGCTGAGAATCTGG

TABLE 26

<u>Carboplatin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
99	ITGA5	0.43	AAATGTTCCCTTGTGCCTGCTCCTG
100	TNFAIP3	0.4	TGCCTGCTCCTGTACTTGTCTCAG
101	WNT5A	0.34	TCCTCCATCACCTGAAACACTGGAC
102	FOXF2	0.36	TGCCTGCTCCTGTACTTGTCTCAG
103	LOC94105	0.32	AAATGTTCCCTTGTGCCTGCTCCTG
104	IFI16	0.38	TCCTCCATCACCTGAAACACTGGAC
105	LRRN3	0.33	TTGGACATCTCTAGTGTAGCTGCCA
106	DOCK10	0.4	TCCTGTACTTGTCTCAGCTTGGC
107	LEPRE1	0.32	GCCCCACTGGACAACACTCATTCCT
108	ADAMTS1	0.34	TGGACCCCCTGGCTGAGAATCTGG

TABLE 27

<u>Adriamycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
109	CD99	0.41	AAGCCTATACTGTTCTGTGGAGTAA
110	ALDOC	0.31	TCCTTGCGCTGCTCCTGTACTTGT
111	SLA	0.35	TGCCTGCTCCTGTACTTGTCTCAG
112	SSBP2	0.34	TCCTCCATCACCTGAAACACTGGAC
113	IL2RG	0.38	TCCTTGCGCTGCTCCTGTACTTGT
114	CXorf9	0.32	TGGACCCCCTGGCTGAGAATCTGG
115	RHOH	0.31	ACTTGTCCTCAGCTTGGGCTTC
116	ZNFN1A1	0.43	TTGGACATCTCTAGTGTAGCTGCCA
117	CENTB1	0.36	AAGCCTATACTGTTCTGTGGAGTAA
118	MAP4K1	0.35	TCCTCCATCACCTGAAACACTGGAC
119	CD3G	0.31	AAATGTTCCCTTGTGCCTGCTCCTG
120	CCR9	0.34	CACCCAGCTGGCCTGTGGATGGGA
121	CXCR4	0.3	TCCTTGCGCTGCTCCTGTACTTGT

TABLE 27-continued

<u>Adriamycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
122	ARHGEF6	0.31	TCCTCCATCACCTGAAACACTGGAC
123	SELPLG	0.31	TGGACCCCCTGGCTGAGAATCTGG
124	SEC31L2	0.33	TGCCTGCTCCTGTACTTGTCTCAG
125	CD3Z	0.37	ACTTGTCCTCAGCTTGGGCTTC
126	SH2D1A	0.37	TTGGACATCTCTAGTGTAGCTGCCA
127	CD1A	0.4	AAGCCTATACTGTTCTGTGGAGTAA
128	LAIR1	0.39	AAGCCTATACTGTTCTGTGGAGTAA
129	TRB@	0.34	TCCTCCATCACCTGAAACACTGGAC
130	CD3D	0.33	TCCTTGCGCTGCTCCTGTACTTGT
131	WBSCR20C	0.34	ACTTGTCCTCAGCTTGGGCTTC
132	ZAP70	0.33	TCCTGTACTTGTCTCAGCTTGGG
133	IFI44	0.32	TGCCTGCTCCTGTACTTGTCTCAG
134	GPR65	0.31	AAGCCTATACTGTTCTGTGGAGTAA
135	AIF1	0.3	CACCCAGCTGGCCTGTGGATGGGA
136	ARHGAP15	0.37	TCCTGTACTTGTCTCAGCTTGGG
137	NARF	0.3	TCCTCCATCACCTGAAACACTGGAC
138	PACAP	0.32	CACCCAGCTGGCCTGTGGATGGGA

TABLE 28

<u>Aclarubicin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
139	RPL12	0.3	AAATGTTCCCTTGTGCCTGCTCCTG
140	RPLP2	0.37	TTGGACATCTCTAGTGTAGCTGCCA
141	MYB	0.31	TCCTTGCGCTGCTCCTGTACTTGT
142	ZNFN1A1	0.34	AAATGTTCCCTTGTGCCTGCTCCTG
143	SCAP1	0.33	TGCCTGCTCCTGTACTTGTCTCAG
144	STAT4	0.31	AAATGTTCCCTTGTGCCTGCTCCTG
145	SP140	0.4	AAGCCTATACTGTTCTGTGGAGTAA
146	AMPD3	0.3	TGCCTGCTCCTGTACTTGTCTCAG
147	TNFAIP8	0.4	AAGCCTATACTGTTCTGTGGAGTAA
148	DDX18	0.31	TCCTTGCGCTGCTCCTGTACTTGT
149	TAF5	0.3	TCCTTGCGCTGCTCCTGTACTTGT
150	RPS2	0.34	CACCCAGCTGGCCTGTGGATGGGA

TABLE 28-continued

<u>Aclarubicin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
151	DOCK2	0.32	AAGCCTATACTGTTCTGTGGAGTAA
152	GPR65	0.35	AAGCCTATACTGTTCTGTGGAGTAA
153	HOXA9	0.33	TCCTTGTGCCTGCTCCTGTACTTGT
154	FLJ12270	0.31	AAATGTTCCCTGTGCCTGCTCCTG
155	HNRPD	0.4	ACTTGTCCCTCAGCTGGGCTCTTC

TABLE 29-continued

<u>Mitoxantrone biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
181	IFI44	0.33	TGGACCCCCTGGCTGAGAATCTGG
182	CUTC	0.33	AAATGTTCCCTGTGCCTGCTCCTG
183	NAP1L2	0.33	AAGCCTATACTGTTCTGTGGAGTAA
184	NME7	0.35	AAATGTTCCCTGTGCCTGCTCCTG
185	FLJ21159	0.33	TCCTGTACTTGTCCCTCAGCTGGG

TABLE 29

<u>Mitoxantrone biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
156	PGAM1	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
157	DPYSL3	0.36	AAATGTTCCCTGTGCCTGCTCCTG
158	INSIG1	0.32	TCCTTGTGCCTGCTCCTGTACTTGT
159	GJA1	0.31	TTGGACATCTCTAGTGTAGCTGCCA
160	BNIP3	0.31	TTGGACATCTCTAGTGTAGCTGCCA
161	PRG1	0.39	GCCCCACTGGACAACACTGATTCCCT
162	G6PD	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
163	PLOD2	0.34	GCCCCACTGGACAACACTGATTCCCT
164	LOXL2	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
165	SSBP2	0.36	TCCTCCATCACCTGAAACACTGGAC
166	C1orf29	0.35	TCCTTGTGCCTGCTCCTGTACTTGT
167	TOX	0.35	TCCTTGTGCCTGCTCCTGTACTTGT
168	STC1	0.39	TCCTGTACTTGTCCCTCAGCTGGGC
169	TNFRSF1A	0.34	AAATGTTCCCTGTGCCTGCTCCTG
170	NCOR2	0.3	TCCTCCATCACCTGAAACACTGGAC
171	NAP1L1	0.32	TCCTTGTGCCTGCTCCTGTACTTGT
172	LOC94105	0.34	AAGCCTATACTGTTCTGTGGAGTAA
173	ARHGEF6	0.34	TCCTCCATCACCTGAAACACTGGAC
174	GATA3	0.35	TCCTTGTGCCTGCTCCTGTACTTGT
175	TFPI	0.31	TCCTGTACTTGTCCCTCAGCTGGGC
176	CD3Z	0.37	AAGCCTATACTGTTCTGTGGAGTAA
177	AF1Q	0.33	GCCCCACTGGACAACACTGATTCCCT
178	MAP1B	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
179	CD3D	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
180	BCAT1	0.32	TCCTGTACTTGTCCCTCAGCTGGGC

TABLE 30

<u>Mitomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
186	STC1	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
187	GPR65	0.32	GCCCCACTGGACAACACTGATTCCCT
188	DOCK10	0.35	ACTTGTCCCTCAGCTGGGCTTCTTC
189	FAM46A	0.36	TCCTTGTGCCTGCTCCTGTACTTGT
190	LOC54103	0.39	ACTTGTCCCTCAGCTGGGCTTCTTC

TABLE 31

<u>Paclitaxel (Taxol) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
191	RPL10	0.31	TCCTCCATCACCTGAAACACTGGAC
192	RPS4X	0.31	TCCTCCATCACCTGAAACACTGGAC
193	DKC1	0.3	TCCTGTGCCTGCTCCTGTACTTGT
194	DKFZP564C186	0.32	ACTTGTCCCTCAGCTGGGCTTCTTC
195	PRP19	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
196	PABP9P40	0.33	GCCCCACTGGACAACACTGATTCCCT
197	HSA9761	0.37	AAATGTTCCCTGTGCCTGCTCCTG
198	GMDS	0.3	AAATGTTCCCTGTGCCTGCTCCTG
199	CEP1	0.3	AAATGTTCCCTGTGCCTGCTCCTG
200	IL13RA2	0.34	AAATGTTCCCTGTGCCTGCTCCTG
201	MAGEB2	0.41	ACTTGTCCCTCAGCTGGGCTTCTTC
202	HMGN2	0.35	CACCCAGCTGGTCCCTGTGGATGGGA
203	ALMS1	0.3	TCCCTCCATCACCTGAAACACTGGAC
204	GPR65	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG

TABLE 31-continued

<u>Paclitaxel (Taxol) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
205	FLJ10774	0.31	TGGACCCCACTGGCTGAGAAATCTGG
206	NOL8	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
207	DAZAP1	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
208	SLC25A15	0.31	TTGGACATCTCTAGTGTAGCTGCCA
209	PAF53	0.36	TCCTCCATCACCTGAAACACTGGAC
210	PITPN1	0.33	TCCTCCATCACCTGAAACACTGGAC
211	SPANXC	0.3	TGGACCCCACTGGCTGAGAAATCTGG
212	KIAA1393	0.33	CACCCAGCTGGCCTGTGGATGGGA

TABLE 32

<u>Gemcitabine (Gemzar) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
213	UBE2L6	0.38	CACCCAGCTGGCCTGTGGATGGGA
214	TAP1	0.33	CACCCAGCTGGCCTGTGGATGGGA
215	F2R	0.3	TCCTGTACTTGTCCCTCAGCTGGGC
216	PSMB9	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
217	IL7R	0.31	AAGCCTATACTGTTCTGTGGAGTAA
218	TNFAIP8	0.33	AAGCCTATACTGTTCTGTGGAGTAA
219	HLA-C	0.33	TGGACCCCACTGGCTGAGAAATCTGG
220	IFI44	0.31	TGGACCCCACTGGCTGAGAAATCTGG

TABLE 33

<u>Taxotere (docetaxel) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
221	ANP32B	0.45	GCCCCACTGGACAACACTGATTCT
222	GTF3A	0.31	TTGGACATCTCTAGTGTAGCTGCCA
223	TRIM14	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
224	SKP2	0.33	GCCCCACTGGACAACACTGATTCT
225	TRIP13	0.36	TCCTGTACTTGTCCCTCAGCTGGGC
226	RFC3	0.45	GCCCCACTGGACAACACTGATTCT
227	CASP7	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
228	TXN	0.36	AAGCCTATACTGTTCTGTGGAGTAA
229	MCM5	0.34	AAATGTTCCCTGTGCCTGCTCCTG

TABLE 33-continued

<u>Taxotere (docetaxel) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
230	PTGES2	0.39	AAATGTTCCCTGTGCCTGCTCCTG
231	OBFC1	0.37	TGGACCCCACTGGCTGAGAAATCTGG
232	EPB41L4B	0.32	GCCCCACTGGACAACACTGATTCCCT
233	CALML4	0.31	TCCTCCATCACCTGAAACACTGGAC

TABLE 34

<u>Dexamethasone biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
234	IFITM2	0.38	ATATATGGACCTAGCTTGAGGCAAT
235	UBE2L6	0.32	AAGCCTATACTGTTCTGTGGAGTAA
236	ITM2A	0.38	CACCCAGCTGGCCTGTGGATGGGA
237	IL2RG	0.36	TCCTCCATCACCTGAAACACTGGAC
238	GPRASP1	0.36	TCCTGTACTTGTCCCTCAGCTTGGGC
239	PTPN7	0.31	TCCTTGCCCTGCTCCTGTACTTGT
240	CXorf9	0.36	GCCCCACTGGACAACACTGATTCCCT
241	RHOH	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
242	GIT2	0.31	ACTTGTCCCTCAGCTGGGCTTCTTC
243	ZNFN1A1	0.35	TCCTTGCCCTGCTCCTGTACTTGT
244	CEP1	0.31	CACCCAGCTGGCCTGTGGATGGGA
245	MAP4K1	0.3	AAGCCTATACTGTTCTGTGGAGTAA
246	CCR7	0.33	AAATGTTCCCTGTGCCTGCTCCTG
247	CD3G	0.35	CACCCAGCTGGCCTGTGGATGGGA
248	UCP2	0.3	AAGCCTATACTGTTCTGTGGAGTAA
249	GATA3	0.37	TGGACCCCACTGGCTGAGAAATCTGG
250	CDKN2A	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
251	TARP	0.3	GCCCCACTGGACAACACTGATTCCCT
252	LAIR1	0.34	TTGGACATCTCTAGTGTAGCTGCCA
253	SH2D1A	0.34	TCCTTGCCCTGCTCCTGTACTTGT
254	SEPT6	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
255	HA-1	0.34	TCCTTGCCCTGCTCCTGTACTTGT
256	CD3D	0.32	TCCTCCATCACCTGAAACACTGGAC
257	LST1	0.39	CACCCAGCTGGCCTGTGGATGGGA
258	AIF1	0.35	AAGCCTATACTGTTCTGTGGAGTAA

TABLE 34-continued

<u>Dexamethasone biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
259	ADA	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
260	DATF1	0.41	CACCCAGCTGGTCCTGTGGATGGGA
261	ARHGAP15	0.3	TCCTGTACTTGTCCCTCAGCTGGC
262	PLAC8	0.31	CACCCAGCTGGTCCTGTGGATGGGA
263	CECR1	0.31	GCCCCACTGGACAACACTGATTCT
264	LOC81558	0.33	TGGACCCCAGCTGGCTGAGAATCTGG
265	EHD2	0.37	ACTTGTCCCTCAGCTTGGGCTCTTC

TABLE 35

<u>Ara-C (Cytarabine hydrochloride) biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
266	ITM2A	0.32	TGGACCCCAGCTGGCTGAGAATCTGG
267	RHOH	0.31	AAATGTTCCCTGTGCTGCCCTG
268	PRIM1	0.3	TCCTCCATCACCTGAAACACTGGAC
269	CENTB1	0.31	TCCTTGTCCTGCTCCTGTACTTGT
270	NAP1L1	0.31	GCCCCACTGGACAACACTGATTCT
271	ATP5G2	0.31	TCCTCCATCACCTGAAACACTGGAC
272	GATA3	0.33	AAATGTTCCCTGTGCTGCCCTG
273	PRKCQ	0.32	AAGCCTATACGTTCTGTGGAGTAA
274	SH2D1A	0.3	GCCCCACTGGACAACACTGATTCT
275	SEPT6	0.42	ACTTGTCCCTCAGCTTGGGCTCTTC
276	NME4	0.33	ACTTGTCCCTCAGCTTGGGCTCTTC
277	CD3D	0.31	AAGCCTATACGTTCTGTGGAGTAA
278	CD1E	0.32	TGGACCCCAGCTGGCTGAGAATCTGG
279	ADA	0.34	GCCCCACTGGACAACACTGATTCT
280	FHOD1	0.31	CACCCAGCTGGTCCTGTGGATGGGA

TABLE 36

<u>Methylprednisolone biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
281	CD99	0.31	GCCCCACTGGACAACACTGATTCT
282	ARHGDIB	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
283	ITM2A	0.35	GCCCCACTGGACAACACTGATTCT

TABLE 36-continued

<u>Methylprednisolone biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
284	LGALS9	0.43	TCCTCCATCACCTGAAACACTGGAC
285	INPP5D	0.34	TGGACCCCAGCTGGCTGAGAATCTGG
286	SATB1	0.32	TCCTTGTCCTGCTCCTGTACTTGT
287	TFDP2	0.4	AAATGTTCCCTGTGCTGCTCCTG
288	SLA	0.31	TGGACCCCAGCTGGCTGAGAATCTGG
289	IL2RG	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
290	MFNG	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
291	SELL	0.33	AAATGTTCCCTGTGCTGCTCCTG
292	CDW52	0.33	TCCTCCATCACCTGAAACACTGGAC
293	LRMP	0.32	TCCTGTACTTGTCCCTCAGCTTGGC
294	ICAM2	0.38	CACCCAGCTGGCCTGTGGATGGGA
295	RIMS3	0.36	TGCCTGCTCCTGTACTTGTCCCTCAG
296	PTPN7	0.39	TGGACCCCAGCTGGCTGAGAATCTGG
297	ARHGAP25	0.37	TCCTGTACTTGTCCCTCAGCTTGGC
298	LCK	0.3	TCCTCCATCACCTGAAACACTGGAC
299	CXorf9	0.3	TTGGACATCTCTAGTGTAGCTGCCA
300	RHOH	0.51	AAGCCTATACGTTCTGTGGAGTAA
301	GIT2	0.33	ACTTGTCCCTCAGCTTGGCTCTTC
302	ZNFN1A1	0.53	TCCTTGTCCTGCTCCTGTACTTGT
303	CENTB1	0.36	TCCTCCATCACCTGAAACACTGGAC
304	LCP2	0.34	TCCTGTACTTGTCCCTCAGCTTGGC
305	SPI1	0.3	TCCTGTACTTGTCCCTCAGCTTGGC
306	GZMA	0.31	AAGCCTATACGTTCTGTGGAGTAA
307	CEP1	0.37	AAGCCTATACGTTCTGTGGAGTAA
308	CD8A	0.38	TGGACCCCAGCTGGCTGAGAATCTGG
309	SCAP1	0.32	TCCTCCATCACCTGAAACACTGGAC
310	CD2	0.48	GCCCCACTGGACAACACTGATTCCCT
311	VAV1	0.41	ACTTGTCCCTCAGCTTGGCTCTTC
312	MAP4K1	0.36	TCCTGTACTTGTCCCTCAGCTTGGC
313	CCR7	0.37	ACTTGTCCCTCAGCTTGGCTCTTC
314	C6orf32	0.38	TCCTTGTCCTGCTCCTGTACTTGT
315	ALOX15B	0.43	TGCCTGCTCCTGTACTTGTCCCTCAG
316	BRDT	0.33	AAGCCTATACGTTCTGTGGAGTAA
317	CD3G	0.51	AAGCCTATACGTTCTGTGGAGTAA
318	LTB	0.32	ACTTGTCCCTCAGCTTGGCTCTTC

TABLE 36-continued

<u>Methylprednisolone biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
319	NVL	0.31	TTGGACATCTCTAGTGTAGCTGCCA
320	RASGRP2	0.35	TGCCTGCTCCTGTACTTGTCCCTCAG
321	LCP1	0.34	AAATGTTCCCTGTGCCTGCTCCTG
322	CXCR4	0.3	AAGCCTATACTACGTTCTGTGGAGTAA
323	PRKD2	0.33	CACCCAGCTGGCCTGTGGATGGGA
324	GATA3	0.39	TCCTGTACTTGTCCCTCAGCTGGGC
325	KIAA0922	0.36	GCCCCACTGGACAACACTGATTCT
326	TARP	0.49	TCCTCCATCACCTGAAAACACTGGAC
327	SEC31L2	0.32	ACTTGCTCTCAGCTGGGCTCTTC
328	PRKCQ	0.37	TTGGACATCTCTAGTGTAGCTGCCA
329	SH2D1A	0.33	AAGCCTATACTACGTTCTGTGGAGTAA
330	CHRNA3	0.5	AAGCCTATACTACGTTCTGTGGAGTAA
331	CD1A	0.44	AAGCCTATACTACGTTCTGTGGAGTAA
332	LST1	0.36	CACCCAGCTGGCCTGTGGATGGGA
333	LAIR1	0.47	CACCCAGCTGGCCTGTGGATGGGA
334	CACNA1G	0.33	GCCCCACTGGACAACACTGATTCT
335	TRB@	0.31	ACTTGCTCTCAGCTGGGCTCTTC
336	SEPT6	0.33	TCCTGTGCCTGCTCCTGTACTTGT
337	HA-1	0.42	CACCCAGCTGGCCTGTGGATGGGA
338	DOCK2	0.32	TCCTGTACTTGTCCCTCAGCTGGC
339	CD3D	0.41	TCCTGTACTTGTCCCTCAGCTGGC
340	TRD@	0.38	TGCCTGCTCCTGTACTTGTCCCTCAG
341	T3JAM	0.37	TGCCTGCTCCTGTACTTGTCCCTCAG
342	FNBP1	0.37	TCCTGTACTTGTCCCTCAGCTGGC
343	CD6	0.4	CACCCAGCTGGCCTGTGGATGGGA
344	AIF1	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
345	FOLH1	0.45	TCCTGTACTTGTCCCTCAGCTGGC
346	CD1E	0.58	CACCCAGCTGGCCTGTGGATGGGA
347	LY9	0.39	TCCTGTGCCTGCTCCTGTACTTGT
348	ADA	0.39	AAATGTTCCCTGTGCCTGCTCCTG
349	CDKL5	0.44	GCCCCACTGGACAACACTGATTCT
350	TRIM	0.38	AAGCCTATACTACGTTCTGTGGAGTAA
351	DATF1	0.31	ACTTGCTCTCAGCTGGGCTCTTC
352	RGC32	0.51	TCCTGTGCCTGCTCCTGTACTTGT
353	ARHGAP15	0.34	CACCCAGCTGGCCTGTGGATGGGA

TABLE 36-continued

<u>Methylprednisolone biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
354	NOTCH1	0.36	TCCTTGCTGCCTGCTCCTGTACTTGT
355	BIN2	0.31	AAATGTTCCCTGTGCCTGCTCCTG
356	SEMA4G	0.35	AAGCCTATACTACGTTCTGTGGAGTAA
357	DPEP2	0.33	CACCCAGCTGGCCTGTGGATGGGA
358	CECR1	0.36	TCCTGTACTTGTCCCTCAGCTGGGC
359	BCL11B	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
360	STAG3	0.41	TTGGACATCTCTAGTGTAGCTGCCA
361	GALNT6	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
362	UBASH3A	0.3	AAATGTTCCCTGTGCCTGCTCCTG
363	PHEMX	0.38	TCCTCCATCACCTGAAACACTGGAC
364	FLJ13373	0.34	TCCTTGCTGCCTGCTCCTGTACTTGT
365	LEF1	0.49	TCCTCCATCACCTGAAACACTGGAC
366	IL21R	0.42	TTGGACATCTCTAGTGTAGCTGCCA
367	MGC17330	0.33	TCCTTGCTGCCTGCTCCTGTACTTGT
368	AKAP13	0.53	TCCTTGCTGCCTGCTCCTGTACTTGT
369	GIMAP5	0.34	AAATGTTCCCTGTGCCTGCTCCTG

TABLE 37

<u>Methotrexate biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
370	PRPF8	0.34	TCCTCCATCACCTGAAACACTGGAC
371	RPL18	0.34	AAGCCTATACTACGTTCTGTGGAGTAA
372	GOT2	0.31	CACCCAGCTGGCCTGTGGATGGGA
373	RPL13A	0.31	TCCTGTACTTGTCCCTCAGCTGGGC
374	RPS15	0.39	CACCCAGCTGGCCTGTGGATGGGA
375	RPLP2	0.32	GCCCCACTGGACAACACTGATTCCCT
376	CSDA	0.39	GCCCCACTGGACAACACTGATTCCCT
377	KHDRBS1	0.32	TCCTCCATCACCTGAAACACTGGAC
378	SNRPA	0.31	TCCTGTACTTGTCCCTCAGCTGGGC
379	IMPDH2	0.39	AAATGTTCCCTGTGCCTGCTCCTG
380	RPS19	0.47	AAATGTTCCCTGTGCCTGCTCCTG
381	NUP88	0.36	CACCCAGCTGGCCTGTGGATGGGA
382	ATP5D	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG

TABLE 37-continued

<u>Methotrexate biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
383	PCBP2	0.32	AAATGTTCCCTTGTGCCTGCTCCTG
384	ZNF593	0.4	AAATGTTCCCTTGTGCCTGCTCCTG
385	HSU79274	0.32	TGGACCCCAGCTGGCTGAGAATCTGG
386	PRIM1	0.3	CACCCAGCTGGCCTGTGGATGGGA
387	PFDN5	0.33	TCCTCCATCACCTGAAACACTGGAC
388	OXA1L	0.37	CACCCAGCTGGCCTGTGGATGGGA
389	ATIC	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
390	CIAPIN1	0.34	ACTTGTCCCTCAGCTGGGCTCTTC
391	RPS2	0.32	CACCCAGCTGGCCTGTGGATGGGA
392	PCCB	0.36	GCCCCACTGGACAACACTGATTCT
393	SHMT2	0.34	ACTTGTCCCTCAGCTGGGCTCTTC
394	RPLP0	0.35	AAGCCTATACTGTTCTGTGGAGTAA
395	HNRPA1	0.35	TGGACCCCAGCTGGCTGAGAATCTGG
396	STOML2	0.32	TGCCTGCTCCTGTACTTGCTCTCAG
397	SKB1	0.33	ACTTGTCCCTCAGCTGGGCTCTTC
398	GLTSCR2	0.37	AAGCCTATACTGTTCTGTGGAGTAA
399	CCNB1IP1	0.3	TCCTTGTGCCCTGCTCTGTACTTGT
400	MRPS2	0.33	TTGGACATCTCTAGTGTAGCTGCCA
401	FLJ20859	0.34	TGCCTGCTCCTGTACTTGCTCTCAG
402	FLJ12270	0.3	ACTTGTCCCTCAGCTGGGCTCTTC

TABLE 38

<u>Bleomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
403	PFN1	0.45	GCCCCACTGGACAACACTGATTCT
404	HK1	0.33	TTGGACATCTCTAGTGTAGCTGCCA
405	MCL1	0.31	TGGACCCCAGCTGGCTGAGAATCTGG
406	ZYX	0.32	TGGACCCCAGCTGGCTGAGAATCTGG
407	RAP1B	0.34	ACTTGTCCCTCAGCTGGGCTCTTC
408	GNB2	0.32	CACCCAGCTGGCCTGTGGATGGGA
409	EPAS1	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
410	PGAM1	0.42	TGCCTGCTCCTGTACTTGCTCTCAG
411	CKAP4	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
412	DUSP1	0.4	AAATGTTCCCTTGTGCCTGCTCCTG

TABLE 38-continued

<u>Bleomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
413	MYL9	0.4	TTGGACATCTCTAGTGTAGCTGCCA
414	K-ALPHA-1	0.37	TTGGACATCTCTAGTGTAGCTGCCA
415	CSDA	0.3	TCCTGTGCCTGCTCTGTACTTGT
416	IFITM2	0.36	TTGGACATCTCTAGTGTAGCTGCCA
417	ITGA5	0.43	GCCCCACTGGACAAACACTGATTCT
418	DPYSL3	0.44	TGGACCCCAGCTGGCTGAGAATCTGG
419	JUNB	0.32	TCCTGTACTTGCTCTCAGCTTGGGC
420	NFKBIA	0.32	TCCTCCATCACCTGAAACACTGGAC
421	LAMB1	0.37	AAATGTTCCCTGTGCCTGCTCCTG
422	FHL1	0.31	TGGACCCCAGCTGGCTGAGAATCTGG
423	INSIG1	0.31	TGGACCCCAGCTGGCTGAGAATCTGG
424	TIMP1	0.48	TGGACCCCAGCTGGCTGAGAATCTGG
425	GJA1	0.54	AAGCCTATACTGTTCTGTGGAGTAA
426	PRG1	0.46	TCCTGTGCCTGCTCTGTACTTGT
427	EXT1	0.35	TCCTTGCTGCCTGCTCTGTACTTGT
428	DKFZP434J154	0.31	GCCCCACTGGACAAACACTGATTCT
429	MVP	0.34	CACCCAGCTGGCCTGTGGATGGGA
430	VASP	0.31	TCCTCCATCACCTGAAACACTGGAC
431	ARL7	0.39	TGGACCCCAGCTGGCTGAGAATCTGG
432	NNMT	0.34	TCCTGTACTTGCTCTCAGCTTGGGC
433	TAP1	0.3	TCCTGTACTTGCTCTCAGCTTGGGC
434	PLOD2	0.37	GCCCCACTGGACAAACACTGATTCT
435	ATF3	0.42	CACCCAGCTGGCCTGTGGATGGGA
436	PALM2-AKAP2	0.33	TGGACCCCAGCTGGCTGAGAATCTGG
437	IL8	0.34	GCCCCACTGGACAAACACTGATTCT
438	LOXL2	0.32	GCCCCACTGGACAAACACTGATTCT
439	IL4R	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
440	DGKA	0.32	GCCCCACTGGACAAACACTGATTCT
441	SEC61G	0.41	CACCCAGCTGGCCTGTGGATGGGA
442	RGS3	0.37	TGGACCCCAGCTGGCTGAGAATCTGG
443	F2R	0.34	CACCCAGCTGGCCTGTGGATGGGA
444	TPM2	0.35	CACCCAGCTGGCCTGTGGATGGGA
445	PSMB9	0.34	CACCCAGCTGGCCTGTGGATGGGA
446	LOX	0.37	TCCTGTACTTGCTCTCAGCTTGGGC
447	STC1	0.35	TCCTCCATCACCTGAAACACTGGAC

TABLE 38-continued

<u>Bleomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
448	PTGER4	0.31	CACCCAGCTGGCCTGTGGATGGGA
449	SMAD3	0.38	TTGGACATCTCTAGTGTAGCTGCCA
450	WNT5A	0.44	TGGACCCCAGTGGCTGAGAACTGG
451	BDNF	0.34	TCCTCCATCACCTGAAACACTGGAC
452	TNFRSF1A	0.46	TCCTCCATCACCTGAAACACTGGAC
453	FLNC	0.34	ACTTGTCCTCAGCTGGGCTCTTC
454	DKFZP564K0822	0.34	TTGGACATCTCTAGTGTAGCTGCCA
455	FLOT1	0.38	TTGGACATCTCTAGTGTAGCTGCCA
456	PTRF	0.39	TGGACCCCAGTGGCTGAGAACTGG
457	HLA-B	0.36	TTGGACATCTCTAGTGTAGCTGCCA
458	MGC4083	0.32	GCCCCACTGGACAACACTGATTCC
459	TNFRSF10B	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
460	PLAGL1	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
461	PNMA2	0.38	GCCCCACTGGACAACACTGATTCC
462	TFPI	0.38	TCCTGTACTTGTCCCTCAGCTGGC
463	GZMB	0.51	TCCTCCATCACCTGAAACACTGGAC
464	PLAUR	0.35	AAGCCTATACTGTTCTGTGGAGTAA
465	FSCN1	0.32	ACTTGTCCTCAGCTGGCTCTTC
466	ERP70	0.32	ACTTGTCCTCAGCTGGCTCTTC
467	AF1Q	0.3	TTGGACATCTCTAGTGTAGCTGCCA
468	HIC	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
469	COL6A1	0.32	AAGCCTATACTGTTCTGTGGAGTAA
470	IFITM3	0.3	GCCCCACTGGACAACACTGATTCC
471	MAP1B	0.38	CACCCAGCTGGCCTGTGGATGGGA
472	FLJ46603	0.37	TCCTCCATCACCTGAAACACTGGAC
473	RAFTLIN	0.34	TGGACCCCAGTGGCTGAGAACTGG
474	RRAS	0.31	TCCTGTACTTGTCCCTCAGCTGGC
475	FTL	0.3	CACCCAGCTGGCCTGTGGATGGGA
476	KIAA0877	0.31	CACCCAGCTGGCCTGTGGATGGGA
477	MT1E	0.31	TCCTGTGCCTGCTCCTGTACTTGT
478	CDC10	0.51	AAATGTTCCCTGTGCCTGCTCCTG
479	DOCK2	0.32	AAGCCTATACTGTTCTGTGGAGTAA
480	RIS1	0.37	ACTTGTCCTCAGCTGGCTCTTC
481	BCAT1	0.42	TTGGACATCTCTAGTGTAGCTGCCA
482	PRF1	0.34	TCCTCCATCACCTGAAACACTGGAC

TABLE 38-continued

<u>Bleomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
483	DBN1	0.36	GCCCCACTGGACAACACTGATTCC
484	MT1K	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
485	TMSB10	0.42	GCCCCACTGGACAACACTGATTCC
486	FLJ10350	0.4	AAATGTTCCCTGTGCCTGCTCCTG
487	Clorf24	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
488	NME7	0.46	TCCTGTACTTGTCCCTCAGCTGGG
489	TMEM22	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
490	TPK1	0.37	TCCTCCATCACCTGAAACACTGGAC
491	ELK3	0.38	TGCCTGCTCCTGTACTTGTCCCTCAG
492	CYLD	0.4	TCCTGTGCCTGCTCCTGTACTTGT
493	ADAMTS1	0.31	AAGCCTATACTGTTCTGTGGAGTAA
494	EHD2	0.41	TCCTCCATCACCTGAAACACTGGAC
495	ACTB	0.33	TCCTGTGCCTGCTCCTGTACTTGT

TABLE 39

<u>Methyl-GAG (methyl glyoxal bis amidinohydrazone dihydrochloride) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
496	SSRP1	0.37	TGCCTGCTCCTGTACTTGTCCCTCAG
497	CTSC	0.35	CACCCAGCTGGCCTGTGGATGGGA
498	LBR	0.38	ACTTGTCCCTCAGCTGGGCTCTTC
499	EFNB2	0.31	AAATGTTCCCTGTGCCTGCTCCTG
500	SERPINAI	0.34	TCCTTGTCCTGCTCCTGTACTTGT
501	SSSCA1	0.32	TCCTGTACTTGTCCCTCAGCTGGG
502	EZH2	0.36	TTGGACATCTCTAGTGTAGCTGCCA
503	MYB	0.33	GCCCCACTGGACAACACTGATTCC
504	PRIM1	0.39	TCCTCCATCACCTGAAACACTGGAC
505	H2AFX	0.33	TCCTTGTCCTGCTCCTGTACTTGT
506	HMGA1	0.35	TTGGACATCTCTAGTGTAGCTGCCA
507	HMMR	0.33	TCCTTGTCCTGCTCCTGTACTTGT
508	TK2	0.42	CACCCAGCTGGCCTGTGGATGGGA
509	WHSC1	0.35	AAATGTTCCCTGTGCCTGCTCCTG
510	DIAPH1	0.34	GCCCCACTGGACAACACTGATTCC
511	LAMB3	0.31	GCCCCACTGGACAACACTGATTCC

TABLE 39-continued

<u>Methyl-GAG (methyl glyoxal bis amidinohydrazone dihydrochloride) biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Median	probe
512	DPAGT1	0.42	TGCCTGCTCCTGTACTTGTCCTCAG	
513	UCK2	0.31	GCCCCACTGGACAAACACTGATTCCCT	
514	SERPINB1	0.31	TCCCTGTGCCTGCTCCTGTACTTGT	
515	MDN1	0.35	TGCCTGCTCCTGTACTTGTCCTCAG	
516	GOS2	0.43	CACCCAGCTGGTCCTGTGGATGGGA	
517	MGC21654	0.36	TGGACCCCCTGGCTGAGAATCTGG	
518	GTSE1	0.35	ACTTGTCCTCAGCTGGGCTTCCTC	
519	TACC3	0.31	TCCTCCATCACCTGAAACACTGGAC	
520	PLAC8	0.31	CACCCAGCTGGTCCTGTGGATGGGA	
521	HNRPD	0.35	TTGGACATCTCTAGTGTAGCTGCCA	
522	PNAS-4	0.3	TTGGACATCTCTAGTGTAGCTGCCA	

TABLE 40

HDAC inhibitors biomarkers.

SEQ ID NO	Gene	Corre- lation	Median	probe
523	FAU	0.33	TTGGACATCTCTAGTGTAGCTGCCA	
524	NOL5A	0.33	TGGACCCCCTGGCTGAGAATCTGG	
525	ANP32A	0.32	CACCCAGCTGGTCCTGTGGATGGGA	
526	ARHGDIB	0.3	ACTTGTCCTCAGCTGGGCTTCCTC	
527	LBR	0.31	ACTTGTCCTCAGCTGGGCTTCCTC	
528	FABP5	0.33	TCCTCCATCACCTGAAACACTGGAC	
529	ITM2A	0.32	TTGGACATCTCTAGTGTAGCTGCCA	
530	SFRS5	0.34	TCCTCCATCACCTGAAACACTGGAC	
531	IQGAP2	0.4	CACCCAGCTGGCCTGTGGATGGGA	
532	SLC7A6	0.35	AAGCCTATACGTTCTGTGGAGTAA	
533	SLA	0.31	TGCCTGCTCCTGTACTTGTCCTCAG	
534	IL2RG	0.31	TCCTCCATCACCTGAAACACTGGAC	
535	MFNG	0.39	TCCTGTACTTGTCCTCAGCTGGGC	
536	GPSM3	0.32	TTGGACATCTCTAGTGTAGCTGCCA	
537	PIM2	0.3	TTGGACATCTCTAGTGTAGCTGCCA	
538	EVER1	0.35	GCCCCACTGGACAAACACTGATTCCCT	
539	LRMP	0.35	TGCCTGCTCCTGTACTTGTCCTCAG	
540	ICAM2	0.44	TCCTGTACTTGTCCTCAGCTGGGC	

TABLE 40-continued

<u>HDAC inhibitors biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Median	probe
541	RIMS3	0.43	TGGACCCCCTGGCTGAGAATCTGG	
542	FMNL1	0.35	TTGGACATCTCTAGTGTAGCTGCCA	
543	MYB	0.37	TGCCTGCTCCTGTACTTGTCCTCAG	
544	PTPN7	0.36	TCCTGTGCCTGCTCCTGTACTTGT	
545	LCK	0.48	CACCCAGCTGGCCTGTGGATGGGA	
546	CXorf9	0.3	ACTTGTCCTCAGCTGGGCTTCCTC	
547	RHOH	0.31	TCCTGTGCCTGCTCCTGTACTTGT	
548	ZNFN1A1	0.33	AAATTTCCCTGTGCCTGCTCCTG	
549	CENTB1	0.45	CACCCAGCTGGCCTGTGGATGGGA	
550	LCP2	0.31	TGCCTGCTCCTGTACTTGTCCTCAG	
551	DBT	0.32	TCCTGTACTTGTCCTCAGCTGGG	
552	CEP1	0.31	TTGGACATCTCTAGTGTAGCTGCCA	
553	IL6R	0.31	TGGACCCCCTGGCTGAGAATCTGG	
554	VAV1	0.32	TCCTGTGCCTGCTCCTGTACTTGT	
555	MAP4K1	0.3	AAGCCTATACGTTCTGTGGAGTAA	
556	CD28	0.36	TCCTGTGCCTGCTCCTGTACTTGT	
557	PTP4A3	0.3	TTGGACATCTCTAGTGTAGCTGCCA	
558	CD3G	0.33	CACCCAGCTGGCCTGTGGATGGGA	
559	LTB	0.4	TCCTGTACTTGTCCTCAGCTGGG	
560	USP34	0.44	GCCCCACTGGACAAACACTGATTCC	
561	NVL	0.41	TCCTGTGCCTGCTCCTGTACTTGT	
562	CD8B1	0.33	ACTTGTCCTCAGCTGGGCTTCCTC	
563	SFRS6	0.31	GCCCCACTGGACAAACACTGATTCC	
564	LCP1	0.34	TCCTGTACTTGTCCTCAGCTGGG	
565	CXCR4	0.36	TGCCTGCTCCTGTACTTGTCCTCAG	
566	PSCDBP	0.33	TGGACCCCCTGGCTGAGAATCTGG	
567	SELPLG	0.33	TTGGACATCTCTAGTGTAGCTGCCA	
568	CD3Z	0.3	TCCTGTGCCTGCTCCTGTACTTGT	
569	PRKCQ	0.33	TTGGACATCTCTAGTGTAGCTGCCA	
570	CD1A	0.34	GCCCCACTGGACAAACACTGATTCC	
571	GATA2	0.31	TTGGACATCTCTAGTGTAGCTGCCA	
572	P2RX5	0.32	TGCCTGCTCCTGTACTTGTCCTCAG	
573	LAIR1	0.35	TGGACCCCCTGGCTGAGAATCTGG	
574	C1orf38	0.4	GCCCCACTGGACAAACACTGATTCC	
575	SH2D1A	0.44	TCCTGTGCCTGCTCCTGTACTTGT	

TABLE 40-continued

<u>HDAC inhibitors biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
576	TRB@	0.33	CACCCAGCTGGTCCTGTGGATGGGA
577	SEPT6	0.34	GCCCCACTGGACAACACTGATTCT
578	HA-1	0.32	AAGCCTATACTGTTCTGTGGAGTAA
579	DOCK2	0.3	TCC TTGTGCCTGCTCCTGTACTTGT
580	WBSCR20C	0.31	TGCCTGCTCCTGTACTTGTCCAG
581	CD3D	0.3	ACTTGTCCTCAGCTGGGCTCTTC
582	RNASE6	0.31	GCCCCACTGGACAACACTGATTCT
583	SFRS7	0.32	AAATGTTCCCTTGTGCCTGCTCCTG
584	WBSCR20A	0.3	AAGCCTATACTGTTCTGTGGAGTAA
585	NUP210	0.31	TTGGACATCTCTAGTGTAGCTGCCA
586	CD6	0.34	TCC TTGTGCCTGCTCCTGTACTTGT
587	HNRPA1	0.3	GCCCCACTGGACAACACTGATTCT
588	AIF1	0.34	AAGCCTATACTGTTCTGTGGAGTAA
589	CYFIP2	0.38	TGGACCCC ACTGGCTGAGAATCTGG
590	GLTSCR2	0.38	TCC TTGTGCCTGCTCCTGTACTTGT
591	C11orf2	0.31	AAGCCTATACTGTTCTGTGGAGTAA
592	ARHGAP15	0.33	TGGACCCC ACTGGCTGAGAATCTGG
593	BIN2	0.35	TTGGACATCTCTAGTGTAGCTGCCA
594	SH3TC1	0.35	ACTTGTCCTCAGCTGGGCTCTTC
595	STAG3	0.32	AAATGTTCCCTTGTGCCTGCTCCTG
596	TM6SF1	0.34	ACTTGTCCTCAGCTGGGCTCTTC
597	C15orf25	0.33	TCCTCCATCACCTGAAACACTGGAC
598	FLJ22457	0.36	AAATGTTCCCTTGTGCCTGCTCCTG
599	PACAP	0.34	TGCCTGCTCCTGTACTTGTCCAG
600	MGC2744	0.31	GCCCCACTGGACAACACTGATTCT

TABLE 41

5-Fluorouracil biomarkers.

SEQ ID NO	Gene	Corre- lation	Medianprobe
601	RPL18	0.38	AAATGTTCCCTTGTGCCTGCTCCTG
602	RPL10A	0.39	TGGACCCC ACTGGCTGAGAATCTGG
603	ANAPC5	0.37	ACTTGTCCTCAGCTGGGCTCTTC
604	EEF1B2	0.3	TCCTGTACTTGTCCCTCAGCTGGGC

TABLE 41-continued

<u>5-Fluorouracil biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
605	RPL13A	0.5	TGCCTGCTCCTGTACTTGTCCCTCAG
606	RPS15	0.4	ACTTGTCCTCAGCTGGCTTCTTC
607	NDUFAB1	0.38	GCCCCACTGGACAACACTGATTCT
608	APRT	0.32	AAATGTTCCCTGTGCCTGCTCCTG
609	ZNF593	0.34	TCCTCCATCACCTGAAACACTGGAC
610	MRP63	0.32	AAATGTTCCCTGTGCCTGCTCCTG
611	IL6R	0.41	TGGACCCC ACTGGCTGAGAATCTGG
612	SART3	0.37	TCCTCCATCACCTGAAACACTGGAC
613	UCK2	0.32	GCCCCACTGGACAACACTGATTCT
614	RPL17	0.31	AAGCCTATACTGTTCTGTGGAGTAA
615	RPS2	0.35	CACCCAGCTGGCCTGTGGATGGGA
616	PCCB	0.38	TCCTGTGCCTGCTCCTGTACTTGT
617	TOMM20	0.32	TGGACCCC ACTGGCTGAGAATCTGG
618	SHMT2	0.32	TTGGACATCTCTAGTGTAGCTGCCA
619	RPLP0	0.31	TCCTGTGCCTGCTCCTGTACTTGT
620	GTF3A	0.32	CACCCAGCTGGCCTGTGGATGGGA
621	STOML2	0.33	TGGACCCC ACTGGCTGAGAATCTGG
622	DKFZp564J157	0.4	AAATGTTCCCTGTGCCTGCTCCTG
623	MRPS2	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
624	ALG5	0.3	TTGGACATCTCTAGTGTAGCTGCCA
625	CALML4	0.33	CACCCAGCTGGCCTGTGGATGGGA

TABLE 42

<u>Radiation sensitivity biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
626	TRA1	0.36	TGGACCCC ACTGGCTGAGAATCTGG
627	ACTN4	0.36	ACTTGTCCTCAGCTGGCTTCTTC
628	CALM1	0.32	TCCTCCATCACCTGAAACACTGGAC
629	CD63	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
630	FKBP1A	0.38	TGGACCCC ACTGGCTGAGAATCTGG
631	CALU	0.47	ACTTGTCCTCAGCTGGCTTCTTC
632	IQGAP1	0.37	TTGGACATCTCTAGTGTAGCTGCCA
633	MGC8721	0.35	AAATGTTCCCTGTGCCTGCTCCTG
634	STAT1	0.37	TGGACCCC ACTGGCTGAGAATCTGG

TABLE 42-continued

Radiation sensitivity biomarkers.			
SEQ ID NO	Gene	Corre-lation Medianprobe	
635	TACC1	0.41 ACTTGTCCCTCAGCTTGGGCTTC	
636	TM4SF8	0.33 AAGCCTATACTGTTCTGTGGAGTAA	
637	CD59	0.31 TCCTCCATCACCTGAAACACTGGAC	
638	CKAP4	0.45 TCCTGTGCCTGCTCCCTGTACTTGT	
639	DUSP1	0.38 TCCTGTACTTGTCCCTCAGCTGGC	
640	RCN1	0.31 TGCCCTGCTCCTGTACTTGTCCCTCAG	
641	MGC8902	0.35 TGCCCTGCTCCTGTACTTGTCCCTCAG	
642	RRBP1	0.31 ACTTGTCCCTCAGCTTGGGCTTC	
643	PRNP	0.42 TTGGACATCTCTAGTGTAGCTGCCA	
644	IER3	0.34 GCCCCACTGGACAACACTGATTCC	
645	MARCKS	0.43 GCCCCACTGGACAACACTGATTCC	
646	FER1L3	0.47 TGCCCTGCTCCTGTACTTGTCCCTCAG	
647	SLC20A1	0.41 ACTTGTCCCTCAGCTTGGGCTTC	
648	HEXB	0.46 AAATGTTTCCCTGTGCCTGCTCTG	
649	EXT1	0.47 CACCCAGCTGGCCTGTGGATGGGA	
650	TJP1	0.32 AAATGTTTCCCTGTGCCTGCTCTG	
651	CTSL	0.38 TCCTGTACTTGTCCCTCAGCTGGC	
652	SLC39A6	0.36 TCCTGTACTTGTCCCTCAGCTGGC	
653	RIOK3	0.38 TCCTCCATCACCTGAAACACTGGAC	
654	CRK	0.37 TGCCCTGCTCCTGTACTTGTCCCTCAG	
655	NNMT	0.37 TGCCCTGCTCCTGTACTTGTCCCTCAG	
656	TRAM2	0.35 TTGGACATCTCTAGTGTAGCTGCCA	
657	ADAM9	0.52 TCCTGTACTTGTCCCTCAGCTGGC	
658	PLSCR1	0.35 TGGACCCCCTGGCTGAGAACACTGG	
659	PRSS23	0.3 TGCCCTGCTCCTGTACTTGTCCCTCAG	
660	PLOD2	0.36 TGCCCTGCTCCTGTACTTGTCCCTCAG	
661	NPC1	0.39 TGCCCTGCTCCTGTACTTGTCCCTCAG	
662	TOB1	0.37 CACCCAGCTGGCCTGTGGATGGGA	
663	GFPT1	0.47 CACCCAGCTGGCCTGTGGATGGGA	
664	IL8	0.36 AAATGTTTCCCTGTGCCTGCTCTG	
665	PYGL	0.46 TCCTCCATCACCTGAAACACTGGAC	
666	LOXL2	0.49 TTGGACATCTCTAGTGTAGCTGCCA	
667	KIAA0355	0.36 TCCTTGCCCTGCTCCTGTACTTGT	
668	UGDH	0.49 TTGGACATCTCTAGTGTAGCTGCCA	
669	PURA	0.32 TGCCCTGCTCCTGTACTTGTCCCTCAG	

TABLE 42-continued

Radiation sensitivity biomarkers.			
SEQ ID NO	Gene	Corre-lation Medianprobe	
670	ULK2	0.37 AAGCCTATACTGTTCTGTGGAGTAA	
671	CENTG2	0.35 GCCCCACTGGACAACACTGATTCC	
672	CAP350	0.31 GCCCCACTGGACAACACTGATTCC	
673	CXCL1	0.36 TCCTGTACTTGTCCCTCAGCTTGGC	
674	BTN3A3	0.35 AAGCCTATACTGTTCTGTGGAGTAA	
675	WNT5A	0.3 AAGCCTATACTGTTCTGTGGAGTAA	
676	FOXF2	0.44 AAATGTTTCCCTGTGCCTGCTCCTG	
677	LPHN2	0.34 GCCCCACTGGACAACACTGATTCC	
678	CDH11	0.39 TGGACCCCCTGGCTGAGAACACTGG	
679	P4HA1	0.33 TCCTCCATCACCTGAAACACTGGAC	
680	GRP58	0.44 CACCCAGCTGGCCTGTGGATGGGA	
681	DSIPI	0.44 TGGACCCCCTGGCTGAGAACACTGG	
682	MAP1LC3B	0.5 AAGCCTATACTGTTCTGTGGAGTAA	
683	GALIG	0.36 AAATGTTTCCCTGTGCCTGCTCCTG	
684	IGSF4	0.4 TCCTCCATCACCTGAAACACTGGAC	
685	IRS2	0.35 TGGACCCCCTGGCTGAGAACACTGG	
686	ATP2A2	0.35 CACCCAGCTGGCCTGTGGATGGGA	
687	OGT	0.3 TCCTGTACTTGTCCCTCAGCTTGGC	
688	TNFRSF10B	0.31 AAGCCTATACTGTTCTGTGGAGTAA	
689	KIAA1128	0.35 CACCCAGCTGGCCTGTGGATGGGA	
690	TM4SF1	0.35 CACCCAGCTGGCCTGTGGATGGGA	
691	RIPK2	0.42 TGCCCTGCTCCTGTACTTGTCCCTCAG	
692	NR1D2	0.47 TTGGACATCTCTAGTGTAGCTGCCA	
693	SSA2	0.36 TTGGACATCTCTAGTGTAGCTGCCA	
694	NQO1	0.4 AAGCCTATACTGTTCTGTGGAGTAA	
695	ASPH	0.36 TGCCCTGCTCCTGTACTTGTCCCTCAG	
696	ASAHI	0.33 ACTTGTCCCTCAGCTGGCTTCTTC	
697	MGLL	0.35 TGGACCCCCTGGCTGAGAACACTGG	
698	SERPINB6	0.51 AAGCCTATACTGTTCTGTGGAGTAA	
699	HSPA5	0.33 TCCTTGCCCTGCTCCTGTACTTGT	
700	ZFP36L1	0.39 TCCTTGCCCTGCTCCTGTACTTGT	
701	COL4A1	0.3 ACTTGTCCCTCAGCTGGCTTCTTC	
702	NIPA2	0.36 ACTTGTCCCTCAGCTGGCTTCTTC	
703	FKBP9	0.48 AAATGTTTCCCTGTGCCTGCTCCTG	
704	IL6ST	0.4 GCCCCACTGGACAACACTGATTCC	

TABLE 42-continued

<u>Radiation sensitivity biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
705	DKFZP564G2022	0.39	TTGGACATCTCTAGTGTAGCTGCCA
706	PPAP2B	0.33	TGGACCCACTGGCTGAGAACATCTGG
707	MAP1B	0.3	CACCCAGCTGGCCTGTGGATGGGA
708	MAPK1	0.3	TGGACCCACTGGCTGAGAACATCTGG
709	MYO1B	0.38	ACTTGTCCCTCAGCTTGGGCTTC
710	CAST	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
711	RRAS2	0.52	AAATGTTCCCTGTGCCTGCTCCTG
712	QKI	0.31	ACTTGTCCCTCAGCTTGGGCTTC
713	LHFPL2	0.36	TCCTGTGCCCTGCTCCTGTACTTGT
714	SEPT10	0.38	GCCCCACTGGACAAACACTGATTCT
715	ARHE	0.5	AAGCCTATACTGTTCTGTGGAGTAA
716	KIAA1078	0.34	AAGCCTATACTGTTCTGTGGAGTAA
717	FTL	0.38	TCCTGTACTTGTCCCTCAGCTGGC
718	KIAA0877	0.41	AAATGTTCCCTGTGCCTGCTCCTG
719	PLCB1	0.3	AAGCCTATACTGTTCTGTGGAGTAA
720	KIAA0802	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
721	RAB3GAP	0.43	TGCCTGCTCCTGTACTTGTCCCTCAG
722	SERPINB1	0.46	TGCCTGCTCCTGTACTTGTCCCTCAG
723	TIMM17A	0.38	AAATGTTCCCTGTGCCTGCTCCTG
724	SOD2	0.35	TTGGACATCTCTAGTGTAGCTGCCA
725	HLA-A	0.33	TTGGACATCTCTAGTGTAGCTGCCA
726	NOMO2	0.43	CACCCAGCTGGCCTGTGGATGGGA
727	LOC55831	0.32	TCCTGTACTTGTCCCTCAGCTGGC
728	PHLDA1	0.32	CACCCAGCTGGCCTGTGGATGGGA
729	TMEM2	0.47	TGGACCCACTGGCTGAGAACATCTGG
730	MLPH	0.35	ACTTGTCCCTCAGCTTGGGCTTC
731	FAD104	0.34	ACTTGTCCCTCAGCTTGGGCTTC
732	LRRC5	0.42	CACCCAGCTGGCCTGTGGATGGGA
733	RAB7L1	0.41	TTGGACATCTCTAGTGTAGCTGCCA
734	FLJ35036	0.36	TCCTGTACTTGTCCCTCAGCTGGC
735	DOCK10	0.41	TCCTCCATCACCTGAAACACTGGAC
736	LRP12	0.36	AAGCCTATACTGTTCTGTGGAGTAA
737	TXNDC5	0.4	ACTTGTCCCTCAGCTTGGGCTTC
738	CDC14B	0.39	TGCCTGCTCCTGTACTTGTCCCTCAG
739	HRMT1L1	0.38	CACCCAGCTGGCCTGTGGATGGGA

TABLE 42-continued

<u>Radiation sensitivity biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
740	DNAJC10	0.31	TTGGACATCTCTAGTGTAGCTGCCA
741	TNPO1	0.33	GCCCCACTGGACAAACACTGATTCT
742	LONP	0.32	AAATGTTCCCTGTGCCTGCTCCTG
743	AMIGO2	0.38	AAGCCTATACTGTTCTGTGGAGTAA
744	DNAPTP6	0.31	TGCCTGCTCCTGTACTTGTCCCTAG
745	ADAMTS1	0.37	TTGGACATCTCTAGTGTAGCTGCCA

TABLE 43

<u>Rituximab (e.g., Mabthera) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
746	PSMB2	0.89	TCCTCCATCACCTGAAACACTGGAC
747	BAT1	0.88	AAGCCTATACTGTTCTGTGGAGTAA
748	ASCC3L1	0.89	TCCTGTGCCCTGCTCCTGTACTTGT
749	SET	0.94	AAATGTTCCCTGTGCCTGCTCCTG
750	YWHAZ	0.83	TCCTGTGCCCTGCTCCTGTACTTGT
751	GLUL	0.8	TGGACCCACTGGCTGAGAACATCTGG
752	LDHA	0.8	TCCTGTGCCCTGCTCCTGTACTTGT
753	HMGB1	0.84	AAATGTTCCCTGTGCCTGCTCCTG
754	SFRS2	0.87	AAATGTTCCCTGTGCCTGCTCCTG
755	DPYSL2	0.82	TCCTGTACTTGTCCCTCAGCTTGGC
756	MGC8721	0.82	CACCCAGCTGGCCTGTGGATGGGA
757	NOL5A	0.86	TGCCTGCTCCTGTACTTGTCCCTCAG
758	SFRS10	0.88	AAATGTTCCCTGTGCCTGCTCCTG
759	SF3B1	0.82	TCCTGTACTTGTCCCTCAGCTTGGC
760	K-ALPHA-1	0.86	TGCCTGCTCCTGTACTTGTCCCTCAG
761	TXNRD1	0.86	TGGACCCACTGGCTGAGAACATCTGG
762	ARHGDI	0.83	CACCCAGCTGGCCTGTGGATGGGA
763	ZFP36L2	0.92	TTGGACATCTCTAGTGTAGCTGCCA
764	DHX15	0.81	TGGACCCACTGGCTGAGAACATCTGG
765	SOX4	0.85	CACCCAGCTGGCCTGTGGATGGGA
766	GRSF1	0.81	TGGACCCACTGGCTGAGAACATCTGG
767	MCM3	0.85	GCCCCACTGGACAAACACTGATTCT
768	IFITM1	0.82	TCCTCCATCACCTGAAACACTGGAC

TABLE 43-continued

Rituximab (e.g., Mabthera) biomarkers.		
SEQ ID NO	Gene	Corre-lation Medianprobe
769	RPA2	0.86 TCCTCCATCACCTGAAACACTGGAC
770	LBR	0.87 ACTTGTCTCAGCTGGGCTCTTC
771	CKS1B	0.85 AAGCCTATACTGTTCTGTGGAGTAA
772	NASP	0.82 TGGACCCCCTGGCTGAGAACACTGG
773	HNRPDL	0.81 TCCTCCATCACCTGAAACACTGGAC
774	CUGBP2	0.81 TGCTGCTCCTGTACTTGCTCTCAG
775	PTBP1	0.87 TCCTGTGCCTGCTCCTGTACTTGT
776	ARL7	0.83 TTGGACATCTCTAGTGTAGCTGCCA
777	CTCF	0.83 ACTTGTCTCAGCTGGGCTCTTC
778	HMGCR	0.86 TCCTGTGCCTGCTCCTGTACTTGT
779	ITM2A	0.88 AAATGTTCCCTGTGCCTGCTCCTG
780	SFRS3	0.93 TCCTGTGCCTGCTCCTGTACTTGT
781	SRPK2	0.82 TCCTGTGCCTGCTCCTGTACTTGT
782	JARID2	0.92 CACCCAGCTGGCCTGTGGATGGGA
783	M96	0.84 TCCTGTACTTGTCCTCAGCTGGGC
784	MAD2L1	0.87 TCCTCCATCACCTGAAACACTGGAC
785	SATB1	0.81 ACTTGTCTCAGCTGGGCTCTTC
786	TMPO	0.9 ACTTGTCTCAGCTGGGCTCTTC
787	SIVA	0.84 ACTTGTCTCAGCTGGGCTCTTC
788	SEMA4D	0.9 TCCTCCATCACCTGAAACACTGGAC
789	TFDP2	0.87 TCCTGTGCCTGCTCCTGTACTTGT
790	SKP2	0.86 AAGCCTATACTGTTCTGTGGAGTAA
791	SH3YL1	0.88 GCCCCACTGGACAACACTGATTCC
792	RFC4	0.87 TCCTCCATCACCTGAAACACTGGAC
793	PCBP2	0.83 AAGCCTATACTGTTCTGTGGAGTAA
794	IL2RG	0.84 GCCCCACTGGACAACACTGATTCC
795	CDC45L	0.89 TCCTGTACTTGTCCTCAGCTGGGC
796	GTSE1	0.83 TTGGACATCTCTAGTGTAGCTGCCA
797	KIF11	0.85 AAGCCTATACTGTTCTGTGGAGTAA
798	FEN1	0.88 TTGGACATCTCTAGTGTAGCTGCCA
799	MYB	0.9 TGGACCCCCTGGCTGAGAACACTGG
800	LCK	0.87 TCCTCCATCACCTGAAACACTGGAC
801	CENPA	0.84 GCCCCACTGGACAACACTGATTCC
802	CCNE2	0.84 GCCCCACTGGACAACACTGATTCC
803	H2AFX	0.88 TTGGACATCTCTAGTGTAGCTGCCA

TABLE 43-continued

Rituximab (e.g., Mabthera) biomarkers.		
SEQ ID NO	Gene	Corre-lation Medianprobe
804	SNRPG	0.84 TCCTCCATCACCTGAAACACTGGAC
805	CD3G	0.94 TCCTGTGCCTGCTCCTGTACTTGT
806	STK6	0.9 ACTTGTCTCAGCTGGGTTCTC
807	PTP4A2	0.81 TGCTGCTCCTGTACTTGCTCAG
808	FDFT1	0.91 AAATGTTCCCTGTGCCTGCTCCTG
809	HSPA8	0.84 AAATGTTCCCTGTGCCTGCTCCTG
810	HNPR	0.94 TCCTGTGCCTGCTCCTGTACTTGT
811	MCM7	0.92 AAATGTTCCCTGTGCCTGCTCCTG
812	SFRS6	0.85 TGGACCCCCTGGCTGAGAACACTGG
813	PAK2	0.8 CACCCAGCTGGCCTGTGGATGGGA
814	LCP1	0.85 TCCTGTACTTGTCCTCAGCTGGGC
815	STAT3	0.81 ACTTGTCTCAGCTGGGCTCTTC
816	OK/SW-cl.56	0.8 TCCTGTGCCTGCTCCTGTACTTGT
817	WHSC1	0.81 TGGACCCCCTGGCTGAGAACACTGG
818	DIAPH1	0.88 AAGCCTATACTGTTCTGTGGAGTAA
819	KIF2C	0.88 TCCTGTACTTGTCCTCAGCTGGGC
820	HDGFRP3	0.89 CACCCAGCTGGCCTGTGGATGGGA
821	PNMA2	0.93 TTGGACATCTCTAGTGTAGCTGCCA
822	GATA3	0.93 TCCTGTACTTGTCCTCAGCTGGGC
823	BUB1	0.88 AAATGTTCCCTGTGCCTGCTCCTG
824	TPX2	0.8 CACCCAGCTGGCCTGTGGATGGGA
825	SH2D1A	0.86 TCCTGTGCCTGCTCCTGTACTTGT
826	TNFAIP8	0.9 TCCTCCATCACCTGAAACACTGGAC
827	CSE1L	0.83 AAATGTTCCCTGTGCCTGCTCCTG
828	MCAM	0.8 TCCTGTACTTGTCCTCAGCTGGGC
829	AF1Q	0.83 GCCCCACTGGACAACACTGATTCC
830	CD47	0.86 CACCCAGCTGGCCTGTGGATGGGA
831	SFRS1	0.85 AAGCCTATACTGTTCTGTGGAGTAA
832	FYB	0.92 TCCTGTACTTGTCCTCAGCTGGGC
833	TRB@	0.84 ACTTGTCTCAGCTGGGCTCTTC
834	CXCR4	0.94 GCCCCACTGGACAACACTGATTCC
835	H3F3B	0.84 TCCTCCATCACCTGAAACACTGGAC
836	MKI67	0.83 ACTTGTCTCAGCTGGGCTCTTC
837	MAC30	0.82 TCCTGTGCCTGCTCCTGTACTTGT
838	ARID5B	0.88 AAGCCTATACTGTTCTGTGGAGTAA

TABLE 43-continued

<u>Rituximab (e.g., Mabthera) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
839	LOC339287	0.81	AAGCCTATAACGTTCTGTGGAGTAA
840	CD3D	0.82	TCCTTGCGCTCGCTCTGTACTTGT
841	ZAP70	0.87	AAGCCTATAACGTTCTGTGGAGTAA
842	LAPTM4B	0.83	TCCTCCATCACCTGAACACTGGAC
843	SFRS7	0.87	TCCTTGCGCTGCCTGTACTTGT
844	HNRPA1	0.9	AAGCCTATAACGTTCTGTGGAGTAA
845	HSPCA	0.88	AAGCCTATAACGTTCTGTGGAGTAA
846	AIF1	0.82	TCCTTGCGCTGCCTGTACTTGT
847	GTF3A	0.87	AAGCCTATAACGTTCTGTGGAGTAA
848	MCM5	0.91	TTGGACATCTCTAGTGTAGCTGCCA
849	GTL3	0.85	AAGCCTATAACGTTCTGTGGAGTAA
850	ZNF22	0.89	TGCCCTGCTCCTGTACTTGTCCAG
851	FLJ22794	0.83	GCCCCACTGGACAACACTGATTCC
852	LZTFL1	0.89	ACTTGTCCTCAGCTGGGCTCTTC
853	e(y)2	0.87	TCCTCCATCACCTGAACACTGGAC
854	FLJ20152	0.92	TCCTCCATCACCTGAACACTGGAC
855	C10orf3	0.86	ACTTGTCCTCAGCTGGGCTCTTC
856	NRN1	0.86	AAATGTTCCCTGTGCCTGCTCCTG
857	FLJ10858	0.81	GCCCCACTGGACAACACTGATTCC
858	BCL11B	0.89	GCCCCACTGGACAACACTGATTCC
859	ASPM	0.91	AAGCCTATAACGTTCTGTGGAGTAA
860	LEF1	0.9	TTGGACATCTCTAGTGTAGCTGCCA
861	LOC146909	0.83	ACTTGTCCTCAGCTGGGCTCTTC

TABLE 44

<u>5-Aza-2'-deoxycytidine (decitabine) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
862	CD99	0.31	TTGGACATCTCTAGTGTAGCTGCCA
863	SNRPA	0.32	TCCTGTACTTGTCCCTCAGCTGGC
864	CUGBP2	0.32	TCCTGTACTTGTCCCTCAGCTGGC
865	STAT5A	0.32	GCCCCACTGGACAACACTGATTCC
866	SLA	0.38	TTGGACATCTCTAGTGTAGCTGCCA
867	IL2RG	0.33	TGGACCCACTGGCTGAGAACACTGG
868	GTSE1	0.32	ACTTGTCCTCAGCTGGGCTCTTC

TABLE 44-continued

<u>5-Aza-2'-deoxycytidine (decitabine) biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
869	MYB	0.36	TGGACCCCCTGGCTGAGAACACTGG
870	PTPN7	0.33	TCCTGTACTTGTCCCTCAGCTGGC
871	CXorf9	0.42	TCCTGTACTTGTCCCTCAGCTGGC
872	RHOH	0.38	AAATGTTCCCTGTGCCTGCTCCTG
873	ZNFN1A1	0.33	AAGCCTATAACGTTCTGTGGAGTAA
874	CENTB1	0.35	CACCCAGCTGGCCTGTGGATGGGA
875	LCP2	0.3	AAATGTTCCCTGTGCCTGCTCCTG
876	HIST1H4C	0.33	TGGACCCCCTGGCTGAGAACACTGG
877	CCR7	0.37	TGCCTGCTCCTGTACTTGTCCCTG
878	APOBEC3B	0.31	TCCTGTGCCTGCTCCTGTACTTGT
879	MCM7	0.31	TGGACCCCCTGGCTGAGAACACTGG
880	LCP1	0.31	AAGCCTATAACGTTCTGTGGAGTAA
881	SELPLG	0.4	TGGACCCCCTGGCTGAGAACACTGG
882	CD3Z	0.35	TCCTGTACTTGTCCCTCAGCTGGC
883	PRKCQ	0.39	TGCCTGCTCCTGTACTTGTCCCTG
884	GZMB	0.32	GCCCCACTGGACAACACTGATTCC
885	SCN3A	0.4	AAGCCTATAACGTTCTGTGGAGTAA
886	LAIR1	0.35	TGCCTGCTCCTGTACTTGTCCCTG
887	SH2D1A	0.35	GCCCCACTGGACAACACTGATTCC
888	SEPT6	0.35	ACTTGTCCTCAGCTGGGCTCTTC
889	CG018	0.32	ACTTGTCCTCAGCTGGGCTCTTC
890	CD3D	0.31	TGGACCCCCTGGCTGAGAACACTGG
891	C18orf10	0.33	TCCTGTGCCTGCTCCTGTACTTGT
892	PRF1	0.31	TCCTCCATCACCTGAACACTGGAC
893	AIF1	0.31	TTGGACATCTCTAGTGTAGCTGCCA
894	MCM5	0.31	ACTTGTCCTCAGCTGGGCTCTTC
895	LPXN	0.35	TCCTCCATCACCTGAACACTGGAC
896	C22orf18	0.33	AAATGTTCCCTGTGCCTGCTCCTG
897	ARHGAP15	0.31	AAATGTTCCCTGTGCCTGCTCCTG
898	LEF1	0.43	GCCCCACTGGACAACACTGATTCC

TABLE 45

<u>Idarubicin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
899	SLC9A3R1	0.31	TGGACCCCCTGGCTGAGAACATCTGG
900	RPS19	0.32	TGGACCCCCTGGCTGAGAACATCTGG
901	ITM2A	0.34	TCCTGTACTTGTCCCTCAGCTGGGC
902	SSBP2	0.31	AAGCCTATACTGTTCTGTGGAGTAA
903	CXorf9	0.31	TCCTGTACTTGTCCCTCAGCTGGGC
904	RHOH	0.32	TCCTCCATCACCTGAAACACTGGAC
905	ZNFN1A1	0.36	AAATGTTCCCTTGCGCTGCTCCTG
906	FXYD2	0.35	CACCCAGCTGGCCTGTGGATGGGA
907	CCR9	0.39	TGGACCCCCTGGCTGAGAACATCTGG
908	NAP1L1	0.3	TTGGACATCTCTAGTGTAGCTGCCA
909	CXCR4	0.31	AAATGTTCCCTTGCGCTGCTCCTG
910	SH2D1A	0.3	TCCTGTACTTGTCCCTCAGCTGGGC
911	CD1A	0.3	AAGCCTATACTGTTCTGTGGAGTAA
912	TRB@	0.32	AAATGTTCCCTTGCGCTGCTCCTG
913	SEPT6	0.32	GCCCCACTGGACAACACTGATTCT
914	RPS2	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
915	DOCK2	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
916	CD3D	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
917	CD6	0.3	GCCCCACTGGACAACACTGATTCT
918	ZAP70	0.34	ACTTGTCCCTCAGCTGGGCTCTTC
919	AIF1	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
920	CD1E	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
921	CYFIP2	0.3	TTGGACATCTCTAGTGTAGCTGCCA
922	ADA	0.41	TCCTGTACTTGTCCCTCAGCTGGGC
923	TRIM	0.31	TCCTGTGCCTGCTCCTGTACTTGT
924	GLTSCR2	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
925	FLJ10858	0.35	GCCCCACTGGACAACACTGATTCT
926	BCL11B	0.34	TCCTGTACTTGTCCCTCAGCTGGGC
927	GIMAP6	0.36	TGCCTGCTCCTGTACTTGTCCCTCAG
928	STAG3	0.34	TTGGACATCTCTAGTGTAGCTGCCA
929	UBASH3A	0.39	ACTTGTCCCTCAGCTGGGCTCTTC

TABLE 46

<u>Melphalan biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
930	CD99	0.31	TGGACCCCCTGGCTGAGAACATCTGG
931	HLA-DPB1	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
932	ARHGDI	0.35	TGCCTGCTCCTGTACTTGTCCCTCAG
933	IFITM1	0.33	CACCCAGCTGGCCTGTGGATGGGA
934	UBE2L6	0.32	TCCTGTGCCTGCTCCTGTACTTGT
935	ITM2A	0.37	TCCTGTGCCTGCTCCTGTACTTGT
936	SERPINA1	0.31	AAATGTTCCCTGTGCCTGCTCCTG
937	STAT5A	0.38	AAATGTTCCCTGTGCCTGCTCCTG
938	INPP5D	0.37	TCCTGTGCCTGCTCCTGTACTTGT
939	DGKA	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
940	SATB1	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
941	SEMA4D	0.37	AAATGTTCCCTGTGCCTGCTCCTG
942	TFDP2	0.31	CACCCAGCTGGCCTGTGGATGGGA
943	SLA	0.49	TCCTCCATCACCTGAAACACTGGAC
944	IL2RG	0.42	CACCCAGCTGGCCTGTGGATGGGA
945	CD48	0.33	TCCTGTGCCTGCTCCTGTACTTGT
946	MFNG	0.48	ACTTGTCCCTCAGCTGGGCTCTTC
947	ALOX5AP	0.3	CACCCAGCTGGCCTGTGGATGGGA
948	GPSM3	0.31	AAGCCTATACTGTTCTGTGGAGTAA
949	PSMB9	0.34	GCCCCACTGGACAACACTGATTCT
950	KIAA0711	0.37	TGGACCCCCTGGCTGAGAACATCTGG
951	SELL	0.32	AAATGTTCCCTGTGCCTGCTCCTG
952	ADA	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
953	EDG1	0.49	TTGGACATCTCTAGTGTAGCTGCCA
954	RIMS3	0.3	CACCCAGCTGGCCTGTGGATGGGA
955	FMNL1	0.33	AAGCCTATACTGTTCTGTGGAGTAA
956	MYB	0.3	GCCCCACTGGACAACACTGATTCT
957	PTPN7	0.34	AAATGTTCCCTGTGCCTGCTCCTG
958	LCK	0.31	AAATGTTCCCTGTGCCTGCTCCTG
959	CXorf9	0.55	CACCCAGCTGGCCTGTGGATGGGA
960	RHOH	0.35	TGGACCCCCTGGCTGAGAACATCTGG
961	ZNFN1A1	0.31	ACTTGTCCCTCAGCTGGGCTCTTC
962	CENTB1	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
963	LCP2	0.32	TCCTGTGCCTGCTCCTGTACTTGT
964	FXYD2	0.55	CACCCAGCTGGCCTGTGGATGGGA

TABLE 46-continued

<u>Melphalan biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
965	CD1D	0.44	AAGCCTATACTACGTTCTGTGGAGTAA
966	BATF	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
967	STAT4	0.33	TCCTCCATCACCTGAAACACTGGAC
968	VAV1	0.31	TCCTCCATCACCTGAAACACTGGAC
969	MAP4K1	0.39	CACCCAGCTGGCCTGTGGATGGGA
970	CCR7	0.44	TCCTGTACTTGTCCCTCAGCTTGGC
971	PDE4C	0.32	TCCTGTACTTGTCCCTCAGCTTGGC
972	CD3G	0.32	AAGCCTATACTACGTTCTGTGGAGTAA
973	CCR9	0.36	TTGGACATCTCTAGTGTAGGTGCCA
974	SP110	0.34	TCCTGTACTTGTCCCTCAGCTTGGC
975	LCP1	0.35	AAATGTTCCCTTGCCCTGCTCCTG
976	IFI16	0.32	GCCCCACTGGACAACACTGATTCCCT
977	CXCR4	0.36	ACTTGTCCCTCAGCTTGGGCTTCTC
978	ARHGEF6	0.47	AAGCCTATACTACGTTCTGTGGAGTAA
979	GATA3	0.55	TTGGACATCTCTAGTGTAGCTGCCA
980	SELPLG	0.47	TTGGACATCTCTAGTGTAGCTGCCA
981	SEG31L2	0.36	TGGACCCCCTGGCTGAGAACACTGG
982	CD3Z	0.5	TTGGACATCTCTAGTGTAGCTGCCA
983	PRKCQ	0.56	GCCGCACTGGACAACACTGATTCCCT
984	SH2D1A	0.33	TCCTCCATCACCTGAAACACTGGAC
985	GZMB	0.39	TGCCTGCTCCTGTACTTGTCCCTCAG
986	CD1A	0.55	TGCCTGCTCCTGTACTTGTCCCTCAG
987	SCN3A	0.64	CACCCAGCTGGCCTGTGGATGGGA
988	LAIR1	0.32	CACCCAGCTGGCCTGTGGATGGGA
989	FYB	0.49	TTGGACATCTCTAGTGTAGCTGCCA
990	TRB@	0.37	TTGGACATCTCTAGTGTAGCTGCCA
991	SEPT6	0.32	GCCCCACTGGACAACACTGATTCCCT
992	HA-1	0.48	GCCCCACTGGACAACACTGATTCCCT
993	DOCK2	0.33	TTGGACATCTCTAGTGTAGCTGCCA
994	CG018	0.37	AAATGTTCCCTGTGCCTGCTCCTG
995	CD3D	0.32	TCCTCCATCACCTGAAACACTGGAC
996	T3JAM	0.41	TGCCTGCTCCTGTACTTGTCCCTCAG
997	FNBP1	0.36	TCCTGTACTTGTCCCTCAGCTTGGC
998	CD6	0.36	AAGCCTATACTACGTTCTGTGGAGTAA
999	ZAP70	0.36	TGGACCCCCTGGCTGAGAACACTGG

TABLE 46-continued

<u>Melphalan biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
1000	LST1	0.31	ACTTGTCCCTCAGCTGGCTTCTTC
1001	GPR65	0.42	TTGGACATCTCTAGTGTAGCTGCCA
1002	PRF1	0.41	GCCCCACTGGACAAACACTGATTCCCT
1003	AIF1	0.32	GCCCCACTGGACAAACACTGATTCCCT
1004	FLJ20331	0.42	TCCTCCATCACCTGAAACACTGGAC
1005	RAG2	0.31	CACCCAGCTGGCCTGTGGATGGGA
1006	WDR45	0.37	TCCTGTACTTGTCCCTCAGCTTGGC
1007	CD1E	0.31	TCCTGTACTTGTCCCTCAGCTTGGC
1008	CYFIP2	0.4	TCCTCCATCACCTGAAACACTGGAC
1009	TARP	0.36	CACCCAGCTGGCCTGTGGATGGGA
1010	TRIM	0.31	ACTTGTCCCTCAGCTGGCTTCTTC
1011	RPL10L	0.3	TTGGACATCTCTAGTGTAGCTGCCA
1012	GLTSCR2	0.46	CACCCAGCTGGCCTGTGGATGGGA
1013	GIMAP5	0.32	AAGCCTATACTACGTTCTGTGGAGTAA
1014	ARHGAP15	0.36	TGCCTGCTCCTGTACTTGTCCCTCAG
1015	NOTCH1	0.34	GCCCCACTGGACAAACACTGATTCCCT
1016	BIN2	0.36	TGGACCCCACTGGCTGAGAACACTGG
1017	C13orf18	0.34	TCCTTGTGCCTGCTCCTGTACTTGT
1018	CECR1	0.32	TCCTGTACTTGTCCCTCAGCTTGGC
1019	BCL11B	0.32	TCCTTGTGCCTGCTCCTGTACTTGT
1020	GIMAP6	0.3	TCCTTGTGCCTGCTCCTGTACTTGT
1021	STAG3	0.58	TTGGACATCTCTAGTGTAGCTGCCA
1022	TM6SF1	0.31	TCCTGTACTTGTCCCTCAGCTTGGC
1023	HSD17B7	0.32	GCCCCACTGGACAAACACTGATTCCCT
1024	UBASH3A	0.3	ACTTGTCCCTCAGCTGGCTTCTTC
1025	MGC5566	0.45	TCCTCCATCACCTGAAACACTGGAC
1026	FLJ22457	0.39	AAGCCTATACTACGTTCTGTGGAGTAA
1027	TPK1	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
1028	PHF11	0.3	AAATGTTCCCTGTGCCTGCTCCTG
1029	DKFZP434B0335	0.4	TCCTTGTGCCTGCTCCTGTACTTGT

TABLE 47

<u>IL4-PR38 fusion protein biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Median	probe
1030	MCL1	0.3	TCCTGCATCACCTGAAACACTGGAC	
1031	DDX23	0.35	CACCCAGCTGGCTCTGTGGATGGGA	
1032	JUNB	0.31	TGCCTGCTCCTGTACTTGTCTCAG	
1033	ZFP36	0.33	CACCCAGCTGGCTCTGTGGATGGGA	
1034	IFITM1	0.32	ACTTGTCTCAGCTTGGCTTCTTC	
1035	CKS1B	0.3	TGGACCCCAGCTGGCTGAGAACACTGG	
1036	SERPINA1	0.31	GCCCCACTGGACAACACTGGATTCT	
1037	IL4R	0.3	ACTTGTCTCAGCTTGGCTTCTTC	
1038	CLDN3	0.31	ACTTGTCTCAGCTTGGCTTCTTC	
1039	ARL4A	0.33	AAATGTTCCCTGTGCCTGCTCTG	
1040	HMMR	0.31	TCCTTGCTGCCCTGCTCCTGTACTTGT	
1041	FLJ12671	0.42	TCCTTGCTGCCCTGCTCCTGTACTTGT	
1042	ANKHD1	0.42	GCCCCACTGGACAACACTGATTCT	
1043	KIF2C	0.37	ACTTGTCTCAGCTTGGCTTCTTC	
1044	RPA3	0.34	CACCCAGCTGGCTCTGTGGATGGGA	
1045	MCCC2	0.34	TGCCTGCTCCTGTACTTGTCTCAG	
1046	CDH17	0.32	TCCTTGCTGCCCTGCTCCTGTACTTGT	
1047	LSM5	0.33	TTGGACATCTCTAGTGTAGCTGCCA	
1048	PRF1	0.32	GCCCCACTGGACAACACTGATTCT	
1049	ROD1	0.34	TCCTCCATCACCTGAAACACTGGAC	
1050	FLJ12666	0.37	TCCTCCATCACCTGAAACACTGGAC	
1051	SUV420H1	0.31	TTGGACATCTCTAGTGTAGCTGCCA	
1052	MUC13	0.36	TCCTCCATCACCTGAAACACTGGAC	
1053	C13orf18	0.35	GCCCCACTGGACAACACTGATTCT	
1054	CDCA8	0.35	TGCCTGCTCCTGTACTTGTCTCAG	

TABLE 48

<u>Valproic acid (VPA) biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Median	probe
1055	STOM	0.32	AAATGTTCCCTGTGCCTGCTCTG	
1056	TNFAIP3	0.32	TGGACCCCAGCTGGCTGAGAACACTGG	
1057	ASNS	0.31	GCCCCACTGGACAACACTGATTCT	
1058	GARS	0.37	TGCCTGCTCCTGTACTTGTCTCAG	
1059	CXCR4	0.32	AAGCCTATACTGTTCTGTGGAGTAA	

TABLE 48-continued

<u>Valproic acid (VPA) biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Median	probe
1060	EGLN3	0.31	TGGACCCCAGCTGGCTGAGAACACTGG	
1061	LBH	0.35	TCCTGTACTTGTCTCAGCTTGGGGC	
1062	GDF15	0.3	TGCCTGCTCCTGTACTTGTCTCAG	
<u>TABLE 49</u>				
<u>All-trans retinoic acid (ATRA) biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Median	probe
1063	PPIB	0.31	AAGCCTATACTGTTCTGTGGAGTAA	
1064	ZFP36L2	0.48	AAGCCTATACTGTTCTGTGGAGTAA	
1065	IFI30	0.46	ACTTGTCTCAGCTTGGCTTCTTC	
1066	USP7	0.35	TCCTCCATCACCTGAAACACTGGAC	
1067	SRM	0.43	TCCTCCATCACCTGAAACACTGGAC	
1068	SH3BP5	0.32	TGCCTGCTCCTGTACTTGTCTCAG	
1069	ALDOC	0.41	TTGGACATCTCTAGTGTAGCTGCCA	
1070	FADS2	0.33	ACTTGTCTCAGCTTGGCTTCTTC	
1071	GUSB	0.38	TTGGACATCTCTAGTGTAGCTGCCA	
1072	PSCD1	0.48	TCCTGTACTTGTCTCAGCTTGGGC	
1073	IQGAP2	0.34	TCCTGTACTTGTCTCAGCTTGGGC	
1074	STS	0.34	GCCCCACTGGACAACACTGATTCT	
1075	MFNG	0.36	TGGACCCCAGCTGGCTGAGAACACTGG	
1076	FLI1	0.33	ACTTGTCTCAGCTTGGCTTCTTC	
1077	PIM2	0.35	TGGACCCCAGCTGGCTGAGAACACTGG	
1078	INPP4A	0.54	TCCTGTACTTGTCTCAGCTTGGGC	
1079	LRMP	0.51	GCCCCACTGGACAACACTGATTCT	
1080	ICAM2	0.3	AAATGTTCCCTGTGCCTGCTCTG	
1081	EVI2A	0.33	CACCCAGCTGGCTCTGTGGATGGGA	
1082	MAL	0.46	AAATGTTCCCTGTGCCTGCTCTG	
1083	BTN3A3	0.43	TTGGACATCTCTAGTGTAGCTGCCA	
1084	PTPN7	0.4	TTGGACATCTCTAGTGTAGCTGCCA	
1085	IL10RA	0.42	TTGGACATCTCTAGTGTAGCTGCCA	
1086	SPI1	0.41	AAGCCTATACTGTTCTGTGGAGTAA	
1087	TRAF1	0.3	TGCCTGCTCCTGTACTTGTCTCAG	
1088	ITGB7	0.33	TCCTGTGCCTGCTCTGTACTTGT	

TABLE 49-continued

<u>All-trans retinoic acid (ATRA) biomarkers.</u>				
SEQ ID NO	Gene	Corre-lation	Median	probe
1089	ARHGAP6	0.32	TGGACCCCACTGGCTGAGAAATCTGG	
1090	MAP4K1	0.52	GCCCCACTGGACAACACTGGATTCCCT	
1091	CD28	0.34	AAGCCTATACTGTTCTGTGGAGTAA	
1092	PTP4A3	0.3	TCCTCCATCACCTGAAACACTGGAC	
1093	LTB	0.32	ACTTGTCCCTCAGCTTGGGCTTCTTC	
1094	Clorf38	0.4	TGCCTGCTCCTGTACTTGTCCCTCAG	
1095	WBSCR22	0.53	TCCTCCATCACCTGAAACACTGGAC	
1096	CD8B1	0.35	TCCTCCATCACCTGAAACACTGGAC	
1097	LCP1	0.35	ACTTGTCCCTCAGCTTGGGCTTCTTC	
1098	FLJ13052	0.31	TCCTCCATCACCTGAAACACTGGAC	
1099	MEF2C	0.71	TTGGACATCTCTAGTGTAGCTGCCA	
1100	PSCDBP	0.41	AAATGTTCCCTGTGCCTGCTCCTG	
1101	IL16	0.51	TGGACCCCACTGGCTGAGAAATCTGG	
1102	SELPLG	0.53	TGCCTGCTCCTGTACTTGTCCCTCAG	
1103	MAGEA9	0.6	AAATGTTCCCTGTGCCTGCTCCTG	
1104	LAIR1	0.43	TCCTCCATCACCTGAAACACTGGAC	
1105	TNFRSF25	0.53	TCCTCCATCACCTGAAACACTGGAC	
1106	EVI2B	0.42	ACTTGTCCCTCAGCTTGGGCTTCTTC	
1107	IGJ	0.37	TCCTTGCCCTGCTCCTGTACTTGT	
1108	PDCD4	0.47	AAATGTTCCCTGTGCCTGCTCCTG	
1109	RASA4	0.52	CACCCAGCTGGCCTGTGGATGGGA	
1110	HA-1	0.73	AAGCCTATACTGTTCTGTGGAGTAA	
1111	PLCL2	0.47	TCCTGTACTTGTCCCTCAGCTTGGC	
1112	RNASE6	0.31	AAGCCTATACTGTTCTGTGGAGTAA	
1113	WBSCR20C	0.35	TTGGACATCTCTAGTGTAGCTGCCA	
1114	NUP210	0.36	AAGCCTATACTGTTCTGTGGAGTAA	
1115	RPL10L	0.39	ACTTGTCCCTCAGCTTGGGCTTCTTC	
1116	C11orf2	0.33	TGGACCCCACTGGCTGAGAAATCTGG	
1117	CABC1	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG	
1118	ARHGEF3	0.37	TCCTGTACTTGTCCCTCAGCTTGGGC	
1119	TAPBPL	0.42	TGCCTGCTCCTGTACTTGTCCCTCAG	
1120	CHST12	0.35	AAATGTTCCCTGTGCCTGCTCCTG	
1121	FKBP11	0.54	TGCCTGCTCCTGTACTTGTCCCTCAG	
1122	FLJ35036	0.42	TTGGACATCTCTAGTGTAGCTGCCA	
1123	MYLIP	0.38	CACCCAGCTGGCCTGTGGATGGGA	

TABLE 49-continued

<u>All-trans retinoic acid (ATRA) biomarkers.</u>				
SEQ ID NO	Gene	Corre-lation	Median	probe
1124	TXNDC5	0.31	ACTTGTCCCTCAGCTGGCTTGTTC	
1125	PACAP	0.3	TCCTCCATCACCTGAAACACTGGAC	
1126	TOSO	0.34	TCCTGTACTTGTCCCTCAGCTTGGGC	
1127	PNAS-4	0.37	TGGACCCCACTGGCTGAGAAATCTGG	
1128	IL21R	0.57	AAGCCTATACTGTTCTGTGGAGTAA	
1129	TCF4	0.64	TCCTTGCCCTGCTCCTGTACTTGT	

TABLE 50

<u>Cytoxin biomarkers.</u>				
SEQ ID NO	Gene	Corre-lation	Median	probe
1130	C6orf29	0.31	AAGCCTATACTGTTCTGTGGAGTAA	
1131	TRIM31	0.31	AAATGTTCCCTGTGCCTGCTCCTG	
1132	CD69	0.37	GCCCCACTGGACAAACACTGATTCT	
1133	LRRN3	0.31	ACTTGTCCCTCAGCTTGGCTTCTTC	
1134	GPR35	0.41	TCCTCCATCACCTGAAACACTGGAC	
1135	CDW52	0.48	TTGGACATCTCTAGTGTAGCTGCCA	

TABLE 51

<u>Topotecan (Hycamtin) biomarkers.</u>				
SEQ ID NO	Gene	Corre-lation	Median	probe
1136	K-ALPHA-1	0.32	AAGCCTATACTGTTCTGTGGAGTAA	
1137	CSDA	0.32	AAGCCTATACTGTTCTGTGGAGTAA	
1138	UCHL1	0.32	TTGGACATCTCTAGTGTAGCTGCCA	
1139	NAP1L1	0.3	TCCTCCATCACCTGAAACACTGGAC	
1140	ATP5G2	0.3	TCCTGTACTTGTCCCTCAGCTTGGGC	
1141	HDGFRP3	0.3	AAGCCTATACTGTTCTGTGGAGTAA	
1142	IFI44	0.3	GCCCCACTGGACAAACACTGATTCC	

TABLE 52

<u>Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza) biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1143	NOL5A	0.35	TCCTTGTGCCTGCTCCTGTACTTG		
1144	STOM	0.35	TGCCTGCTCCTGTACTTGCTCAG		
1145	SIAT1	0.36	AAATGTTCCCTGTGCCTGCTCCTG		
1146	CUGBP2	0.39	GCCCCACTGGACAACACTGATTCC		
1147	GUSB	0.33	TGGACCCCCTGGCTGAGAACACTGG		
1148	ITM2A	0.34	TCCTTGTGCCTGCTCCTGTACTTG		
1149	JARID2	0.32	ACTTGTCCAGCTTGGGCTTCTTC		
1150	RUNX3	0.32	CACCCAGCTGGCCTGTGGATGGGA		
1151	ICAM2	0.35	TGCCTGCTCCTGTACTTGCTCAG		
1152	PTPN7	0.37	AAGCCTATACTGTTCTGTGGAGTAA		
1153	VAV1	0.35	TTGGACATCTCTAGTGTAGCTGCCA		
1154	PTP4A3	0.42	AAGCCTATACTGTTCTGTGGAGTAA		
1155	MCAM	0.35	ACTTGTCCAGCTTGGGCTTCTTC		
1156	MEF2C	0.32	ACTTGTCCAGCTTGGGCTTCTTC		
1157	IDH3B	0.3	TGCCTGCTCCTGTACTTGCTCAG		
1158	RFP	0.31	TCCTCCATCACCTGAAACACTGGAC		
1159	SEPT6	0.31	TCCTGTACTTGTCCAGCTTGGGC		
1160	SLC43A3	0.34	GCCCCACTGGACAACACTGATTCC		
1161	WBSCR20C	0.46	TGGACCCCCTGGCTGAGAACACTGG		
1162	SHMT2	0.34	TCCTGTACTTGTCCAGCTTGGGC		
1163	GLTSCR2	0.31	TCCTGTACTTGTCCAGCTTGGGC		
1164	CABC1	0.33	TCCTGTACTTGTCCAGCTTGGGC		
1165	FLJ20859	0.42	ACTTGTCCAGCTTGGGCTTCTTC		
1166	FLJ20010	0.51	TCCTGTACTTGTCCAGCTTGGGC		
1167	MGC10993	0.33	TCCTTGTGCCTGCTCCTGTACTTG		
1168	FKBP11	0.31	TCCTCCATCACCTGAAACACTGGAC		

TABLE 53

<u>Depsipeptide (FR901228) biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1169	ZFP36L2	0.32	AAATGTTCCCTGTGCCTGCTCCTG		
1170	TRIB2	0.35	TGCCTGCTCCTGTACTTGCTCAG		
1171	LCP2	0.37	ACTTGTCCAGCTTGGGCTTCTTC		

TABLE 53-continued

<u>Depsipeptide (FR901228) biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1172	C6orf32	0.35	TGCCTGCTCCTGTACTTGCTCCTG		
1173	IL16	0.34	CACCCAGCTGGCCTGTGGATGGGA		
1174	CACNA1G	0.31	AAGCCTATACTGTTCTGTGGAGTAA		
1175	SPDEF	0.31	GCCCCACTGGACAACACTGATTCC		
1176	HAB1	0.39	TCCTCCATCACCTGAAACACTGGAC		
1177	TOSO	0.31	TGGACCCCCTGGCTGAGAACACTGG		
1178	ARHGAP25	0.33	AAGCCTATACTGTTCTGTGGAGTAA		

TABLE 54

<u>Bortezomib biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1179	PLEKHB2	0.32	AAATGTTCCCTGTGCCTGCTCCTG		
1180	ARPC1B	0.32	TGGACCCCCTGGCTGAGAACACTGG		
1181	MX1	0.39	TCCTGTGCCTGCTCCTGTACTTG		
1182	CUGBP2	0.37	AAGCCTATACTGTTCTGTGGAGTAA		
1183	IFI16	0.33	AAGCCTATACTGTTCTGTGGAGTAA		
1184	TNFRSF14	0.3	AAATGTTCCCTGTGCCTGCTCCTG		
1185	SP110	0.39	TGGACCCCCTGGCTGAGAACACTGG		
1186	ELF1	0.33	TGGACCCCCTGGCTGAGAACACTGG		
1187	LPXN	0.33	TCCTGTACTTGTCCAGCTTGGGC		
1188	IFRG28	0.31	TCCTGTACTTGTCCAGCTTGGGC		
1189	LEF1	0.33	GCCCCACTGGACAACACTGATTCC		
1190	PYCARD	0.31	TCCTGTACTTGTCCAGCTTGGGC		

TABLE 55

<u>Leukeran biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1191	SSRP1	0.31	GCCCCACTGGACAACACTGATTCC		
1192	ALDOC	0.36	AAATGTTCCCTGTGCCTGCTCCTG		
1193	C1QR1	0.31	TGCCTGCTCCTGTACTTGCTCAG		
1194	TTF1	0.31	TCCTGTACTTGTCCAGCTTGGGC		
1195	PRIM1	0.31	GCCCCACTGGACAACACTGATTCC		
1196	USP34	0.38	TCCTCCATCACCTGAAACACTGGAC		

TABLE 55-continued

<u>Leukeran biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1197	TK2	0.33	TCCTGTACTTGTCCCTCAGCTGGGC
1198	GOLGIN-67	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1199	NPD014	0.35	ACTTGTCCCTCAGCTTGGGCTTCTTC
1200	KIAA0220	0.31	TCCTCCATCACCTGAAACACTGGAC
1201	SLC43A3	0.3	TTGGACATCTCTAGTGTAGCTGCCA
1202	WBSCR20C	0.3	CACCCAGCTGGCCTGTGGATGGGA
1203	ICAM2	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
1204	TEX10	0.32	TGGACCCCCTGGCTGAGAACACTGG
1205	CHD7	0.3	ACTTGTCCCTCAGCTTGGGCTTCTTC
1206	SAMSN1	0.34	TTGGACATCTCTAGTGTAGCTGCCA
1207	TPRT	0.35	ACTTGTCCCTCAGCTTGGGCTTCTTC

TABLE 56

<u>Fludarabine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1208	HLA-E	0.3	ACTTGTCCCTCAGCTTGGGCTTCTTC
1209	BAT3	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
1210	ENO2	0.37	TGGACCCCCTGGCTGAGAACACTGG
1211	UBE2L6	0.3	TCCTGTACTTGTCCCTCAGCTTGGGC
1212	CUGBP2	0.35	TGCCTGCTCCTGTACTTGTCCCTCAG
1213	ITM2A	0.32	GCCCCACTGGACAACACTGATTCCCT
1214	PALM2-AKAP2	0.41	GCCCCACTGGACAACACTGATTCCCT
1215	JARID2	0.33	GCCCCACTGGACAACACTGATTCCCT
1216	DGKA	0.33	TGGACCCCCTGGCTGAGAACACTGG
1217	SLC7A6	0.4	AAGCCTATACTGTTCTGTGGAGTAA
1218	TFDP2	0.35	AAATGTTCCCTGTGCCTGCTCCTG
1219	ADA	0.41	TGCCTGCTCCTGTACTTGTCCCTCAG
1220	EDG1	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
1221	ICAM2	0.46	AAGCCTATACTGTTCTGTGGAGTAA
1222	PTPN7	0.33	TCCTCCATCACCTGAAACACTGGAC
1223	CXorf9	0.35	AAGCCTATACTGTTCTGTGGAGTAA
1224	RHOH	0.31	CACCCAGCTGGCCTGTGGATGGGA
1225	MX2	0.32	AAATGTTCCCTGTGCCTGCTCCTG

TABLE 56-continued

<u>Fludarabine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1226	ZNFN1A1	0.31	TCCTCCATCACCTGAAACACTGGAC
1227	COCH	0.33	TGGACCCCCTGGCTGAGAACACTGG
1228	LCP2	0.34	TGGACCCCCTGGCTGAGAACACTGG
1229	CLGN	0.31	TCCTCCATCACCTGAAACACTGGAC
1230	BNC1	0.38	GCCCCACTGGACAACACTGATTCCCT
1231	FLNC	0.3	TCCTGTACTTGTCCCTCAGCTTGGC
1232	HLA-DRB3	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
1233	UCP2	0.34	TGGACCCCCTGGCTGAGAACACTGG
1234	HLA-DRB1	0.3	GCCCCACTGGACAACACTGATTCCCT
1235	GATA3	0.37	TCCTGTGCCTGCTCCTGTACTTGT
1236	PRKCQ	0.39	AAATGTTCCCTGTGCCTGCTCCTG
1237	SH2D1A	0.37	ACTTGTCCCTCAGCTTGGCTTCTTC
1238	NFATC3	0.33	ACTTGTCCCTCAGCTTGGCTTCTTC
1239	TRB@	0.35	AAATGTTCCCTGTGCCTGCTCCTG
1240	FNBP1	0.34	TCCTCCATCACCTGAAACACTGGAC
1241	SEPT6	0.33	ACTTGTCCCTCAGCTTGGCTTCTTC
1242	NME4	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1243	DKFZP434C171	0.3	TCCTGTGCCTGCTCCTGTACTTGT
1244	ZC3HAV1	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
1245	SLC43A3	0.37	AAATGTTCCCTGTGCCTGCTCCTG
1246	CD3D	0.31	AAATGTTCCCTGTGCCTGCTCCTG
1247	AIF1	0.35	TCCTCCATCACCTGAAACACTGGAC
1248	SPTAN1	0.34	TCCTCCATCACCTGAAACACTGGAC
1249	CD1E	0.31	TCCTGTGCCTGCTCCTGTACTTGT
1250	TRIM	0.31	AAGCCTATACTGTTCTGTGGAGTAA
1251	DATF1	0.31	TCCTGTGCCTGCTCCTGTACTTGT
1252	FHOD1	0.37	TCCTGTACTTGTCCCTCAGCTTGGGC
1253	ARHGAP15	0.3	CACCCAGCTGGCCTGTGGATGGGA
1254	STAG3	0.34	AAGCCTATACTGTTCTGTGGAGTAA
1255	SAP130	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
1256	CYLD	0.3	ACTTGTCCCTCAGCTTGGCTTCTTC

TABLE 57

<u>Vinblastine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1257	CD99	0.33	ACTTGTCCCTCAGCTTGGGTTCTTC

TABLE 58

<u>Busulfan biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1258	RPLP2	0.37	TCCTCCATCACCTGAAACACTGGAC
1259	BTG1	0.36	ACTTGTCCCTCAGCTTGGGTTCTTC
1260	CSDA	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1261	ARHGDIB	0.38	AAGCCTATACTGTTCTGTGGAGTAA
1262	INSIG1	0.41	TCCTCCATCACCTGAAACACTGGAC
1263	ALDOC	0.36	TTGGACATCTCTAGTGTAGCTGCCA
1264	WASPIP	0.31	TCCTCCATCACCTGAAACACTGGAC
1265	C1QR1	0.46	TCCTGTACTTGTCCCTCAGCTTGGGC
1266	EDEM1	0.36	TGGACCCCCTGGCTGAGAACACTGG
1267	SLA	0.35	TCCTTGTCCTGCCTCCTGTACTTGT
1268	MFNG	0.4	TCCTTGTCCTGCCTCCTGTACTTGT
1269	GPSM3	0.75	GCCCCACTGGACAACACTGATTCT
1270	ADA	0.53	ACTTGTCCCTCAGCTTGGGTTCTTC
1271	LRMP	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
1272	EVI2A	0.52	TCCTCCATCACCTGAAACACTGGAC
1273	FMNL1	0.45	ACTTGTCCCTCAGCTTGGGTTCTTC
1274	PTPN7	0.3	ACTTGTCCCTCAGCTTGGGTTCTTC
1275	RHOH	0.39	ACTTGTCCCTCAGCTTGGGTTCTTC
1276	ZNFX1A1	0.36	AAGCCTATACTGTTCTGTGGAGTAA
1277	CENTB1	0.33	TTGGACATCTCTAGTGTAGCTGCCA
1278	MAP4K1	0.31	TGGACCCCCTGGCTGAGAACACTGG
1279	CD28	0.51	TCCTGTACTTGTCCCTCAGCTTGGGC
1280	SP110	0.38	TCCTTGTCCTGCCTCCTGTACTTGT
1281	NAP1L1	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1282	IFI16	0.35	TCCTCCATCACCTGAAACACTGGAC
1283	ARHGEF6	0.42	AAATGTTCCCTGTGCCTGCTCCTG
1284	SELPLG	0.45	TCCTGTACTTGTCCCTCAGCTTGGGC
1285	CD3Z	0.35	CACCCAGCTGGCTCTGTGGATGGGA
1286	SH2D1A	0.38	CACCCAGCTGGCTCTGTGGATGGGA

TABLE 58-continued

<u>Busulfan biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1287	LAIR1	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
1288	RAFTLIN	0.36	GCCCCACTGGACAAACACTGATTCCCT
1289	HA-1	0.61	ACTTGTCCCTCAGCTTGGGTTCTTC
1290	DOCK2	0.4	TGCCTGCTCCTGTACTTGTCCCTCAG
1291	CD3D	0.31	GCCCCACTGGACAAACACTGATTCCCT
1292	T3JAM	0.35	ACTTGTCCCTCAGCTTGGGTTCTTC
1293	ZAP70	0.36	TGGACCCCCTGGCTGAGAACACTGG
1294	GPR65	0.32	TCCTCCATCACCTGAAACACTGGAC
1295	CYFIP2	0.58	CACCCAGCTGGCTCTGTGGATGGGA
1296	LPXN	0.34	TTGGACATCTCTAGTGTAGCTGCCA
1297	RPL10L	0.41	TCCTGTACTTGTCCCTCAGCTTGGGC
1298	GLTSCR2	0.33	AAATGTTCCCTGTGCCTGCTCCTG
1299	ARHGAP15	0.47	CACCCAGCTGGCTCTGTGGATGGGA
1300	BCL11B	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1301	TM6SF1	0.39	AAGCCTATACTGTTCTGTGGAGTAA
1302	PACAP	0.33	ACTTGTCCCTCAGCTTGGGTTCTTC
1303	TCF4	0.32	TGGACCCCCTGGCTGAGAACACTGG

TABLE 59

<u>Dacarbazine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1304	ARHGDIB	0.31	TCCTGTGCCTGCTCCTGTACTTGT
1305	ITM2A	0.4	TCCTCCATCACCTGAAACACTGGAC
1306	SSBP2	0.33	CACCCAGCTGGCTCTGTGGATGGGA
1307	PIM2	0.39	GCCCCACTGGACAAACACTGATTCCCT
1308	SELL	0.31	GCCCCACTGGACAAACACTGATTCCCT
1309	ICAM2	0.43	TCCTGTACTTGTCCCTCAGCTTGGGC
1310	EVI2A	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1311	MAL	0.32	TTGGACATCTCTAGTGTAGCTGCCA
1312	PTPN7	0.34	ACTTGTCCCTCAGCTTGGGTTCTTC
1313	ZNFX1A1	0.32	TCCTGTGCCTGCTCCTGTACTTGT
1314	LCP2	0.3	GCCCCACTGGACAAACACTGATTCCCT
1315	ARHGAP6	0.33	TGGACCCCCTGGCTGAGAACACTGG

TABLE 59-continued

<u>Dacarbazine biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
1316	CD28	0.33	ACTTGTCTCAGCTTGGGCTTC
1317	CD8B1	0.32	TCCTCCATCACCTGAAACACTGGAC
1318	LCP1	0.34	TCCTTGTGCCTGCTCCTGTACTTGT
1319	NPD014	0.31	TGCCTGCTCCTGTACTTGTCCAG
1320	CD69	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1321	NFATC3	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1322	TRB@	0.32	AAATGTTTCTGTGCCTGCTCTG
1323	IGJ	0.33	AAGCCTATACTGTTCTGTGGAGTAA
1324	SLC43A3	0.3	TTGGACATCTCTAGTGTAGCTGCCA
1325	DOCK2	0.36	TCCTCCATCACCTGAAACACTGGAC
1326	FHOD1	0.33	TGGACCCCCTGGCTGAGAACATCTGG
1327	PACAP	0.31	AAGCCTATACTGTTCTGTGGAGTAA

TABLE 60

Oxaliplatin biomarkers.

<u>Oxaliplatin biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
1328	RPL18	0.38	TTGGACATCTCTAGTGTAGCTGCCA
1329	RPL10A	0.32	AAATGTTTCTGTGCCTGCTCTG
1330	RPS3A	0.34	TGGACCCCCTGGCTGAGAACATCTGG
1331	EEF1B2	0.39	CACCCAGTGGCTCTGTGGATGGGA
1332	GOT2	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1333	RPL13A	0.33	AAATGTTTCTGTGCCTGCTCTG
1334	RPS15	0.41	GCCCCACTGGACAACACTGATTCC
1335	NOLSA	0.37	TGCCTGCTCCTGTACTTGTCCAG
1336	RPLP2	0.36	TGCCTGCTCCTGTACTTGTCCAG
1337	SLC9A3R1	0.43	TGGACCCCCTGGCTGAGAACATCTGG
1338	E1F3S3	0.43	GCCCCACTGGACAACACTGATTCC
1339	MTHFD2	0.33	TGCCTGCTCCTGTACTTGTCCAG
1340	IMPDH2	0.34	ACTTGTCTCAGCTTGGGCTTC
1341	ALDOC	0.44	TGCCTGCTCCTGTACTTGTCCAG
1342	FABP5	0.33	CACCCAGTGGCTCTGTGGATGGGA
1343	ITM2A	0.35	TCCTTGTGCCTGCTCCTGTACTTGT
1344	PCK2	0.36	ACTTGTCTCAGCTTGGGCTTC
1345	MFNG	0.33	GCCCCACTGGACAACACTGATTCC

TABLE 60-continued

<u>Oxaliplatin biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
1346	GCH1	0.37	TGGACCCCCTGGCTGAGAACATCTGG
1347	PIM2	0.39	CACCCAGCTGGCCTGTGGATGGGA
1348	ADA	0.32	TCCTTGTGCCTGCTCCTGTACTTGT
1349	ICAM2	0.31	TCCTCCATCACCTGAAACACTGGAC
1350	TTF1	0.47	TTGGACATCTCTAGTGTAGCTGCCA
1351	MYB	0.36	TGCCTGCTCCTGTACTTGTCCAG
1352	PTPN7	0.37	CACCCAGCTGGCCTGTGGATGGGA
1353	RHOH	0.42	TCCTCCATCACCTGAAACACTGGAC
1354	ZNFN1A1	0.39	ACTTGTCTCAGCTGGGCTTC
1355	PRIM1	0.36	TCCTTGTGCCTGCTCCTGTACTTGT
1356	FHIT	0.48	TCCTCCATCACCTGAAACACTGGAC
1357	ASS	0.45	TGGACCCCCTGGCTGAGAACATCTGG
1358	SYK	0.31	TGCCTGCTCCTGTACTTGTCCAG
1359	OXA1L	0.32	TTGGACATCTCTAGTGTAGCTGCCA
1360	LCP1	0.31	TGCCTGCTCCTGTACTTGTCCAG
1361	DDX18	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1362	NOLA2	0.35	AAATGTTTCTGTGCCTGCTCCTG
1363	KIAA0922	0.41	TCCTCCATCACCTGAAACACTGGAC
1364	PRKCQ	0.34	TCCTTGTGCCTGCTCCTGTACTTGT
1365	NFATC3	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
1366	ANAPC5	0.34	TCCTCCATCACCTGAAACACTGGAC
1367	TRB@	0.4	TGGACCCCCTGGCTGAGAACATCTGG
1368	CXCR4	0.32	TCCTTGTGCCTGCTCCTGTACTTGT
1369	FNBP4	0.3	TCCTGTACTTGTCCCTCAGCTTGGGC
1370	SEPT6	0.53	TTGGACATCTCTAGTGTAGCTGCCA
1371	RPS2	0.35	TCCTCCATCACCTGAAACACTGGAC
1372	MDN1	0.41	ACTTGTCTCAGCTGGGCTTC
1373	PCCB	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1374	RASA4	0.33	TGGACCCCCTGGCTGAGAACATCTGG
1375	WBSCR20C	0.31	CACCCAGCTGGCCTGTGGATGGGA
1376	SFRS7	0.32	TTGGACATCTCTAGTGTAGCTGCCA
1377	WBSCR20A	0.3	TGCCTGCTCCTGTACTTGTCCAG
1378	NUP210	0.43	TGGACCCCCTGGCTGAGAACATCTGG
1379	SHMT2	0.36	TCCTTGTGCCTGCTCCTGTACTTGT
1380	RPLP0	0.33	TTGGACATCTCTAGTGTAGCTGCCA

TABLE 60-continued

<u>Oxaliplatin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1381	MAP4K1	0.31	CACCCAGCTGGCCTGTGGATGGAA
1382	HNRPA1	0.37	TCCTCCATCACCTGAAACACTGGAC
1383	CYFIP2	0.3	GCCCCACTGGACAACACTGATTCCCT
1384	RPL10L	0.32	TCGTCATCACCTGAAACACTGGAC
1385	GLTSCR2	0.39	TGGACCCCCTGGCTGAGAATCTGG
1386	MRPL16	0.38	TCCTGTACTTGTCCCTCAGCTGGGC
1387	MRPS2	0.34	GCCCCACTGGACAACACTGATTCCCT
1388	FLJ12270	0.31	AAGCCTATACTGTTCTGTGGAGTAA
1389	CDK5RAP3	0.32	TTGGACATCTCTAGTGTAGCTGCCA
1390	ARHGAP15	0.32	TCCTGTACTTGTCCCTCAGCTGGGC
1391	CUTC	0.33	TCCTTGCCCTGCTCCCTGTACTTGT
1392	FKBP11	0.32	ACTTGTCCCTCAGCTTGGGCTTCTTC
1393	ADPGK	0.41	AAGCCTATACTGTTCTGTGGAGTAA
1394	FLJ22457	0.32	GCCCCACTGGACAACACTGATTCCCT
1395	PUS3	0.31	TCCTTGCCCTGCTCCCTGTACTTGT
1396	PACAP	0.36	TGCCTGCTCCTGTACTTGTCCCTCAG
1397	CALML4	0.31	TCCTGTACTTGTCCCTCAGCTGGGC

TABLE 61

Hydroxyurea biomarkers.

SEQ ID NO	Gene	Corre- lation	Medianprobe
1398	CSDA	0.31	TCCTCCATCACCTGAAACACTGGAC
1399	INSIG1	0.38	AAGCCTATACTGTTCTGTGGAGTAA
1400	UBE2L6	0.33	CACCCAGCTGGCCTGTGGATGGGA
1401	PRG1	0.36	GCCCCACTGGACAACACTGATTCCCT
1402	ITM2A	0.3	ACTTGTCCCTCAGCTTGGGCTTCTTC
1403	DGKA	0.31	CACCCAGCTGGCCTGTGGATGGGA
1404	SLA	0.47	CACCCAGCTGGCCTGTGGATGGGA
1405	PCBP2	0.51	TGGACCCCCTGGCTGAGAATCTGG
1406	IL2RG	0.42	ACTTGTCCCTCAGCTTGGGCTTCTTC
1407	ALOX5AP	0.31	AAGCCTATACTGTTCTGTGGAGTAA
1408	PSMB9	0.33	GCCCCACTGGACAACACTGATTCCCT
1409	LRMP	0.36	TTGGACATCTCTAGTGTAGCTGCCA

TABLE 61-continued

<u>Hydroxyurea biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1410	ICAM2	0.31	TGGACCCCCTGGCTGAGAATCTGG
1411	PTPN7	0.36	TCCTCCATCACCTGAAACACTGGAC
1412	CXorf9	0.38	TCCTTGCCCTGCTCTGTACTTGT
1413	RHOH	0.41	TGCCTGCTCCTGTACTTGTCCCTAG
1414	ZNFN1A1	0.31	AAATGTTCCCTGTGCCTGCTCCTG
1415	CENTB1	0.36	TTGGACATCTCTAGTGTAGCTGCCA
1416	LCP2	0.37	CACCCAGCTGGCCTGTGGATGGGA
1417	STAT4	0.32	GCCCCACTGGACAACACTGATTCCCT
1418	CCR7	0.31	TCCTTGCCCTGCTCCTGTACTTGT
1419	CD3G	0.33	AAATGTTCCCTGTGCCTGCTCCTG
1420	SP110	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
1421	TNFAIP8	0.31	TCCTCCATCACCTGAAACACTGGAC
1422	IFI16	0.4	TGGACCCCCTGGCTGAGAATCTGG
1423	CXCR4	0.37	ACTTGTCCCTCAGCTGGGCTTCTTC
1424	ARHGEF6	0.37	TTGGACATCTCTAGTGTAGCTGCCA
1425	SELPLG	0.3	TCCTCCATCACCTGAAACACTGGAC
1426	CD3Z	0.38	TCCTCCATCACCTGAAACACTGGAC
1427	PRKCQ	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
1428	SH2D1A	0.3	TCCTTGCCCTGCTCCTGTACTTGT
1429	CD1A	0.31	TTGGACATCTCTAGTGTAGCTGCCA
1430	NFATC3	0.33	TGGACCCCCTGGCTGAGAATCTGG
1431	LAIR1	0.34	TCCTCCATCACCTGAAACACTGGAC
1432	TRB@	0.3	CACCCAGCTGGCCTGTGGATGGGA
1433	SEPT6	0.34	CACCCAGCTGGCCTGTGGATGGGA
1434	RAFTLIN	0.3	TCCTTGCCCTGCTCCTGTACTTGT
1435	DOCK2	0.32	TGGACCCCCTGGCTGAGAATCTGG
1436	CD3D	0.37	TGCCTGCTCCTGTACTTGTCCCTAG
1437	CD6	0.42	AAGCCTATACTGTTCTGTGGAGTAA
1438	AIF1	0.4	TGCCTGCTCCTGTACTTGTCCCTAG
1439	CD1E	0.41	GCCCCACTGGACAACACTGATTCCCT
1440	CYFIP2	0.35	TCCTGTACTTGTCCCTCAGCTTGGGC
1441	TARP	0.38	AAATGTTCCCTGTGCCTGCTCCTG
1442	ADA	0.33	AAGCCTATACTGTTCTGTGGAGTAA
1443	ARHGAP15	0.32	TGGACCCCCTGGCTGAGAATCTGG
1444	GIMAP6	0.34	GCCCCACTGGACAACACTGATTCCCT

TABLE 61-continued

<u>Hydroxyurea biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1445	STAG3	0.31	ACTTGTCCCTCAGCTTGGGCTTC		
1446	FLJ22457	0.31	AAGCCTATACGTTCTGTGGAGTAA		
1447	PACAP	0.35	AAGCCTATACGTTCTGTGGAGTAA		
1448	TCF4	0.4	TCCTGTACTTGTCCCTCAGCTGGC		

TABLE 62

<u>Tegafur biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1449	RPL11	0.31	GCCCCACTGGACAACACTGATTCT		
1450	RPL17	0.38	TGCCTGCTCCTGTACTTGTCCCTCAG		
1451	ANAPC5	0.34	CACCCAGCTGGCCTGTGGATGGGA		
1452	RPL13A	0.34	TCCTGTACTTGTCCCTCAGCTTGGC		
1453	STOM	0.37	TCCTCCATCACCTGAAACACTGGAC		
1454	TUFM	0.38	GCCCCACTGGACAACACTGATTCT		
1455	SCARB1	0.35	TCCTGTACTTGTCCCTCAGCTTGGC		
1456	FABP5	0.33	CACCCAGCTGGCCTGTGGATGGGA		
1457	KIAA0711	0.35	TCCTTGCTCCTGCTCCTGTACTTGT		
1458	ILGR	0.33	TCCTCCATCACCTGAAACACTGGAC		
1459	WBSCR22	0.3	AAATGTTCCCTGTGCCTGCTCCTG		
1460	UCK2	0.4	TGCCTGCTCCTGTACTTGTCCCTCAG		
1461	GZMB	0.3	AAGCCTATACGTTCTGTGGAGTAA		
1462	Clorf38	0.32	CACCCAGCTGGCCTGTGGATGGGA		
1463	PCBP2	0.31	TCCTGTACTTGTCCCTCAGCTTGGC		
1464	GPR65	0.44	TGCCTGCTCCTGTACTTGTCCCTCAG		
1465	GLTSCR2	0.38	TCCTTGCTCCTGCTCCTGTACTTGT		
1466	FKBP11	0.38	TGGACCCACTGGCTGAGAACACTGG		

TABLE 63

<u>Daunorubicin biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1467	ALDOC	0.41	TGCCTGCTCCTGTACTTGTCCCTCAG		
1468	ITM2A	0.32	GCCCCACTGGACAACACTGATTCT		
1469	SLA	0.41	TCCTTGCTCCTGCTCCTGTACTTGT		

TABLE 63-continued

<u>Daunorubicin biomarkers.</u>					
SEQ ID NO	Gene	Corre- lation	Median	probe	
1470	SSBP2	0.31	TCCTTGCTCCTGTACTTGT		
1471	IL2RG	0.31	TGGACCCCCTGGCTGAGAACACTGG		
1472	MFNG	0.47	TTGGACATCTCTAGTGTAGCTGCCA		
1473	SELL	0.33	TCCTCCATCACCTGAAACACTGGAC		
1474	STC1	0.31	AAATGTTCCCTGTGCCTGCCCTG		
1475	LRMP	0.33	AAGCCTATACGTTCTGTGGAGTAA		
1476	MYB	0.41	GCCCCACTGGACAAACACTGATTCCCT		
1477	PTPN7	0.31	AAATGTTCCCTGTGCCTGCCCTG		
1478	CXorf9	0.38	TGGACCCCCTGGCTGAGAACACTGG		
1479	RHOH	0.32	AAATGTTCCCTGTGCCTGCCCTG		
1480	ZNFN1A1	0.36	CACCCAGCTGGCCTGTGGATGGGA		
1481	CENTB1	0.37	TGGACCCCCTGGCTGAGAACACTGG		
1482	MAP4K1	0.32	TGGACCCCCTGGCTGAGAACACTGG		
1483	CCR7	0.3	TCCTGTACTTGTCCCTCAGCTTGGC		
1484	CD3G	0.33	AAATGTTCCCTGTGCCTGCCCTG		
1485	CCR9	0.33	TGGACCCCCTGGCTGAGAACACTGG		
1486	CBFA2T3	0.31	CACCCAGCTGGCCTGTGGATGGGA		
1487	CXGR4	0.41	AAATGTTCCCTGTGCCTGCCCTG		
1488	ARHGEF6	0.4	TCCTGTACTTGTCCCTCAGCTTGGC		
1489	SELPLG	0.45	TCCTTGCTCCTGTCCCTGTACTTGT		
1490	SEC31L2	0.38	TCCTGTACTTGTCCCTCAGCTTGGC		
1491	CD3Z	0.33	CACCCAGCTGGCCTGTGGATGGGA		
1492	SH2D1A	0.33	TCCTGTACTTGTCCCTCAGCTTGGC		
1493	CD1A	0.35	TGGACCCCCTGGCTGAGAACACTGG		
1494	SCN3A	0.33	CACCCAGCTGGCCTGTGGATGGGA		
1495	LAIR1	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG		
1496	TRB®	0.3	AAATGTTCCCTGTGCCTGCCCTG		
1497	DOCK2	0.35	AAGCCTATACGTTCTGTGGAGTAA		
1498	WBSCR20C	0.38	CACCCAGCTGGCCTGTGGATGGGA		
1499	CD3D	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG		
1500	T3JAM	0.34	CACCCAGCTGGCCTGTGGATGGGA		
1501	CD6	0.33	ACTTGTCCCTCAGCTGGGCTTCTC		
1502	ZAP70	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG		
1503	GPR65	0.33	TCCTGTACTTGTCCCTCAGCTTGGC		
1504	AIF1	0.3	GCCCCACTGGACAAACACTGATTCCCT		

TABLE 63-continued

<u>Daunorubicin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1505	WDR45	0.3	TCCTCCATCACCTGAAACACTGGAC
1506	CD1E	0.3	TCCTTGTGCCTGCTCCTGTACTTGT
1507	CYFIP2	0.39	AAGCCTATACTACGTTCTGTGGAGTAA
1508	TARP	0.38	TTGGACATCTCTAGTGTAGCTGCCA
1509	TRIM	0.34	TCCTGTACTTGTCCCTCAGCTGGGC
1510	ARHGAP15	0.37	ACTTGTCCCTCAGCTGGGCTTCTTC
1511	NOTCH1	0.39	AAGCCTATACTACGTTCTGTGGAGTAA
1512	STAG3	0.35	AAGCCTATACTACGTTCTGTGGAGTAA
1513	UBASH3A	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1514	MGC5566	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
1515	PACAP	0.33	TCCTTGTGCCTGCTCCTGTACTTGT

TABLE 64

<u>Bleomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1516	PFN1	0.32	CACCCAGCTGGCCTGTGGATGGGA
1517	CALU	0.32	ACTTGTCCCTCAGCTTGGGCTTCTTC
1518	ZYX	0.34	CACCCAGCTGGCCTGTGGATGGGA
1519	PSMD2	0.36	GCCCCACTGGACAACACTGATTCC
1520	RAP1B	0.32	TCCTGTACTTGTCCCTCAGCTGGGC
1521	EPAS1	0.35	TCCTGTACTTGTCCCTCAGCTGGC
1522	PGAM1	0.36	GCCCCACTGGACAACACTGATTCC
1523	STAT1	0.38	TGCCTGCTCCTGTACTTGTCCCTCAG
1524	CKAP4	0.38	GCCCCACTGGACAACACTGATTCC
1525	DUSP1	0.32	TCCTGTACTTGTCCCTCAGCTGGC
1526	RCN1	0.32	TCCTCCATCACCTGAAACACTGGAC
1527	UCHL1	0.44	TGGACCCCAGCTGGCTGAGAACACTGG
1528	ITGA5	0.33	AAATGTTCCCTGTGCCTGCTCCTG
1529	NFKBIA	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
1530	LAMB1	0.4	GCCCCACTGGACAACACTGATTCC
1531	TGFBI	0.37	TTGGACATCTCTAGTGTAGCTGCCA
1532	FHL1	0.31	GCCCCACTGGACAACACTGATTCC
1533	GJA1	0.32	TCCTCCATCACCTGAAACACTGGAC

TABLE 64-continued

<u>Bleomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1534	PRG1	0.33	CACCCAGCTGGCCTGTGGATGGGA
1535	EXT1	0.35	CACCCAGCTGGCCTGTGGATGGGA
1536	MVP	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
1537	NNMT	0.38	TGGACCCCAGCTGGCTGAGAACACTGG
1538	TAP1	0.37	TCCTCCATCACCTGAAACACTGGAC
1539	CRIM1	0.41	TGGACCCCAGCTGGCTGAGAACACTGG
1540	PLD2	0.36	GCCCCACTGGACAAACACTGATTCC
1541	RPS19	0.34	TCCTGTACTTGTCCCTCAGCTTGGGC
1542	AXL	0.43	GCCCCACTGGACAAACACTGATTCC
1543	PALM2-AKAP2	0.42	TCCTCCATCACCTGAAACACTGGAC
1544	IL8	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
1545	LOXL2	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
1546	PAPSS2	0.31	CACCCAGCTGGCCTGTGGATGGGA
1547	CAV1	0.31	TCCTGTGCCTGCTCCTGTACTTGT
1548	F2R	0.32	ACTTGTCCCTCAGCTTGGGCTTCTTC
1549	PSMB9	0.38	CACCCAGCTGGCCTGTGGATGGGA
1550	LOX	0.36	TGGACCCCAGCTGGCTGAGAACACTGG
1551	Clorf29	0.36	TCCTGTACTTGTCCCTCAGCTTGGGC
1552	STC1	0.32	TTGGACATCTCTAGTGTAGCTGCCA
1553	LIF	0.34	TCCTGTGCCTGCTCCTGTACTTGT
1554	KCNJ8	0.46	GCCCCACTGGACAAACACTGATTCC
1555	SMAD3	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
1556	HPCAL1	0.45	AAATGTTCCCTGTGCCTGCTCCTG
1557	WNT5A	0.34	TCCTGTGCCTGCTCCTGTACTTGT
1558	BDNF	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
1559	TNFRSF1A	0.38	TCCTGTACTTGTCCCTCAGCTTGGGC
1560	NCOR2	0.45	CACCCAGCTGGCCTGTGGATGGGA
1561	FLNC	0.44	TTGGACATCTCTAGTGTAGCTGCCA
1562	HMGA2	0.41	AAATGTTCCCTGTGCCTGCTCCTG
1563	HLA-B	0.42	AAAGCCTATACTACGTTCTGTGGAGTAA
1564	FLOT1	0.3	AAATGTTCCCTGTGCCTGCTCCTG
1565	PTRF	0.36	CACCCAGCTGGCCTGTGGATGGGA
1566	IFI16	0.32	TCCTGTGCCTGCTCCTGTACTTGT
1567	MGC4083	0.34	TCCTCCATCACCTGAAACACTGGAC
1568	TNFRSF10B	0.4	ACTTGTCCCTCAGCTTGGGCTTCTTC

TABLE 64-continued

<u>Bleomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
1569	PNMA2	0.38	TCCTGTACTTGTCCCTCAGCTGGGC
1570	TFPII	0.32	TCCTGTGCCTGCTCCCTGTACTTGT
1571	CLECSF2	0.33	TGCCTGCTCCGTACTTGTCCCTCAG
1572	SP110	0.34	GCCCCACTGGACAACACTGATTCCCT
1573	PLAUR	0.35	ACTTGTCCCTCAGCTTGGGCTTCTTC
1574	ASPH	0.42	TCCTTGCTGCCTGCTCCGTACTTGT
1575	FSCN1	0.38	TGCCTGCTCCGTACTTGTCCCTCAG
1576	HIC	0.46	TCCTCCATCACCTGAAACACTGGAC
1577	HLA-C	0.34	TGGACCCCCTGGCTGAGAACATCTGG
1578	COL6A1	0.34	TCCTCCATCACCTGAAACACTGGAC
1579	IL6ST	0.45	AAATGTTCCCTTGTGCCTGCTCCTG
1580	IFITM3	0.36	GCCCCACTGGACAACACTGATTCCCT
1581	MAP1B	0.31	TCCTCCATCACCTGAAACACTGGAC
1582	FLJ46603	0.3	ACTTGTCCCTCAGCTTGGGCTTCTTC
1583	RAFTLIN	0.32	GCCCCACTGGACAACACTGATTCCCT
1584	FTL	0.37	CACCCAGCTGGCCTGTGGATGGGA
1585	KIAA0877	0.43	TCCTCCATCACCTGAAACACTGGAC
1586	MT1E	0.41	TGGACCCCCTGGCTGAGAACATCTGG
1587	CDC10	0.32	TGCCTGCTCCGTACTTGTCCCTCAG
1588	ZNF258	0.31	AAATGTTCCCTTGTGCCTGCTCCTG
1589	BCAT1	0.39	TTGGACATCTCTAGTGTAGCTGCCA
1590	IFI44	0.36	AAATGTTCCCTTGTGCCTGCTCCTG
1591	SOD2	0.36	GCCCCACTGGACAACACTGATTCCCT
1592	TMSB10	0.33	TCCTCCATCACCTGAAACACTGGAC
1593	FLJ10350	0.3	TTGGACATCTCTAGTGTAGCTGCCA
1594	Clorf24	0.34	CACCCAGCTGGCCTGTGGATGGGA
1595	EFHD2	0.36	AAATGTTCCCTTGTGCCTGCTCCTG
1596	RPS27L	0.33	AAGCCTATACTGTTCTGTGGAGTAA
1597	TNFRSF12A	0.43	CACCCAGCTGGCCTGTGGATGGGA
1598	FAD104	0.38	TTGGACATCTCTAGTGTAGCTGCCA
1599	RAB7L1	0.58	ACTTGTCCCTCAGCTTGGGCTTCTTC
1600	NME7	0.36	TTGGACATCTCTAGTGTAGCTGCCA
1601	TMEM22	0.34	TTGGACATCTCTAGTGTAGCTGCCA
1602	TPK1	0.31	GCCCCACTGGACAACACTGATTCCCT
1603	ELK3	0.36	TGGACCCCCTGGCTGAGAACATCTGG

TABLE 64-continued

<u>Bleomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
1604	CYLD	0.3	AAGCCTATACTGTTCTGTGGAGTAA
1605	AMIGO2	0.31	ACTTGTCCCTCAGCTTGGCTTCTTC
1606	ADAMTS1	0.43	ACTTGTCCCTCAGCTTGGCTTCTTC
1607	ACTB	0.36	ACTTGTCCCTCAGCTTGGCTTCTTC

TABLE 65

<u>Estramustine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
1608	HSPCB	0.31	ACTTGTCCCTCAGCTTGGCTTCTTC
1609	LDHA	0.42	TGCCTGCTCCGTACTTGTCCCTCAG
1610	TM4SF7	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC

TABLE 66

<u>Chlorambucil biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
1611	CSDA	0.33	TGGACCCCCTGGCTGAGAACATCTGG
1612	INSIG1	0.32	TCCTGTGCCTGCTCCTGTACTTGT
1613	UBE2L6	0.39	TGGACCCCCTGGCTGAGAACATCTGG
1614	PRG1	0.37	TCCTGTGCCTGCTCCTGTACTTGT
1615	ITM2A	0.3	GCCCCACTGGACAACACTGATTCCCT
1616	DGKA	0.38	TCCTTGCGCTGCTCCTGTACTTGT
1617	TFDP2	0.32	AAATGTTCCCTTGTGCCTGCTCCTG
1618	SLA	0.32	TCCTCCATCACCTGAAACACTGGAC
1619	IL2RG	0.44	AAGCCTATACTGTTCTGTGGAGTAA
1620	ALOX5AP	0.45	GCCCCACTGGACAACACTGATTCCCT
1621	GPSM3	0.34	TTGGACATCTCTAGTGTAGCTGCCA
1622	PSMB9	0.36	ACTTGTCCCTCAGCTTGGCTTCTTC
1623	SELL	0.42	TGCCTGCTCCTGTACTTGTCCCTCAG
1624	ADA	0.35	ACTTGTCCCTCAGCTTGGCTTCTTC
1625	EDG1	0.33	AAATGTTCCCTTGTGCCTGCTCCTG
1626	FMNL1	0.3	TCCTCCATCACCTGAAACACTGGAC
1627	PTPN7	0.5	TCCTTGCGCTGCTCCTGTACTTGT
1628	CXorf9	0.41	TGGACCCCCTGGCTGAGAACATCTGG

TABLE 66-continued

<u>Chlorambucil biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1629	RHOH	0.35	TTGGACATCTCTAGTGTAGCTGCCA
1630	ZNFN1A1	0.32	TCCTTGCCCTGCTCCTGTACTTGT
1631	CENTB1	0.47	TCCTGTACTTGTCCCTCAGCTGGGC
1632	LCP2	0.37	TCCTCCATCACCTGAAACACTGGAC
1633	CD1D	0.36	AAGCCTATACTGTTCTGTGGAGTAA
1634	STAT4	0.31	AAATGTTCCCTGTGCCTGCTCCTG
1635	VAV1	0.35	AAATGTTCCCTGTGCCTGCTCCTG
1636	MAP4K1	0.36	TTGGACATCTCTAGTGTAGCTGCCA
1637	CCR7	0.31	TCCTGTACTTGTCCCTCAGCTGGGC
1638	PDE4C	0.42	GCCCCACTGGACAACACTGATTCC
1639	CD3G	0.41	AAGCCTATACTGTTCTGTGGAGTAA
1640	CCR9	0.43	AAGCCTATACTGTTCTGTGGAGTAA
1641	SP110	0.43	TTGGACATCTCTAGTGTAGCTGCCA
1642	TNFAIP8	0.48	TTGGACATCTCTAGTGTAGCTGCCA
1643	LCP1	0.31	AAATGTTCCCTGTGCCTGCTCCTG
1644	IFI16	0.5	TCCTCCATCACCTGAAACACTGGAC
1645	CXCR4	0.37	ACTTGTCCCTCAGCTTGGGCTTCTC
1646	ARHGEF6	0.37	ACTTGTCCCTCAGCTTGGGCTTCTC
1647	SELPLG	0.43	TTGGACATCTCTAGTGTAGCTGCCA
1648	SEC31L2	0.32	TGGACCCCCTGGCTGAGAACACTGG
1649	CD3Z	0.3	AAGCCTATACTGTTCTGTGGAGTAA
1650	PRKCQ	0.31	GGCCCCTGGACAACACTGATTCC
1651	SH2D1A	0.47	ACTTGTCCCTCAGCTTGGGCTTCTC
1652	GZMB	0.48	TGGACCCCCTGGCTGAGAACACTGG
1653	CD1A	0.3	AAGCCTATACTGTTCTGTGGAGTAA
1654	LAIR1	0.32	TTGGACATCTCTAGTGTAGCTGCCA
1655	AF1Q	0.41	TTGGACATCTCTAGTGTAGCTGCCA
1656	TRB@	0.35	TCCTCCATCACCTGAAACACTGGAC
1657	SEPT6	0.35	TGGACCCCCTGGCTGAGAACACTGG
1658	DOCK2	0.39	AAGCCTATACTGTTCTGTGGAGTAA
1659	RPS19	0.41	TTGGACATCTCTAGTGTAGCTGCCA
1660	CD3D	0.4	TTGGACATCTCTAGTGTAGCTGCCA
1661	T3JAM	0.32	TGGACCCCCTGGCTGAGAACACTGG
1662	FNBPI	0.31	GGCCCCTGGACAACACTGATTCC
1663	CD6	0.33	TGGACCCCCTGGCTGAGAACACTGG

TABLE 66-continued

<u>Chlorambucil biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1664	ZAP70	0.52	CACCCAGCTGGCCCTGTGGATGGGA
1665	LST1	0.34	AAATGTTCCCTGTGCCTGCTCCTG
1666	BCAT1	0.35	AAGCCTATACTGTTCTGTGGAGTAA
1667	PRF1	0.4	AAGCCTATACTGTTCTGTGGAGTAA
1668	AIF1	0.3	TTGGACATCTCTAGTGTAGCTGCCA
1669	RAG2	0.38	TGGACCCCCTGGCTGAGAACACTGG
1670	CD1E	0.35	ACTTGTCCCTCAGCTTGGGCTTCTC
1671	CYFIP2	0.38	TTGGACATCTCTAGTGTAGCTGCCA
1672	TARP	0.3	TGGACCCCCTGGCTGAGAACACTGG
1673	TRIM	0.36	CACCCAGCTGGCCCTGTGGATGGGA
1674	GLTSCR2	0.37	TCCTCCATCACCTGAAACACTGGAC
1675	GIMAP5	0.3	ACTTGTCCCTCAGCTTGGGCTTCTC
1676	ARHGAP15	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1677	NOTCH1	0.31	CACCCAGCTGGCCCTGTGGATGGGA
1678	BCL11B	0.3	TCCTTGCCCTGCTCCTGTACTTGT
1679	GIMAP6	0.34	TTGGACATCTCTAGTGTAGCTGCCA
1680	STAG3	0.4	TCCTGTACTTGTCCCTCAGCTTGGG
1681	TM6SF1	0.39	TTGGACATCTCTAGTGTAGCTGCCA
1682	UBASH3A	0.37	TCCTGTACTTGTCCCTCAGCTTGGG
1683	MGC5566	0.36	CACCCAGCTGGCCCTGTGGATGGGA
1684	FLJ22457	0.31	TCCTCCATCACCTGAAACACTGGAC
1685	TPK1	0.33	AAATGTTCCCTGTGCCTGCTCCTG

TABLE 67

<u>Mechlorethamine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1686	PRG1	0.37	GCCCCACTGGACAACACTGATTCC
1687	SLC2A3	0.35	ACTTGTCCCTCAGCTTGGGCTTCTC
1688	RPS19	0.32	ACTTGTCCCTCAGCTTGGGCTTCTC
1689	PSMB10	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
1690	ITM2A	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
1691	DGKA	0.37	TGCCTGCTCCTGTACTTGTCCCTCAG
1692	SEMA4D	0.34	TCCTCCATCACCTGAAACACTGGAC

TABLE 67-continued

<u>Mechlorethamine biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Median	probe
1693	SLA	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG	
1694	IL2RG	0.3	TGGACCCCCTGGCTGAGAAATCTGG	
1695	MFNG	0.42	AAGCCTATACTACGTTCTGTGGAGTAA	
1696	ALOX5AP	0.31	AAGCCTATACTACGTTCTGTGGAGTAA	
1697	GPSM3	0.34	AAGCCTATACTACGTTCTGTGGAGTAA	
1698	PSMB9	0.36	ACTTGTCCTCAGCTTGGCCTCTTC	
1699	SELL	0.34	CACCCAGCTGGCCTGTGGATGGGA	
1700	ADA	0.35	AAATGTTCCCTGTGCCTGCTCCTG	
1701	FMNL1	0.4	CACCCAGCTGGCCTGTGGATGGGA	
1702	MYB	0.34	TGGACCCCCTGGCTGAGAAATCTGG	
1703	PTPN7	0.43	AAGCCTATACTACGTTCTGTGGAGTAA	
1704	CXorf9	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG	
1705	RHOH	0.33	TTGGACATCTCTAGTGTAGCTGCCA	
1706	ZNFXN1A1	0.31	TCCTCCCATCACCTGAAACACTGGAC	
1707	CENTB1	0.43	TCCTCCCATCACCTGAAACACTGGAC	
1708	FXYD2	0.35	TTGGACATCTCTAGTGTAGCTGCCA	
1709	CD1D	0.4	TTGGACATCTCTAGTGTAGCTGCCA	
1710	STAT4	0.44	TTGGACATCTCTAGTGTAGCTGCCA	
1711	MAP4K1	0.34	GCCCCACTGGACAACACTGATTCCCT	
1712	CCR7	0.39	TGGACCCCCTGGCTGAGAAATCTGG	
1713	PDE4C	0.33	TCCTTGTGCCTGCTCCTGTACTTGT	
1714	CD3G	0.4	GCCCCACTGGACAACACTGATTCCCT	
1715	CCR9	0.34	TGGACCCCCTGGCTGAGAAATCTGG	
1716	SP110	0.31	AAATGTTCCCTGTGCCTGCTCCTG	
1717	TK2	0.33	TCCTGTACTTGTCCCTCAGCTTGGGC	
1718	TNFAIP8	0.34	GCCCCACTGGACAACACTGATTCCCT	
1719	NAP1L1	0.35	TCCTGTACTTGTCCCTCAGCTTGGGC	
1720	SELPLG	0.35	TCCTGTACTTGTCCCTCAGCTTGGGC	
1721	SEC31L2	0.38	TGCCTGCTCCTGTACTTGTCCCTCAG	
1722	CD3Z	0.44	TTGGACATCTCTAGTGTAGCTGCCA	
1723	PRKCQ	0.37	TCCTGTACTTGTCCCTCAGCTTGGGC	
1724	SH2D1A	0.41	GCCCCACTGGACAACACTGATTCCCT	
1725	GZMB	0.43	TGGACCCCCTGGCTGAGAAATCTGG	
1726	CD1A	0.39	TGGACCCCCTGGCTGAGAAATCTGG	
1727	LAIR1	0.35	TGGACCCCCTGGCTGAGAAATCTGG	

TABLE 67-continued

<u>Mechlorethamine biomarkers.</u>				
SEQ ID NO	Gene	Corre- lation	Median	probe
1728	TRB [@]	0.33	TTGGACATCTCTAGTGTAGCTGCCA	
1729	SEPT6	0.3	CACCCAGCTGGCCTGTGGATGGGA	
1730	DOCK2	0.34	TGGACCCCCTGGCTGAGAAATCTGG	
1731	CG018	0.33	TGGACCCCCTGGCTGAGAAATCTGG	
1732	WBSCR20C	0.34	TCCTGTACTTGTCCCTCAGCTTGGGC	
1733	CD3D	0.33	ACTTGTCCTCAGCTGGCCTCTTC	
1734	CD6	0.32	AAGCCTATACTACGTTCTGTGGAGTAA	
1735	LST1	0.33	TTGGACATCTCTAGTGTAGCTGCCA	
1736	GPR65	0.42	AAGCCTATACTACGTTCTGTGGAGTAA	
1737	PRF1	0.34	CACCCAGCTGGCCTGTGGATGGGA	
1738	ALMS1	0.41	TCCTGTACTTGTCCCTCAGCTTGGGC	
1739	AIF1	0.31	GCCCCACTGGACAAACACTGATTCCCT	
1740	CD1E	0.31	CACCCAGCTGGCCTGTGGATGGGA	
1741	CYFIP2	0.33	GCCCCACTGGACAAACACTGATTCCCT	
1742	TARP	0.31	AAATGTTCCCTGTGCCTGCTCCTG	
1743	GLTSCR2	0.31	AAGCCTATACTACGTTCTGTGGAGTAA	
1744	FLJ12270	0.34	TGGACCCCCTGGCTGAGAAATCTGG	
1745	ARHGAP15	0.33	GCCCCACTGGACAAACACTGATTCCCT	
1746	NAP1L2	0.32	GCCCCACTGGACAAACACTGATTCCCT	
1747	CECR1	0.34	TCCTTGTGCCTGCTCCTGTACTTGT	
1748	GIMAP6	0.35	TCCTGTACTTGTCCCTCAGCTTGGGC	
1749	STAG3	0.33	CACCCAGCTGGCCTGTGGATGGGA	
1750	TM6SF1	0.3	CACCCAGCTGGCCTGTGGATGGGA	
1751	C15orf25	0.36	TTGGACATCTCTAGTGTAGCTGCCA	
1752	MGC5566	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC	
1753	FLJ22457	0.34	AAATGTTCCCTGTGCCTGCTCCTG	
1754	ET	0.32	CACCCAGCTGGCCTGTGGATGGGA	
1755	TPK1	0.34	CACCCAGCTGGCCTGTGGATGGGA	
1756	PHF11	0.36	TTGGACATCTCTAGTGTAGCTGCCA	

TABLE 68

<u>Streptozocin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1757	PGK1	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
1758	SCD	0.31	TGGACCCCACCTGGCTGAGAACATCTGG
1759	INSIG1	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
1760	IGBP1	0.39	TCCTCCATCACCTGAAACACTGGAC
1761	TNFAIP3	0.31	TCCTCCATCACCTGAAACACTGGAC
1762	TNFSF10	0.31	CACCCAGCTGGTCCTGTGGATGGGA
1763	ABCA1	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
1764	AGA	0.31	TGGACCCCACCTGGCTGAGAACATCTGG
1765	ABCA8	0.31	CACCCAGCTGGTCCTGTGGATGGGA
1766	DBC1	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1767	PTGER2	0.32	ACTTGTCCCTCAGCTTGGGCTTCTTC
1768	UGT1A3	0.32	TCCTCCATCACCTGAAACACTGGAC
1769	C10orf10	0.3	CACCCAGCTGGTCCTGTGGATGGGA
1770	TM4SF13	0.34	TGGACCCCACCTGGCTGAGAACATCTGG
1771	CGI-90	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
1772	LXN	0.31	AAGCCTATACTGTTCTGTGGAGTAA
1773	DNAJC12	0.35	TTGGACATCTCTAGTGTAGCTGCCA
1774	HIPK2	0.31	CACCCAGCTGGTCCTGTGGATGGGA
1775	C9orf95	0.36	ACTTGTCCCTCAGCTTGGGCTTCTTC

TABLE 69

<u>Carmustine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1776	RPLP2	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
1777	CD99	0.31	ACTTGTCCCTCAGCTTGGGCTTCTTC
1778	IFITM1	0.36	TCCTCCATCACCTGAAACACTGGAC
1779	INSIG1	0.31	TCCTCCATCACCTGAAACACTGGAC
1780	ALDOC	0.4	TGCCTGCTCCTGTACTTGTCCCTCAG
1781	ITM2A	0.33	TCCTTGTGCCTGCTCCTGTACTTGT
1782	SERPINAI1	0.39	TTGGACATCTCTAGTGTAGCTGCCA
1783	C1QR1	0.35	AAGCCTATACTGTTCTGTGGAGTAA
1784	STAT5A	0.39	TTGGACATCTCTAGTGTAGCTGCCA
1785	INPP5D	0.44	TCCTTGTGCCTGCTCCTGTACTTGT
1786	SATB1	0.36	AAATGTTCCCTGTGCCTGCTCCTG

TABLE 69-continued

<u>Carmustine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1787	VPS16	0.31	AAATGTTCCCTGTGCCTGCTCCTG
1788	SLA	0.37	TCCTGTACTTGTCCCTCAGCTTGGGC
1789	IL2RG	0.45	TCCTCCATCACCTGAAACACTGGAC
1790	MFNG	0.33	TCCTCCATCACCTGAAACACTGGAC
1791	SELL	0.38	AAGCCTATACTGTTCTGTGGAGTAA
1792	LRMP	0.41	GCCCCACTGGACAAACACTGATTCC
1793	ICAM2	0.54	TCCTCCATCACCTGAAACACTGGAC
1794	MYB	0.31	ACTTGTCCCTCAGCTTGGGCTTCTTC
1795	PTPN7	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
1796	ARHGAP25	0.42	TTGGACATCTCTAGTGTAGCTGCCA
1797	LCK	0.41	TGGACCCCACGGCTGAGAACATCTGG
1798	CXorf9	0.35	TGGACCCCACGGCTGAGAACATCTGG
1799	RHOH	0.41	AAATGTTCCCTGTGCCTGCTCCTG
1800	ZNFN1A1	0.37	TGCCTGCTCCTGTACTTGTCCCTCAG
1801	CENTB1	0.59	ACTTGTCCCTCAGCTTGGGCTTCTTC
1802	ADD2	0.34	TCCTTGTGCCTGCTCCTGTACTTGT
1803	LCP2	0.33	TTGGACATCTCTAGTGTAGCTGCCA
1804	SFI1	0.39	TGCCTGCTCCTGTACTTGTCCCTCAG
1805	DBT	0.42	ACTTGTCCCTCAGCTTGGGCTTCTTC
1806	GZMA	0.34	CACCCAGCTGGCCTGTGGATGGGA
1807	CD2	0.36	TGGACCCCACGGCTGAGAACATCTGG
1808	BATF	0.38	ACTTGTCCCTCAGCTTGGGCTTCTTC
1809	HIST1H4C	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
1810	ARHGAP6	0.4	TCCTCCATCACCTGAAACACTGGAC
1811	VAV1	0.42	TGGACCCCACGGCTGAGAACATCTGG
1812	MAP4K1	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
1813	CCR7	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
1814	PDE4C	0.57	TCCTCCATCACCTGAAACACTGGAC
1815	CD3G	0.44	AAGCCTATACTGTTCTGTGGAGTAA
1816	CCR9	0.37	AAATGTTCCCTGTGCCTGCTCCTG
1817	SP140	0.48	TCCTGTACTTGTCCCTCAGCTTGGGC
1818	TK2	0.31	TGGACCCCACGGCTGAGAACATCTGG
1819	LCP1	0.38	TCCTCCATCACCTGAAACACTGGAC
1820	IFI16	0.34	GCCCCACTGGACAAACACTGATTCC
1821	CXCR4	0.42	GCCCCACTGGACAAACACTGATTCC

TABLE 69-continued

<u>Carmustine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1822	ARHGEF6	0.45	AAATGTTCCCTGTGCCTGCTCCTG
1823	PSCDBP	0.42	TCCTGTACTTGTCCCTCAGCTGGC
1824	SELPLG	0.52	TGGACCCCACGGCTGAGAACACTGG
1825	SEC31L2	0.42	TCCTGTACTTGTCCCTCAGCTGGC
1826	CD3Z	0.34	TCCTCCATCACCTGAAACACTGGAC
1827	PRKCQ	0.46	TCCTTGCGCTGCTCCCTGACTTGT
1828	SH2D1A	0.46	TGCCTGCTCCTGTACTTGTCCAG
1829	GZMB	0.55	TTGGACATCTCTAGTGTAGCTGCCA
1830	CD1A	0.34	TCCTTGCGCTGCTCCCTGACTTGT
1831	GATA2	0.41	TTGGACATCTCTAGTGTAGCTGCCA
1832	LY9	0.54	TCCTCCATCACCTGAAACACTGGAC
1833	LAIR1	0.3	TTGGACATGTCTAGTGTAGCTGCCA
1834	TRB@	0.33	TCCTCCATCACCTGAAACACTGGAC
1835	SEPT6	0.32	ACTTGTCCCTCAGCTTGGGCTTCTC
1836	HA-1	0.32	TCCTCCATCACCTGAAACACTGGAC
1837	SLC43A3	0.32	TCCTGTACTTGTCCCTCAGCTTGGC
1838	DOCK2	0.31	TCCTCCATCACCTGAAACACTGGAC
1839	CG018	0.42	ACTTGTCCCTCAGCTTGGGCTTCTC
1840	MLC1	0.33	TGCCTGCTCCTGTACTTGTCCAG
1841	CD3D	0.35	TCCTGTACTTGTCCCTCAGCTTGGC
1842	T3JAM	0.34	CACCCAGTGGCTCTGTGGATGGGA
1843	CD6	0.43	TCCTGTACTTGTCCCTCAGCTTGGC
1844	ZAP70	0.43	GCCCCACTGGACAACACTGATTCT
1845	DOK2	0.3	TCCTGTACTTGTCCCTCAGCTTGGC
1846	LST1	0.36	TCCTGTACTTGTCCCTCAGCTTGGC
1847	GPR65	0.31	TCCTGTACTTGTCCCTCAGCTTGGC
1848	PRF1	0.32	TCCTTGCGCTGCTCCCTGACTTGT
1849	ALMS1	0.38	TTGGACATCTCTAGTGTAGCTGCCA
1850	AIF1	0.31	TCCTTGCGCTGCTCCCTGACTTGT
1851	PRDX2	0.48	GCCCCACTGGACAACACTGATTCT
1852	FLJ12151	0.36	AAATGTTCCCTGTGCCTGCTCCTG
1853	FBXW12	0.37	TGCCTGCTCCTGTACTTGTCCAG
1854	CD1E	0.34	AAGCCTATACGTTCTGTGGAGTAA
1855	CYFIP2	0.3	TCCTTGCGCTGCTCCCTGACTTGT
1856	TARP	0.33	TCCTGTACTTGTCCCTCAGCTTGGC

TABLE 69-continued

<u>Carmustine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1857	TRIM	0.38	CACCCAGCTGGCCTGTGGATGGGA
1858	RPL10L	0.43	AAATGTTCCCTGTGCCTGCTCCTG
1859	GLTSCR2	0.43	CACCCAGCTGGCCTGTGGATGGGA
1860	CKIP-1	0.33	TCCTGTGCCTGCTCCTGACTTGT
1861	NRN1	0.3	TCCTTGCGCTGCTCCTGACTTGT
1862	ARHGAP15	0.4	TCCTCCATCACCTGAAACACTGGAC
1863	NOTCH1	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
1864	PSCD4	0.4	CACCCAGCTGGCCTGTGGATGGGA
1865	C13orf18	0.31	AAGCCTATACGTTCTGTGGAGTAA
1866	BCL11B	0.35	ACTTGTCCCTCAGCTTGGGCTTCTC
1867	GIMAP6	0.33	ACTTGTCCCTCAGCTTGGGCTTCTC
1868	STAG3	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
1869	NARF	0.34	TCCTGTACTTGTCCCTCAGCTTGGGC
1870	TM6SF1	0.48	TCCTCCATCACCTGAAACACTGGAC
1871	C15orf25	0.32	TCCTGTACTTGTCCCTCAGCTTGGC
1872	FLJ11795	0.35	GCCCCACTGGACAAACACTGATTCC
1873	SAMSN1	0.37	GCCCCACTGGACAAACACTGATTCC
1874	UBASH3A	0.4	TCCTGTACTTGTCCCTCAGCTTGGC
1875	PACAP	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1876	LEF1	0.3	CACCCAGCTGGCCTGTGGATGGGA
1877	IL21R	0.34	AAGCCTATACGTTCTGTGGAGTAA
1878	TCF4	0.41	GCCCCACTGGACAAACACTGATTCC
1879	DKFZP434B0335	0.33	TCCTGTACTTGTCCCTCAGCTTGGC

TABLE 70

<u>Lomustine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1880	RPS15	0.43	TCCTGTACTTGTCCCTCAGCTTGGC
1881	INSIG1	0.31	TGGACCCCACGGCTGAGAACACTGG
1882	ALDOC	0.39	TGCCTGCTCCTGTACTTGTCCCTCAG
1883	ITM2A	0.32	TCCTCCATCACCTGAAACACTGGAC
1884	C1QR1	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
1885	STAT5A	0.37	TGCCTGCTCCTGTACTTGTCCCTCAG

TABLE 70-continued

<u>Lomustine biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
1886	INPP5D	0.32	TCCTGTACTTGTCCCTCAGCTGGGC
1887	VPS16	0.32	TGCGCTGCCCTGTACTTGTCCCTCAG
1888	SLA	0.32	TGCGCTGCCCTGTACTTGTCCCTCAG
1889	USP20	0.41	ACTTGTCCCTCAGCTTGGGCTTCTTC
1890	IL2RG	0.31	TCCTGTACTTGTCCCTCAGCTGGGC
1891	MFNG	0.4	ACTTGTCCCTCAGCTTGGGCTTCTTC
1892	LRMP	0.43	GCCCCACTGGACAACACTGATTCCCT
1893	EVI2A	0.35	ACTTGTCCCTCAGCTTGGGCTTCTTC
1894	PTPN7	0.35	TCCTTGCCCTGCTCCCTGTACTTGT
1895	ARHGAP25	0.39	TCCTGTACTTGTCCCTCAGCTGGGC
1896	RHOH	0.31	AAGCCTATACTGTTCTGTGGAGTAA
1897	ZNFN1A1	0.31	TCCTGTACTTGTCCCTCAGCTGGGC
1898	CENTB1	0.35	TCCTGTACTTGTCCCTCAGCTGGGC
1899	LCP2	0.41	TGGACCCCCACTGGCTGAGAACACTGG
1900	SPI1	0.35	TGCGCTGCCCTGTACTTGTCCCTCAG
1901	ARHGAP6	0.33	TTGGACATCTCTAGTGTAGCTGCCA
1902	MAP4K1	0.34	CACCCAGCTGGCCCTGTGGATGGGA
1903	CCR7	0.35	TCCTCCATCACCTGAAACACTGGAC
1904	LY96	0.35	GCCCCACTGGACAACACTGATTCCCT
1905	C6orf32	0.32	ACTTGTCCCTCAGCTTGGGCTTCTTC
1906	MAGEA1	0.31	AAATGTTCCCTGTGCCTGCTCCTG
1907	SP140	0.35	TTGGACATCTCTAGTGTAGCTGCCA
1908	LCP1	0.36	TCCTCCATCACCTGAAACACTGGAC
1909	IFI16	0.39	TGCGCTGCCCTGTACTTGTCCCTCAG
1910	ARHGEF6	0.33	TCCTCCATCACCTGAAACACTGGAC
1911	PSCDBP	0.43	AAGCCTATACTGTTCTGTGGAGTAA
1912	SEPLPLG	0.3	ACTTGTCCCTCAGCTTGGGCTTCTTC
1913	CD3Z	0.35	AAGCCTATACTGTTCTGTGGAGTAA
1914	PRKCQ	0.4	TCCTTGCCCTGCTCCCTGTACTTGT
1915	GZMB	0.31	AAGCCTATACTGTTCTGTGGAGTAA
1916	LAIR1	0.38	TGGACCCCCACTGGCTGAGAACACTGG
1917	SH2D1A	0.36	TCCTGTACTTGTCCCTCAGCTGGGC
1918	TRB@	0.39	TTGGACATCTCTAGTGTAGCTGCCA
1919	RFP	0.35	TGCGCTGCCCTGTACTTGTCCCTCAG
1920	SEPT6	0.41	TCCTCCATCACCTGAAACACTGGAC

TABLE 70-continued

<u>Lomustine biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
1921	HA-1	0.43	TCCTGTACTTGTCCCTCAGCTTGGGC
1922	SLC43A3	0.4	ACTTGTCCCTCAGCTGGCTTCTTC
1923	CD3D	0.32	TCCTTGCCCTGCTCCCTGTACTTGT
1924	T3JAM	0.3	TGGACCCCCACTGGCTGAGAACACTGG
1925	GPR65	0.34	GCCCCACTGGACAAACACTGATTCCCT
1926	PRF1	0.36	TGCCTGCTCCTGTACTTGTCCCTCAG
1927	AIF1	0.33	TGGACCCCCACTGGCTGAGAACACTGG
1928	LPXN	0.38	AAATGTTCCCTGTGCCTGCTCCTG
1929	RPL10L	0.3	TGGACCCCCACTGGCTGAGAACACTGG
1930	SITPEC	0.36	CACCCAGCTGGCCTGTGGATGGGA
1931	ARHGAP15	0.33	TGGACCCCCACTGGCTGAGAACACTGG
1932	C13orf18	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC
1933	NARF	0.35	TGGACCCCCACTGGCTGAGAACACTGG
1934	TM6SF1	0.34	TCCTTGCCCTGCTCCCTGTACTTGT
1935	PACAP	0.31	AAGCCTATACTGTTCTGTGGAGTAA
1936	TCF4	0.33	TCCTTGCCCTGCTCCCTGTACTTGT
<u>Mercaptopurine biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
1937	SSRP1	0.31	GCCCCACTGGACAAACACTGATTCCCT
1938	ALDOC	0.36	AAATGTTCCCTGTGCCTGCTCCTG
1939	C1QRL	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1940	TTF1	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
1941	PRIM1	0.31	GCCCCACTGGACAAACACTGATTCCCT
1942	USP34	0.38	TCCTCCATCACCTGAAACACTGGAC
1943	TK2	0.33	TCCTGTACTTGTCCCTCAGCTTGGGC
1944	GOLGIN-67	0.31	TGCCTGCTCCTGTACTTGTCCCTCAG
1945	N2D014	0.35	ACTTGTCCCTCAGCTTGGGCTTCTTC
1946	KIAA0220	0.31	TCCTCCATCACCTGAAACACTGGAC
1947	SLC43A3	0.3	TTGGACATCTCTAGTGTAGCTGCCA
1948	WBSR20C	0.3	CACCCAGCTGGCCTGTGGATGGGA
1949	ICAM2	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
1950	TEX10	0.32	TGGACCCCCACTGGCTGAGAACACTGG

TABLE 71-continued

<u>Mercaptopurine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1951	CHD7	0.3	ACTTGTCCCTCAGCTTGGGCTTC
1952	SAMSN1	0.34	TTGGACATCTCTAGTGTAGCTGCCA
1953	TPRT	0.35	ACTTGTCCCTCAGCTTGGGCTTC

TABLE 72

<u>Teniposide biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1954	CD99	0.35	TGCCTGCTCCTGTACTTGTCC
1955	INSIG1	0.35	AAGCCTATACTGTTCTGTGGAGTAA
1956	PRG1	0.36	TGCCTGCTCCTGTACTTGTCC
1957	ALDOC	0.3	ACTTGTCCCTCAGCTTGGGCTTC
1958	ITM2A	0.33	AAGCCTATACTGTTCTGTGGAGTAA
1959	SLA	0.43	GCCCCACTGGACAACACTGATTCT
1960	SSBP2	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
1961	IL2RG	0.37	AAATGTTCCCTGTGCCTGCTC
1962	MFNG	0.32	TTGGACATCTCTAGTGTAGCTGCCA
1963	ALOX5AP	0.32	TCCTCCATCACCTGAAAACACTGGAC
1964	C1orf29	0.3	TCCTCCATCACCTGAAAACACTGGAC
1965	SELL	0.33	CACCCAGCTGGCCTGTGGATGGGA
1966	STC1	0.47	TGGACCCCAGTGGCTGAGAACACTGG
1967	LRMP	0.33	TCCTCCATCACCTGAAAACACTGGAC
1968	MYB	0.33	TGCCTGCTCCTGTACTTGTCC
1969	PTPN7	0.34	AAGCCTATACTGTTCTGTGGAGTAA
1970	CXorf9	0.42	TTGGACATCTCTAGTGTAGCTGCCA
1971	RHOH	0.31	AAGCCTATACTGTTCTGTGGAGTAA
1972	ZNFN1A1	0.34	CACCCAGCTGGCCTGTGGATGGGA
1973	CENTB1	0.37	TGGACCCCAGTGGCTGAGAACACTGG
1974	ADD2	0.31	TGCCTGCTCCTGTACTTGTCC
1975	CD1D	0.37	ACTTGTCCCTCAGCTTGGGCTTC
1976	BATF	0.32	TCCTGTACTTGTCCCTCAGCTTGGC
1977	MAP4K1	0.3	GCCCCACTGGACAACACTGATTCT
1978	CCR7	0.48	TCCTGTACTTGTCCCTCAGCTTGGC
1979	PDE4C	0.33	TGGACCCCAGTGGCTGAGAACACTGG

TABLE 72-continued

<u>Teniposide biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
1980	CD3G	0.33	TTGGACATCTCTAGTGTAGCTGCCA
1981	CCR9	0.36	ACTTGTCCCTCAGCTTGGCTTC
1982	SP110	0.34	AAATGTTCCCTGTGCCTGCTC
1983	TNFAIP8	0.31	ACTTGTCCCTCAGCTTGGCTTC
1984	NAP1L1	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1985	CXCR4	0.31	ACTTGTCCCTCAGCTTGGCTTC
1986	ARHGEF6	0.31	TCCTGTACTTGTCCCTCAGCTTGGC
1987	GATA3	0.36	TCCTGTACTTGTCCCTCAGCTTGGC
1988	SEPLG	0.38	AAGCCTATACTGTTCTGTGGAGTAA
1989	SEC31L2	0.46	TGGACCCCAGTGGCTGAGAACACTGG
1990	CD3Z	0.35	GCCCCACTGGACAACACTGATTCT
1991	SH2D1A	0.45	AAATGTTCCCTGTGCCTGCTC
1992	GZMB	0.35	TCCTTGTGCCTGCTCCTGTACTTGT
1993	CD1A	0.45	GCCCCACTGGACAACACTGATTCT
1994	SCN3A	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
1995	LAIR1	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
1996	AF1Q	0.3	TCCTCCATCACCTGAAACACTGGAC
1997	TRB@	0.32	AAGCCTATACTGTTCTGTGGAGTAA
1998	DOCK2	0.33	TCCTGTACTTGTCCCTCAGCTTGGC
1999	MLC1	0.31	TCCTCCATCACCTGAAACACTGGAC
2000	CD3D	0.31	TGGACCCCAGTGGCTGAGAACACTGG
2001	T3JAM	0.31	ACTTGTCCCTCAGCTTGGCTTC
2002	CD6	0.38	TCCTGTCCCTGTACTTGTCC
2003	ZAP70	0.34	TCCTCCATCACCTGAAACACTGGAC
2004	IFI44	0.37	TCCTTGTGCCTGCTCCTGTACTTGT
2005	GPR65	0.34	TCCTGTACTTGTCCCTCAGCTTGGC
2006	PRF1	0.34	TCCTCCATCACCTGAAACACTGGAC
2007	AIF1	0.33	ACTTGTCCCTCAGCTTGGCTTC
2008	WDR45	0.41	GCCCCACTGGACAACACTGATTCT
2009	CD1E	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2010	CYFIP2	0.32	TGGACCCCAGTGGCTGAGAACACTGG
2011	TARP	0.42	CACCCAGCTGGCCTGTGGATGGGA
2012	TRIM	0.33	TGGACCCCAGTGGCTGAGAACACTGG
2013	ARHGAP15	0.38	AAGCCTATACTGTTCTGTGGAGTAA
2014	NOTCH1	0.32	TCCTGTCCCTGTACTTGTCC

TABLE 72-continued

<u>Teniposide biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
2015	STAG3	0.32	GCCCCACTGGACAACACTGATTCCCT
2016	NARF	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2017	TM6SF1	0.33	GCCCCACTGGACAACACTGATTCCCT
2018	UBASH3A	0.33	TCCTGTACTTGTCCCTCAGCTTGGGC
2019	MGC5566	0.31	AGTTGTCCCTCAGCTTGGGCTTCTTC

TABLE 73

<u>Dactinomycin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
2020	ALDOC	0.37	GCCCCACTGGACAACACTGATTCCCT
2021	C1QR1	0.36	TGGACCCCAGCTGGCTGAGAACATCTGG
2022	SLA	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
2023	WBSCR20A	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2024	MFNG	0.3	ACTTGTCCCTCAGCTTGGGCTTCTTC
2025	SELL	0.3	GCCCCACTGGACAACACTGATTCCCT
2026	MYB	0.36	TTGGACATCTCTAGTGTAGCTGCCA
2027	RHOH	0.32	TCCTTGTCCTGCTCCTGTACTTGT
2028	ZNFN1A1	0.3	AAATGTTCCCTTGTGCCTGCTCCTG
2029	LCP2	0.3	CACCCAGCTGGCCTGTGGATGGGA
2030	MAP4K1	0.34	AAGCCTATACTGTTCTGTGGAGTAA
2031	CBFA2T3	0.35	TCCTTGTCCTGCTCCTGTACTTGT
2032	LCP1	0.32	GCCCCACTGGACAACACTGATTCCCT
2033	SELPLG	0.33	ACTTGTCCCTCAGCTTGGGCTTCTTC
2034	CD3Z	0.35	GCCCCACTGGACAACACTGATTCCCT
2035	LAIR1	0.33	TGGACCCCAGCTGGCTGAGAACATCTGG
2036	WBSCR20C	0.3	AAGCCTATACTGTTCTGTGGAGTAA
2037	CD3D	0.35	CACCCAGCTGGCCTGTGGATGGGA
2038	GPR65	0.32	AAATGTTCCCTTGTGCCTGCTCCTG
2039	ARHGAP15	0.32	TCCTCCATCACCTGAAACACTGGAC
2040	FLJ10178	0.36	TCCTTGTCCTGCTCCTGTACTTGT
2041	NARF	0.35	TCCTCCATCACCTGAAACACTGGAC
2042	PUS3	0.32	TCCTGTACTTGTCCCTCAGCTTGGGC

TABLE 74

<u>Tretinooin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
2043	PPIB	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2044	ZFP36L2	0.48	AAGCCTATACTGTTCTGTGGAGTAA
2045	IFI30	0.46	ACTTGTCCCTCAGCTGGCTCTTC
2046	USP7	0.35	TCCTCCATCACCTGAAACACTGGAC
2047	SRM	0.43	TCCTCCATCACCTGAAACACTGGAC
2048	SH3BP5	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
2049	ALDOC	0.41	TTGGACATCTCTAGTGTAGCTGCCA
2050	FADS2	0.33	ACTTGTCCCTCAGCTGGCTCTTC
2051	GUSB	0.38	TTGGACATCTCTAGTGTAGCTGCCA
2052	PSCD1	0.48	TCCTGTACTTGTCCCTCAGCTTGGGC
2053	IQGAP2	0.34	TCCTGTACTTGTCCCTCAGCTTGGGC
2054	STS	0.34	GCCCCACTGGACAACACTGATTCCCT
2055	MFNG	0.36	TGGACCCCAGCTGGCTGAGAACATCTGG
2056	FLI1	0.33	ACTTGTCCCTCAGCTGGCTCTTC
2057	PIM2	0.35	TGGACCCCAGCTGGCTGAGAACATCTGG
2058	INPP4A	0.54	TCCTGTACTTGTCCCTCAGCTTGGGC
2059	LRMP	0.51	GCCCCACTGGACAACACTGATTCCCT
2060	ICAM2	0.3	AAATGTTCCCTTGTGCCTGCTCCTG
2061	EVI2A	0.33	CACCCAGCTGGCCTGTGGATGGGA
2062	MAL	0.46	AAATGTTCCCTTGTGCCTGCTCCTG
2063	BTN3A3	0.43	TTGGACATCTCTAGTGTAGCTGCCA
2064	PTPN7	0.4	TTGGACATCTCTAGTGTAGCTGCCA
2065	IL10RA	0.42	TTGGACATCTCTAGTGTAGCTGCCA
2066	SPI1	0.41	AAGCCTATACTGTTCTGTGGAGTAA
2067	TRAF1	0.3	TGCCTGCTCCTGTACTTGTCCCTCAG
2068	ITGB7	0.33	TCCTTGTCCTGCTCCTGTACTTGT
2069	ARHGAP6	0.32	TGGACCCCAGCTGGCTGAGAACATCTGG
2070	MAP4K1	0.52	GCCCCACTGGACAACACTGATTCCCT
2071	CD28	0.34	AAGCCTATACTGTTCTGTGGAGTAA
2072	PTP4A3	0.3	TCCTCCATCACCTGAAACACTGGAC
2073	LTB	0.32	ACTTGTCCCTCAGCTGGCTCTTC
2074	C1orf38	0.4	TGCCTGCTCCTGTACTTGTCCCTCAG
2075	WBSCR22	0.53	TCCTCCATCACCTGAAACACTGGAC
2076	CD8B1	0.35	TCCTCCATCACCTGAAACACTGGAC
2077	LCP1	0.35	ACTTGTCCCTCAGCTGGCTCTTC

TABLE 74-continued

<u>Tretinoind biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
2078	FLJ13052	0.31	TCCTCCATCACCTGAAACACTGGAC
2079	MEF2C	0.71	TTGGACATCTCTAGTGTAGCTGCCA
2080	PSCDBP	0.41	AAATGTTCCCTGTGCCTGCTCCTG
2081	IL16	0.51	TGGACCCCACTGGCTGAGAACACTGG
2082	SELPLG	0.53	TGCCTGCTCCTGTACTTGCCCTCAG
2083	MAGEA9	0.6	AAATGTTCCCTGTGCCTGCTCCTG
2084	LAIR1	0.43	TCCTCCATCACCTGAAACACTGGAC
2085	TNFRSF25	0.53	TCCTCCATCACCTGAAACACTGGAC
2086	EVI2B	0.42	ACTTGTCCCTCAGCTTGGGCTTCTC
2087	IGJ	0.37	TCCTTGTGCCTGCTCCTGTACTTGT
2088	PDCD4	0.47	AAATGTTCCCTGTGCCTGCTCCTG
2089	RASA4	0.52	CACCCAGCTGGCCTGTGGATGGGA
2090	HA-1	0.73	AAGCCTATACTGTTCTGTGGAGTAA
2091	PLCL2	0.47	TCCTGTACTTGTCCCTCAGCTTGGGC
2092	RNASE6	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2093	WBSCR20C	0.35	TTGGACATCTCTAGTGTAGCTGCCA
2094	NUP210	0.36	AAGCCTATACTGTTCTGTGGAGTAA
2095	RPL10L	0.39	ACTTGTCCCTCAGCTTGGGCTTCTC
2096	C11orf2	0.33	TGGACCCACTGGCTGAGAACACTGG
2097	CABC1	0.32	TGCCTGCTCCTGTACTTGCCCTCAG
2098	ARHGEF3	0.37	TCCTGTACTTGTCCCTCAGCTTGGGC
2099	TAPBPL	0.42	TGCCTGCTCCTGTACTTGCCCTCAG
2100	CHST12	0.35	AAATGTTCCCTGTGCCTGCTCCTG
2101	FKBP11	0.54	TGCCTGCTCCTGTACTTGCCCTCAG
2102	FLJ35036	0.42	TTGGACATCTCTAGTGTAGCTGCCA
2103	MYLIP	0.38	CACCCAGCTGGCCTGTGGATGGGA
2104	TXND5	0.31	ACTTGTCCCTCAGCTTGGGCTTCTC
2105	PACAP	0.3	TCCTCCATCACCTGAAACACTGGAC
2106	TOSO	0.34	TCCTGTACTTGTCCCTCAGCTTGGGC
2107	PNAS-4	0.37	TGGACCCACTGGCTGAGAACACTGG
2108	IL21R	0.57	AAGCCTATACTGTTCTGTGGAGTAA
2109	TCF4	0.64	TCCTTGTGCCTGCTCCTGTACTTGT

TABLE 75

<u>Ifosfamide biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
2110	ARHGDI1B	0.36	TGGACCCCCTGGCTGAGAACACTGG
2111	ZFP36L2	0.45	TGGACCCACTGGCTGAGAACACTGG
2112	ITM2A	0.39	AAGCCTATACTGTTCTGTGGAGTAA
2113	LGALS9	0.54	AAATGTTCCCTGTGCCTGCTCCTG
2114	INPP5D	0.53	TCCTGTACTTGTCCCTCAGCTTGGGC
2115	SATB1	0.35	TTGGACATCTCTAGTGTAGCTGCCA
2116	TFDP2	0.32	AAGCCTATACTGTTCTGTGGAGTAA
2117	IL2RG	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
2118	CD48	0.5	ACTTGTCCCTCAGCTTGGGCTTCTC
2119	SELL	0.32	ACTTGTCCCTCAGCTTGGGCTTCTC
2120	ADA	0.32	TGCCTGCTCCTGTACTTGTCCCTCAG
2121	LRMP	0.34	GCCCCACTGGACAAACACTGATTCTC
2122	RIMS3	0.37	AAGCCTATACTGTTCTGTGGAGTAA
2123	LCK	0.37	TCCTGTACTTGTCCCTCAGCTTGGC
2124	CXorf9	0.4	CACCCAGCTGGCCTGTGGATGGGA
2125	RHOH	0.3	GCCCCACTGGACAAACACTGATTCTC
2126	ZNFN1A1	0.31	TTGGACATCTCTAGTGTAGCTGCCA
2127	LCP2	0.37	TCCTGTACTTGTCCCTCAGCTTGGC
2128	CD1D	0.49	TCCTCCATCACCTGAAACACTGGAC
2129	CD2	0.42	CACCCAGCTGGCCTGTGGATGGGA
2130	ZNF91	0.45	AAATGTTCCCTGTGCCTGCTCCTG
2131	MAP4K1	0.32	TCCTGTGCCTGCTCCTGTACTTGT
2132	CCR7	0.44	TTGGACATCTCTAGTGTAGCTGCCA
2133	IGLL1	0.43	TGCCTGCTCCTGTACTTGTCCCTCAG
2134	CD3G	0.3	TCCTCCATCACCTGAAACACTGGAC
2135	ZNF430	0.31	ACTTGTCCCTCAGCTTGGGCTTCTC
2136	CCR9	0.31	TTGGACATCTCTAGTGTAGCTGCCA
2137	CXCR4	0.37	ACTTGTCCCTCAGCTTGGGCTTCTC
2138	KIAA0922	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2139	TARP	0.31	GCCCCACTGGACAAACACTGATTCTC
2140	FYN	0.35	TCCTGTACTTGTCCCTCAGCTTGGGC
2141	SH2D1A	0.34	TTGGACATCTCTAGTGTAGCTGCCA
2142	CD1A	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2143	LST1	0.33	TTGGACATCTCTAGTGTAGCTGCCA
2144	LAIR1	0.36	ACTTGTCCCTCAGCTTGGGCTTCTC

TABLE 75-continued

<u>Ifosfamide biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
2145	TRB@	0.34	TGGACCCCACTGGCTGAGAAATCTGG
2146	SEPT6	0.39	TTGGACATCTCTAGTGTAGCTGCCA
2147	CD3D	0.37	TCCTCCATCACCTGAAACACTGGAC
2148	CD6	0.32	AAATGTTTCCCTGTGCCTGCTCCTG
2149	AIF1	0.34	TGCCTGCTCCTGTACTTGTCCCTCAG
2150	CD1E	0.31	TCCTGTACTTGTCCCTCAGCTGGGC
2151	TRIM	0.32	TCCTGTACTTGTCCCTCAGCTGGGC
2152	GLTSCR2	0.34	TCCTGTACTTGTCCCTCAGCTGGGC
2153	ARHGAP15	0.33	TCCTGTACTTGTCCCTCAGCTGGGC
2154	BIN2	0.33	TGCCTGCTCCTGTACTTGTCCCTCAG
2155	SH3TC1	0.32	TGGACCCCACTGGCTGAGAAATCTGG
2156	CECR1	0.36	TCCTCCATCACCTGAAACACTGGAC
2157	BCL11B	0.38	TCCTCCATCACCTGAAACACTGGAC
2158	GIMAP6	0.32	GCCCCACTGGACAACACTGATTCCCT
2159	STAG3	0.46	TTGGACATCTCTAGTGTAGCTGCCA
2160	GALNT6	0.32	ACTTGTCCCTCAGCTTGGCTTCTTC
2161	MGC5566	0.49	TCCTTGTGCCTGCTCCTGTACTTGT
2162	PACAP	0.48	TCCTGTACTTGTCCCTCAGCTTGGGC
2163	LEF1	0.4	TGCCTGCTCCTGTACTTGTCCCTCAG

TABLE 76

<u>Tamoxifen biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
2164	MLP	0.33	TCCTGTACTTGTCCCTCAGCTTGGGC
2165	GLUL	0.33	TCCTTGTGCCTGCTCCTGTACTTGT
2166	SLC9A3R1	0.37	CACCCAGCTGGCCTGTGGATGGGA
2167	ZFP36L2	0.33	TTGGACATCTCTAGTGTAGCTGCCA
2168	INSIG1	0.31	TCCTCCATCACCTGAAACACTGGAC
2169	TBL1X	0.36	TCCTGTACTTGTCCCTCAGCTTGGGC
2170	NDUFAB1	0.43	AAATGTTTCCCTGTGCCTGCTCCTG
2171	EBP	0.31	TGGACCCCACTGGCTGAGAAATCTGG
2172	TRIM14	0.43	TTGGACATCTCTAGTGTAGCTGCCA
2173	SRPK2	0.41	GCCCCACTGGACAACACTGATTCCCT
2174	PMM2	0.4	AAATGTTTCCCTGTGCCTGCTCCTG

TABLE 76-continued

<u>Tamoxifen biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
2175	CLDN3	0.41	AAGCCTATACTGTTCTGTGGAGTAA
2176	GCH1	0.34	TTGGACATCTCTAGTGTAGCTGCCA
2177	IDI1	0.34	AAATGTTTCCCTGTGCCTGCTCCTG
2178	TFI	0.46	TCCTGTGCCTGCTCCTGTACTTGT
2179	MYB	0.39	CACCCAGCTGGCCTGTGGATGGGA
2180	RASGRP1	0.32	CACCCAGCTGGCCTGTGGATGGGA
2181	HIST1H3H	0.38	TGGACCCCACTGGCTGAGAAATCTGG
2182	CBFA2T3	0.34	AAATGTTTCCCTGTGCCTGCTCCTG
2183	SRRM2	0.43	GCCCCACTGGACAAACACTGATTCCCT
2184	ANAPC5	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
2185	MBD4	0.5	TCCTGTACTTGTCCCTCAGCTTGGGC
2186	GATA3	0.32	TCCTCCATCACCTGAAACACTGGAC
2187	HIST1H2BG	0.32	AAGCCTATACTGTTCTGTGGAGTAA
2188	RAB14	0.31	TGGACCCCACTGGCTGAGAAATCTGG
2189	PIK3R1	0.36	AAGCCTATACTGTTCTGTGGAGTAA
2190	MGC50853	0.37	CACCCAGCTGGCCTGTGGATGGGA
2191	ELF1	0.35	GCCCCACTGGACAAACACTGATTCCCT
2192	ZRF1	0.32	TCCTTGTGCCTGCTCCTGTACTTGT
2193	ZNF394	0.31	AAATGTTTCCCTGTGCCTGCTCCTG
2194	S100A14	0.39	AAATGTTTCCCTGTGCCTGCTCCTG
2195	SLC6A14	0.31	CACCCAGCTGGCCTGTGGATGGGA
2196	GALNT6	0.37	TCCTCCATCACCTGAAACACTGGAC
2197	SPDEF	0.44	AAATGTTTCCCTGTGCCTGCTCCTG
2198	TPRT	0.5	AAGCCTATACTGTTCTGTGGAGTAA
2199	CALML4	0.31	TTGGACATCTCTAGTGTAGCTGCCA

TABLE 77

<u>Flexuridine biomarkers.</u>			
SEQ ID NO	Gene	Corre-lation	Medianprobe
2200	CSDA	0.33	ACTTGTCCCTCAGCTGGCTTCTTC
2201	F8A1	0.31	TGGACCCCACTGGCTGAGAAATCTGG
2202	KYNU	0.32	TGGACCCCACTGGCTGAGAAATCTGG
2203	PHF14	0.31	AAGCCTATACTGTTCTGTGGAGTAA

TABLE 77-continued

<u>Floxuridine biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
2204	SERPINEB2	0.34	TCCTCCATCACCTGAAACACTGGAC
2205	OPHN1	0.31	GCCCCACTGGACAACACTGATTCCCT
2206	HRMT1L2	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2207	TNFRSF1A	0.3	GCCCCACTGGACAACACTGATTCCCT
2208	PPP4C	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2209	CES1	0.3	TCCTCCATCACCTGAAACACTGGAC
2210	TP53AP1	0.3	GCCCCACTGGACAACACTGATTCCCT
2211	TM4SF4	0.32	GCCCCACTGGACAACACTGATTCCCT
2212	RPL5	0.32	TGCCTGCTCCTGTACTTGCCCTCAG
2213	BC008967	0.32	TGGACCCCCTGGCTGAGAACATCTGG
2214	TLK2	0.35	TTGGACATCTCTAGTGTAGCTGCCA
2215	COL4A6	0.31	TCCTTGTGCCTGCTCCTGTACTTGT
2216	PAK3	0.32	CACCCAGCTGGCCTGTGGATGGGA
2217	RECK	0.34	TCCTTGTGCCTGCTCCTGTACTTGT
2218	LOC51321	0.32	AAGCCTATACTGTTCTGTGGAGTAA
2219	MST4	0.36	TCCTCCATCACCTGAAACACTGGAC
2220	DERP6	0.32	TGGACCCCCTGGCTGAGAACATCTGG
2221	SCD4	0.33	TCCTTGTGCCTGCTCCTGTACTTGT
2222	FLJ22800	0.31	TGGACCCCCTGGCTGAGAACATCTGG

TABLE 78

<u>Irinotecan biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
2223	CSDA	0.32	TGCCTGCTCCTGTACTTGCCCTCAG
2224	UBE2L6	0.32	GCCCCACTGGACAACACTGATTCCCT
2225	TAP1	0.44	TGGACCCCCTGGCTGAGAACATCTGG
2226	RPS19	0.32	TGCCTGCTCCTGTACTTGCCCTCAG
2227	SERPINA1	0.32	ACTTGTCCCTCAGCTTGGCTTCTTC
2228	C1QR1	0.31	TTGGACATCTCTAGTGTAGCTGCCA
2229	SLA	0.33	CACCCAGCTGGCCTGTGGATGGGA
2230	GPSM3	0.46	TGCCTGCTCCTGTACTTGCCCTCAG
2231	PSMB9	0.3	TGCCTGCTCCTGTACTTGCCCTCAG
2232	EDG1	0.34	TGCCTGCTCCTGTACTTGCCCTCAG
2233	FMNL1	0.4	GCCCCACTGGACAACACTGATTCCCT

TABLE 78-continued

<u>Irinotecan biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation	Medianprobe
2234	PTPN7	0.39	TTGGACATCTCTAGTGTAGCTGCCA
2235	ZNFN1A1	0.32	AAGCCTATACTGTTCTGTGGAGTAA
2236	CENTB1	0.33	TTGGACATCTCTAGTGTAGCTGCCA
2237	BATF	0.41	ACTTGTCCCTCAGCTTGGCTTCTTC
2238	MAP4K1	0.39	AAATGTTCTTGTGCCTGCTCCTG
2239	PDE4C	0.31	AAGCCTATACTGTTCTGTGGAGTAA
2240	SP110	0.35	TCCTTGTGCCTGCTCCTGTACTTGT
2241	HLA-DRA	0.31	TGGACCCCCTGGCTGAGAACATCTGG
2242	IFI16	0.36	TTGGACATCTCTAGTGTAGCTGCCA
2243	HLA-DRB1	0.32	AAGCCTATACTGTTCTGTGGAGTAA
2244	ARHGEF6	0.43	ACTTGTCCCTCAGCTTGGCTTCTTC
2245	SELPLG	0.35	TCCTTGTGCCTGCTCCTGTACTTGT
2246	SEC31L2	0.35	CACCCAGCTGGCCTGTGGATGGGA
2247	CD3Z	0.51	TCCTCCATCACCTGAAACACTGGAC
2248	PRKCQ	0.39	TTGGACATCTCTAGTGTAGCTGCCA
2249	SH2D1A	0.43	AAGCCTATACTGTTCTGTGGAGTAA
2250	GZMB	0.49	TCCTCCATCACCTGAAACACTGGAC
2251	TRB@	0.43	ACTTGTCCCTCAGCTTGGCTTCTTC
2252	HLA-DPA1	0.47	ACTTGTCCCTCAGCTTGGCTTCTTC
2253	AIM1	0.36	TCCTTGTGCCTGCTCCTGTACTTGT
2254	DOCK2	0.39	TGGACCCCCTGGCTGAGAACATCTGG
2255	CD3D	0.31	TCCTGTACTTGTCCCTCAGCTTGGGC
2256	IFITM1	0.31	TTGGACATCTCTAGTGTAGCTGCCA
2257	ZAP70	0.31	GCCCCACTGGACAACACTGATTCCCT
2258	PRF1	0.47	CACCCAGCTGGCCTGTGGATGGGA
2259	Clorf24	0.39	GCCCCACTGGACAACACTGATTCCCT
2260	ARHGAP15	0.48	TCCTCCATCACCTGAAACACTGGAC
2261	C13orf18	0.33	CACCCAGCTGGCCTGTGGATGGGA
2262	TM6SF1	0.37	TCCTTGTGCCTGCTCCTGTACTTGT

TABLE 79

<u>Satraplatin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
2263	STAT1	0.32	TCCTGTACTTGTCCCTCAGCTGGGC
2264	HSBP1	0.33	AAATGTTCCCTTGTGCCCTGCTCCTG
2265	IFI30	0.35	AAGCTATACTGTTCTGTGGAGTAA
2266	RIOK3	0.36	TCCTCCATCACCTGAAACACTGGAC
2267	TNFSF10	0.31	ACTTGTCCCTCAGCTTGGGCTTCTTC
2268	ALOX5AP	0.3	TCCTTGCTGCCTGCTCCTGTACTTGT
2269	ADFP	0.33	TGGACCCCCACTGGCTGAGAATCTGG
2270	IRS2	0.37	TCCTGTACTTGTCCCTCAGCTGGGC
2271	EFEMP2	0.31	TTGGACATCTCTAGTGTAGCTGCCA

TABLE 79-continued

<u>Satraplatin biomarkers.</u>			
SEQ ID NO	Gene	Corre- lation Medianprobe	
2272	RTPK2	0.35	TGGACCCCACGGCTGAGAACATCTGG
2273	DKFZp564I1922	0.33	TCCTCCATCACCTGAAACACTGGAC
2274	MT1K	0.34	TCCTCCATCACCTGAAACACTGGAC
2275	RNASET2	0.38	ACTTGTCCCTCAGCTTGGGCTTCTTC
2276	EFHD2	0.31	CACCCAGCTGGCCTGTGGATGGGA
2277	TRIB3	0.33	GCCCCACTGGACAACACTGATTCC
2278	ACSL5	0.42	AAATGTTCCCTGTGCCTGCTCCTG
2279	IFIH1	0.37	ACTTGTCCCTCAGCTTGGGCTTCTTC
2280	DNAPTP6	0.42	TGCCTGCTCCTGTACTTGTCCCTCAG

TABLE 80

<u>Vincristine microRNA biomarkers.</u>		
SEQ ID NO	Medianprobe	Corr Sequence
2281	Hcd892 left	0.3 GAGGGCTGGAGAGGTTGGGTGCGCTTGTGCGTTTCACTTT
2282	Hcd678 right	0.27 GCCCTGAAGCTCCGACTACAGCTCCCAGGCCCTCTCAAAG
2283	mir-007-1-prec	0.28 TGTTGGCCTAGTTCTGTGTTGAAAGACTAGTGATTTGTTG
2284	MPR243 left	0.25 GTATTACCTAGTTGTAATGTGGGTTGCCATGGTGTGTTG
2285	Hcd654 left	0.25 AACGAGTAAAAGGCGTACATGGAGCGCGGGCGCAGAG
2286	mir-487No1	0.26 TTATGACGAATCATACAGGGACATCCAGTTTCAGTATC
2287	Hcd794 right	0.35 GGCCACCACAGACACCAACAAGTCAGTCGGTTCTGCAG
2288	Hcd739 right	0.32 TATTAGCTGAGGGAGGGCTGGAGGCAGCTGCATTCCGACT
2289	Hcd562 right	0.28 CGCATGTCCTGGCCCTCGTCTTCCATGGCACTGGCACCG

TABLE 81

<u>Cisplatin microRNA biomarkers.</u>		
SEQ ID NO	Medianprobe	Corr Sequence
2290	HUMTRF	0.34 GATCTAAAGGTCCCTGGTTCGATCCCAGGGTTCCGACCA
2291	HPR187 right	0.25 TATTTATTACAAGGTCCCTTCCCGTAAACTTTGTCC
2292	mir-450-1	0.26 AACGATACTAAACTGTTTGCATGTGTCCTAAATATGC
2293	mir-155-prec	0.31 TTAATGCTAACGTCAGTGTAGGGTTTTGCCTCCAAGTGAC
2294	mir-515-15p	0.25 GATCTCATGCAGTCATTCTCAAAAGAACGACTTCTGT
2295	mir-181b-precNo2	0.25 ACCATCGACCGTTGATTGTACCCATGGCTAACCATCATC

TABLE 81-continued

<u>Cisplatin microRNA biomarkers.</u>		
SEQ	ID	NO Medianprobe Corr Sequence
2296	mir-124a-1-prec1	0.26 ATACAATTAAGGCACGCCGGTGAATGCCAAGAACGGGCTG
2297	mir-450-2No1	0.3 GAAAGATGCTAAACTATTTGCGATGTGTTCTAATATG
2298	Hcd923 right	0.31 CTGGAGATAATGATTCTGCATTTCTAATTAACTCCCAGGT
2299	mir-342No1	0.31 GTCTCACACAGAAATCGCACCCGTACCTTGGCTACTTA
2300	mir-142-prec	0.27 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2301	mir-223-prec	0.26 GAGTGTCAAGTTGTCAAATACCCAAGTGCGGCACATGCT
2302	Hcd754 left	0.38 TCCTCCTCCTCCTTCGTTCCGGCTCCCTGGCTGGCTCC
2303	Hcd213_HPR182 left	0.3 CTGTTTCATACTTGAGGGAGAAATTATCCTTGGTGTGTTCG

TABLE 82

<u>Azaquanine microRNA biomarkers.</u>		
SEQ	ID	NO Medianprobe Corr Sequence
2304	MPR121 left	0.3 CACCTGGCTCTGAGAACTGAATTCCATAGGCTGTGAGCTC
2305	HUMTRS	0.26 TCTAGCGACAGAGTGGTTCAATTCCACCTTCGGCGCCA
2306	mir-213-precNo1	0.26 AACATTGCTATGCTGCGGTGGTTGAACGTGTGAGACAAG
2307	mir-155-prec	0.4 TTAATGCTAATCGTGATAGGGTTTTGCCTCCAAGTGCAC
2308	mir-147-prec	0.47 GACTATGGAAGCCAGTGTGAGGGAAATGCTCTGCTAGATT
2309	mir-100No1	0.26 CCTGTTGCCACAAACCCGTAGATCCGAACCTGTGGTATTA
2310	mir-138-1-prec	0.29 AGCTGGTGTGTGAATCAGGCCGTGCCAACATCAGAGAACG
2311	mir-140No2	0.38 TTCTACCACAGGGTAGAACCAACGGACAGGATACCGGGCA
2312	mir-146-prec	0.51 TGAGAACTGAATTCCATGGGTTGTGTAGTGTGTCAGACCTC
2313	mir-509No1	0.25 ATTAAAAATGATTGGTACGTCTGTGGTAGAGTACTGCAT
2314	mir-146bNo1	0.33 CACCTGGCACTGAGAACTGAATTCCATAGGCTGTGAGCTC
2315	Hcd514 right	0.26 ATTAGAGACTCGTTAAGAGAAGGTGAGAAGGGCTCAGTAA
2316	Hcd397 left	0.34 GTGTGTATCTATGTGTGTATGTGTGAGTGTGAATAT
2317	Hcd731 left	0.27 AATTGTGACAACGTAGTGGGAGGTTGTGTGATGATTATC
2318	mir-034-precNo2	0.32 AGTAAGGAAGCAATCAGCAAGTATACTGCCCTAGAAGTGC
2319	mir-100-1/2-prec	0.3 TGAGGCCCTGTTGCCACAAACCCGTAGATCCGAACCTGTGG

TABLE 83

Etoposide microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2320	Hcd415 right	0.28 GATGTTGGAAACAATGGGAGTGAGAGAATGGGAGAGCT
2321	Hcd768 right	0.37 GCCCTGGCGGAACGCTGAGAAGACAGTCGAACTGACTAT
2322	HUMTRF	0.38 GATCTAAAGGTCCCTGGTCGATCCGGGTTCGGCACCA
2323	Hcd866 right	0.26 GTCATGCTGCCACCAGCAGGCAGAGAAGAACAGAAGAAC
2324	Hcd145 left	0.33 AAAAATCCCAGCGGCCACCTTCCTCCCTGCCATTGGG
2325	HUMTRAB	0.29 ATGGTAGAGCGCTCGCTTGCTGAGAGGTAGCGGGAT
2326	Hcd913 right	0.36 CAAACATCATGTGACGTCTGGAGCGGGCGCGCGCG
2327	HPR163 left	0.29 GCTGCCCTCCCTTAGCAACGTGGCCCCGGCGTTCAA
2328	Hcd697 right	0.27 GCCCTCATGCTGCCAAGGGCTGGCAAGAACGTCCTGCTT
2329	Hcd755 left	0.26 GGAAGTGGAGCAAATGGATGGAAAGCAATTGGAAAGAT
2330	Hcd716 right	0.25 CAATAATGTGCCTATAAGCGCCGCTCCGGCGCG
2331	MPR207 right	0.33 AACAACTTGTGCTGGTGCCGGGAAGTTGTCTCCTA
2332	HSTRNL	0.26 TCCGGATGGAGCGTGGTTCGAATCCACTCTGACACCA
2333	HPR206 left	0.29 CTATATTGGACCGCAGCGCTGAGAGCTTGTGTTAAATG
2334	MPR243 left	0.27 GTATTTACCTAGTTGTAATGTGGTTGCCATGGTGTGTTG
2335	Hcd654 left	0.4 AACGAGTAAAAGCGTACATGGAGCGCGGGCGAGAG
2336	MPR130 left	0.28 AGGCCAAGGTGACGGGTGCGATTCTGTGAGACAATTC
2337	Hcd782 left	0.26 GGAGCCCTGCTGCAAAGAGTGGTGCCTGCGTGTGA
2338	Hcd794 right	0.26 GGCCACCACAGACACCAACAAGTTCACTCCGTTCTGCAG
2339	Hcd739 right	0.3 TATAGCTGAGGGAGGGCTGGAGGCGCTGCATTCCGACT
2340	mir-142-prec	0.29 CCCATAAGTAGAAAGCACTACTAACAGCACTGGAGGTG
2341	HSHELA01	0.29 GGCGCAGCACCTCGGTCGTATCCGAGTCACGGCACCA
2342	HUMTRV1A	0.29 ACGCGAAAGGTCCCCGGTCGAAACGGGCGGAAACACCA
2343	Hcd754 left	0.34 TCCTCCTCCCTTTCGTTCCGGCTCCCTGGCTGGCTCC

TABLE 84

Carboplatin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2344	Hcd829 right	0.27 AAAATGGCGGGGGAAAAGCGAGCGCGAGAGCGAGGGAGG
2345	HUMTRF	0.26 GATCTAAAGGTCCCTGGTCGATCCGGTTCCGCACCA
2346	HPR187 left	0.29 TGTGTGTTGCGGGGGTGGGGGCGGTGAAAGTGATTGAT
2347	Hcd210_HPR205 right	0.32 CGAACATTCGCGGTGCACTTCTTTCACTATCTTATT
2348	mir-379N01	0.26 TTCCGTGGTCTGAAGAGATGGTAGACTATGGAACGTAG
2349	mir-213-precN01	0.26 AACATTCAATTGCTGTCGGTGGGTTGAACTGTGTTGACAAG

TABLE 84-continued

Carboplatin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2350	mir-4325p	0.29 CCAGGTCTTGGAGTAGGTCAATTGGTGGATCCTCTATTTC
2351	mir-450-1	0.3 AACGATACTAAACTGTTTTGCGATGTGTCCTAATATGC
2352	mir-155-prec	0.25 TTAATGCTAACGTGATAGGGTTTGCCTCAAATGAC
2353	Hcd28_HPR39right	0.26 AAGCTCCAAATTAGCTTTAAATAGAAGCTGAGAGTTA
2354	MPR244 right	0.27 TAAACATAGAGGAAATTCACGTTTCAGTGTCAAATGCT
2355	mir-409-3p	0.3 GACGAATGTTGCTCGGTGAACCCCTTTCGGTATCAAATT
2356	mir-124a-1-prec1	0.28 ATACAATTAAGGCACGCCGTGAATGCCAAGAATGGGCTG
2357	mir-154-prec1N01	0.26 GTGGTACTTGAAAGATAAGGTTATCCGTGTTGCCTCGCTTT
2358	mir-495N01	0.32 GTGACGAAACAAACATGGTGCACCTCTTTTCGGTATCAA
2359	mir-515-23p	0.25 CAGAGTGCCTTCTTTGGAGCGTTACTGTTGAGAAAAC
2360	Hcd438 right	0.27 GTGTTTATTGAAATCTCACATCGCTCATAGAATACACGC
2361	Hcd770 left	0.3 CCAGTATAACAATCCGTTTCAGTTAGCTTGAGATCAGA
2362	mir-382	0.32 GGTACTTGAAAGAGAAGTTGTTCGTGGTGGATTGCTTAC
2363	mir-223-prec	0.3 GAGTGTCAAGTTGTCAAATACCCAAAGTGCGGCACATGCT
2364	Hcd754 left	0.48 TCCTCCTCCTCCTTTCGTTCCGGCTCCCTGGCTGGCTCC
2365	Hcd213_HPR182 left	0.31 CTGTTTCATACTTGAGGAGAAATTATCCTGGTGTTCG

TABLE 85

Adriamycin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2366	Hcd768 right	0.25 GCCCTGGCGGAACGCTGAGAAGACAGTCGAACCTGACTAT
2367	mir-483N01	0.28 ATCACGCCCTCCTCACTCCTCTCCTCCCGTCTCTCCTCTC
2368	Hcd145 left	0.28 AAAATCCCAGCGGCCACCTTTCTCCCTGCCCATGGG
2369	mir-197-prec	0.25 TAAGAGCTTCAACCCTTCACCACCTCTCCACCCAGCAT
2370	mir-212-precN01	0.27 CCTCAGTAACAGTCTCCAGTCACGCCACCGACGCCGGC
2371	HPR163 left	0.3 GCTGCCCTCCCTTAGCAACGTGGCCCCGGCGTCCAAA
2372	Hcd654 left	0.26 AACGAGTAAAAGGCGTACATGGGAGGCCGGGGCGCAGAG
2373	mir-342N01	0.32 GTCTCACACAGAAATCGCACCCGTACCTTGGCTACTTA
2374	Hcd794 right	0.32 GGCCACCAAGACACCAACAAGTTCACTCCGTTCTGCAG
2375	mir-142-prec	0.38 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2376	Hcd754 left	0.28 TCCTCCTCCTCCTTTCGTTCCGGCTCCCTGGCTGGCTCC

TABLE 86

Aclarubicin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2377	mir-092-prec-X = 092-2	0.32 GTTCTATATAAAGTATTGCAC TTGTCCCCGCCTGTGGAAG
2378	mir-096-prec-7No2	0.29 TGGCCGATTTGGCACTAGCACATTTGCTTGTCCTCT
2379	Hcd605 left	0.26 ATTACTAGCAGTTAATGATTGGTTGTTAGTTAATGGCCC
2380	mir-007-2-precNo2	0.34 GGACCGGCCTGGCCCATCTGGAAGACTAGTGATTGTTG
2381	mir-019b-2-prec	0.28 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
2382	MPR216 left	0.26 GATCCTAGTAGTGCCAAAGTGCTCATAGTCAGGTAGTT
2383	mir-019b-1-prec	0.25 TTCTGCTGTGCAAATCCATGCAAAACTGACTGTGGTAGTG
2384	mir-135-2-prec	0.26 CACTCTAGTGCTTTATGGCTTTTATTCTATGTGATAGT
2385	HSTRNL	0.26 TCCGGATGGAGCGTGGGTCGAATCCACCTCTGACACCA
2386	mir-025-prec	0.31 ACGCTGCCCTGGCATTGCAC TTGTCTCGGTCTGACAGTG
2387	mir-007-1-prec	0.4 TGTTGGCCTAGTTCTGTGTGGAAGACTAGTGATTGTTG
2388	mir-019a-prec	0.26 TGTAGTTGTGCAAATCTATGCAAAACTGATGGTGGCCTGC
2389	mir-380-5p	0.31 AGGTACCTGAAAAGATGGTGACC ATAGAACATGCGCTAT
2390	mir-093-prec-7.1 = 093-1	0.37 CCAAAAGTGTGCTCGTCAGGTAGTGATTACCCAACCT
2391	mir-106-prec-X	0.37 CCTTGGCCATGTAAGGTGCTTACAGTGAGGTAGCTTT
2392	mir-142-prec	0.32 CCCATAAAAGTAGAAAAGCACTACTAACAGCACTGGAGGGTG
2393	mir-018-prec	0.31 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
2394	mir-020-prec	0.36 TAAAGTGCTTATAGTGCAGGTAGTGTTAGTTATCTACTG

TABLE 87

Mitoxantrone microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2395	Hcd768 left	0.26 GATGGTTAGTGAGGCCCTCGGATCAGCCGCTGGTCAG
2396	HUMTRF	0.31 GATCTAAAGGTCCCTGGTCGATCCGGTTTCGGCACCA
2397	mir-213-precNo1	0.28 AACATT CATTGCTCGTGGTGGTTGAAGTGTGGACAAG
2398	mir-181b-precNo1	0.26 TGAGGTTGCTTCAGTGAACATTCAACGCTGTGGTGAGTT
2399	M2R244 right	0.27 TAAACATAGAGGAATTTCACGTTTCAGTGTCAAATGCT
2400	mir-409-3p	0.29 GACGAATGTGCTCGGTGAACCCCTTTCGGTATCAAATT
2401	HSTRNL	0.33 TCCGGATGGAGCGTGGGTCGAATCCAC TTCTGACACCA
2402	mir-382	0.34 GGTACTTGAAGAGAAGTTGCTGGTGGATTGCTTAC
2403	mir-342No1	0.3 GTCTCACACAGAAATCGCACCCGTCACCTTGGCCTACTTA
2404	mir-142-prec	0.27 CCCATAAAAGTAGAAAAGCACTACTAACAGCACTGGAGGGTG
2405	Hcd200 right	0.29 CAATTAGCCAATTGTGGGTATAATTAGCTGCATGTAGAAT

TABLE 88

<u>Mitomycin microRNA biomarkers.</u>		
SEQ	ID	NO Medianprobe Corr Sequence
		2406 HUMTRF 0.26 GATCTAAAGGTCCCTGGTCGATCCGGTTTGGCACCA
		2407 Hcd148_HPR225left 0.27 AATTAATGACCAAAATGTCAGATGTGTCACAGCTAATTA
		2408 Hcd938 right 0.26 ATTCCCTGCATCACTCTCATGAAATGGCTGAGAAAGTGAG
		2409 MPR174 left 0.32 GAGCCGGTCTCTTACATCTCAAATACCAGGTATTAGGT
		2410 mir-4323p 0.29 CCTTACGTGGGCCACTGGATGGCTCCTCCATGTCTGGAG

TABLE 89

<u>Paclitaxel (Taxol) microRNA biomarkers.</u>		
SEQ	ID	NO Medianprobe Corr Sequence
		2411 mir-092-prec-X = 092-2 0.29 GTTCTATATAAGTATTGCACCTGTCCCCGCTGTGGAAG
		2412 mir-096-prec-7No1 0.36 CTCCGCTCTGAGCAATCATGTGCAGTGCCAATATGGGAAA
		2413 mir-101-prec-9 0.38 GCTGTATATCTGAAAGGTACAGTACTGTGATAACTGAAGA
		2414 mir-20bNo1 0.28 AGTACCAAAGTGCTCATAGTCAGGTAGTTGGCATGAC
		2415 mir-019b-2-prec 0.28 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
		2416 mir-032-precNo2 0.29 GGAGATATTGCACATTACTAAAGTTGCATGTTGTCACGCC
		2417 MPR156 left 0.25 TCCCTCACCTGAACTGACTGCCAGAGTTCACAGACAGCTG
		2418 mir-019b-1-prec 0.28 TTCTGCTGTGCAAATCCATGCAAAACTGACTGTGGTAGTG
		2419 mir-135-2-prec 0.36 CACTCTAGTGCTTTATGGCTTTTATTCTATGTGATAGT
		2420 mir-025-prec 0.36 ACGCTGCCCTGGCATTGCACTTGTCTCGGTCTGACAGTG
		2421 mir-007-1-prec 0.27 TGTTGGCTAGTTCTGTGAGACTAGTGATTTGTTG
		2422 mir-361No1 0.29 GGATTGGGAGCTTATCAGAATCTCCAGGGTACTTTATA
		2423 mir-093-prec-7.1 = 093-1 0.37 CAAAGTGTCTCGTCAGGTAGTGTGATTACCCAACCT
		2424 mir-106-prec-X 0.38 CCTTGGCCATGTAAAAGTGTCTACAGTGCAGGTAGCTTT
		2425 mir-098-prec-X 0.29 TGAGGTAGTAAGTTGTATTGTTGGGTAGGGATATTAG
		2426 mir-142-prec 0.27 CCCATAAAAGTAGAAAAGCACTACTAACAGCACTGGAGGGTG
		2427 HPR169 right 0.26 GTTCTTCTCACGGTAAGTGGCAGCCTCGTTGTGGCTG
		2428 mir-018-prec 0.4 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
		2429 mir-020-prec 0.36 TAAAGTGCTTATAGTGCAGGTAGTGTGTTAGTTATCTACTG

TABLE 90

Gemcitabine (Gemzar) microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2430	mir-123-precNo2	0.27 TGTGACACTTCAAACTCGTACCGTGAGTAATAATGCGCCG
2431	Hcd257 right	0.29 CTTGGTTTTGCAATAATGCTAGCAGAGTACACACAAGAA
2432	mir-155-prec	0.35 TTAATGCTAACATCGTGATAGGGTTTTGCCTCAAUTGAC
2433	ath-MIR180aNo2	0.26 TGAGAATCTTGATGATGCTGCATGGCAATCAACGACTAT
2434	Hcd448 left	0.33 TGTAATTCCATTGAGGGTTCTGGTGACTCCAGCTTCGTA
2435	HSTRNL	0.31 TCCGGATGGAGCGTGGGTTCGAATCCCACCTCTGACACCA
2436	MPR174 right	0.29 CATTAGGGACACGTGTGAGTGTGCCAGGCTATTCTGAG
2437	Hcd200 right	0.29 CAATTAGCCAATTGTGGGTATAATTAGCTGCATGTAGAAT
2438	mir-4323p	0.26 CCTTACGTGGGCCACTGGATGGCTCCTCCATGTCTGGAG
2439	HPR244 right	0.3 TAGTTCATGGCGTCCAGCAGCAGCTTCTGGCAGACCGGGT

TABLE 91

Taxotere (docetaxel) microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2440	mir-096-prec-7No1	0.28 CTCCGCTCTGAGCAATCATGTGCAGTGCAATATGGGAAA
2441	mir-095-prec-4	0.27 CGTTACATTCAACGGGTATTATTGAGCACCCACTCTGTG
2442	HSTRNL	0.26 TCCGGATGGAGCGTGGGTTCGAATCCCACCTCTGACACCA
2443	mir-007-1-prec	0.37 TGTTGGCTAGTTCTGTGTGGAAGACTAGTGATTTGTTG

TABLE 392

Dexamethasone microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2444	MPR141 left	0.42 CTCAGTCGTGCCCTAGCAGCGGGAACAGTACTGCAGTGAG
2445	mir-424No2	0.35 GTTCAAAACGTGAGGCCGCTGCTATAACCCCTCGTGGGAA
2446	Hcd690 right	0.26 GGACAAGGGAGGAGACACGCAGAGGTGACAGAAAGGTTAG
2447	Hcd783 left	0.26 CAGGCTCACACCTCCCTCCCCAACTCTCTGGAATGTATA
2448	mir-150-prec	0.38 CTCCCCATGGCCCTGTCTCCCAACCCTGTACCGAGTGTG
2449	Hcd266 left	0.37 AAGGTCTTGGCTTGAGGAAGGTGTGCTACTGGAGAG
2450	mir-503No1	0.34 CTCAGCCGTGCCCTAGCAGCGGGAACAGTCTGCAGTGAG
2451	mir-128b-precNo1	0.29 TCACAGTGAACCGGTCTTTCCCTACTGTGTACACTCC
2452	Hcd397 left	0.26 GTGTGTATACTTATGTGTGTATGTGTGAGTGTGAATAT
2453	mir-484	0.38 GTCAGGCTCAGTCCCCCTCCGATAAACCCCTAAATAGGGA

TABLE 93

Ara-C (Cytarabine hydrochloride) microRNA biomarkers.

SEQ	ID	NO	Medianprobe	Corr Sequence
2454	HUMTRF		0.33	GATCTAAAGGTCCCTGGTCGATCCGGGTTTCGGCACCA
2455	mir-155-prec		0.28	TTAATGCTAACATCGTGATAGGGGTTTGCCTCCAAGTGAC
2456	mir-515-15p		0.27	GATCTCATGCAGTCATTCTCCAAAAGAAAGCAGTTCTGT
2457	Hcd938 right		0.26	ATTCCTGCATCACTCTCATGAAATGGCTGAGAAAGTGAG
2458	Hcd642 right		0.25	TCAGGGTTATGAAGTTATCAAAGCCCCTGATGGAATTA
2459	Hcd120 left		0.26	CTTGGTGTGTTCTCGGTAGCTATGAAATCCCAGGGTTTC
2460	mir-380-5p		0.25	AGGTACCTGAAAGATGGTTGACCATAGAACATGCGCTAT
2461	mir-342N01		0.25	GTCTCACACAGAAATCGCACCCGTCACCTGGCCTACTTA
2462	mir-142-prec		0.27	CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2463	mir-223-prec		0.31	GAGTGTCAAGTTGTCAAATACCCAAGTGCGGCACATGCT
2464	mir-4323p		0.28	CCTTACGTGGGCCACTGGATGGCTCCTCCATGTCTGGAG

TABLE 94

Methylprednisolone microRNA biomarkers

SEQ	ID	NO	Medianprobe	Corr Sequence
2465	Hcd544 left		0.26	TTCCAGGTGTCACCAAGGACGTGCCCTGGCCTGATGG
2466	mir-181c-precN01		0.28	TGCCAAGGGTTGGGGAACATTCAACCTGTCGGTGAGTT
2467	Hcd517 left		0.25	TTAACGCAGGAGAGGTGAGAGGAAGATTAAATGTGTGCTC
2468	MPR151 left		0.27	GGGATTAATGACCAGCTGGGGAGTTGATAGCCCTCAGTG
2469	mir-213-precN01		0.34	AACATTCAATTGCTGTCGGTGGTTGAACGTGTGGACAAG
2470	mir-181b-precN02		0.36	ACCATCGACCGTTGATTGTACCTATGGCTAACATCATC
2471	mir-150-prec		0.27	CTCCCCATGGCCCTGTCTCCAACCCCTGTACAGTGCTG
2472	mir-153-1-prec1		0.28	CAGTGCATAGTCACAAAAGTGATCATGGCAGGTGTGGC
2473	mir-128b-precN01		0.48	TCACAGTGAACCGGTCTCTTCCCTACTGTGTACACTCC
2474	Hcd812 left		0.25	CTGTGGATCTGGTCTGTAGCTGAGAGCACATCGCTAAA
2475	mir-195-prec		0.3	TCTAGCAGCACAGAAATATTGGCACAGGGAAAGCGAGTCG
2476	mir-342N01		0.38	GTCTCACACAGAAATCGCACCCGTCACCTGGCCTACTTA
2477	mir-370N01		0.28	TTACACAGTCACGAGTCCTGCTGGGGTGGAACCTGGTC
2478	mir-142-prec		0.32	CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2479	mir-223-prec		0.36	GAGTGTCAAGTTGTCAAATACCCAAGTGCGGCACATGCT
2480	mir-484		0.36	GTCAGGCTCAGTCCCCCTCCGATAAACCCCTAAATAGGGA

TABLE 95

Methotrexate microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2481	mir-092-prec-X = 092-2	0.37 GTTCTATATAAAGTATTGCAC TTGTCCCCGCCTGTGGAAAG
2482	mir-096-prec-7No1	0.33 CTCCGCTCTGAGCAATCATGTGCAGTGCAATATGGGAAA
2483	mir-123-precNo1	0.25 GACGGGACATTATTACTTTGGTACGCCTGTGACACTTC
2484	Hcd250 left	0.26 GTTCTGTTGCTAAGACAACAGGATGCTAGCAGGCATATGC
2485	mir-518e/526c	0.3 TCTCAGGCCTGTGACCCCTAGAGGGAAAGCGCTTCTGTTG
2486	HPR232 right	0.3 TGAATTATTGCACAATAAATT CATGCCCTTGTGTCTTA
2487	Hcd263 left	0.29 GAGCATTAAAGATTCCATTCTTGAGGCAAATATTGACC
2488	mir-516-33p	0.35 GTGAAAGAAAAGTGCCTCTTCAGAGGGTTACTCTTGAG
2489	Hcd605 left	0.27 ATTACTAGCAGTTAATGATTGGTTGTAGTTAATGGCCC
2490	Hcd373 right	0.25 CCTGAAAGGTCTGGTGTAAAGCAAATACTCGGTGACCAGA
2491	MPR254 right	0.28 GTTCACAGTGGAGAAATATGCTTCGTATTACTCTTC
2492	MPR215 left	0.3 CAGCTATGTGGACTCTAGCTGCCAAAGGCCTTCCTTC
2493	HUMTRF	0.28 GATCTAAAGGTCCCTGGTCGATCCGGTTCCGCACCA
2494	mir-106aN01	0.27 CCTTG GCCATGTAAAAGTGCTTACAGTG CAGGTAGCTTT
2495	mir-20bN01	0.37 AGTACCAAAGTGCTCATAGTG CAGGTAGTTGGCATGAC
2496	Hcd361 right	0.28 AACTGGCTACAAGGCTTTCCCTCTATGAAGGACAG
2497	Hcd412 left	0.25 AGTTGGGAGAAC TTTATGATTATTCTCATGCATCATCTT
2498	Hcd781 left	0.26 GAGTGTGGATCTAATCTCAGCTGATTAAATGCCCCTCAT
2499	mir-019b-2-prec	0.33 GTGGCTGTGCAAATCCATGC AAAACTGATTGTGATAATGT
2500	HPR214 right	0.29 AGCAAAAGCTATTATTTGCCCTGATGAGCCAATCAGATG
2501	Hcd807 right	0.26 GCGCTGACAAATCTGCCTGATTCTGTATGATCCATGAGA
2502	Hcd817 left	0.37 TAATGAGAATTATGTTGCACATTGAGG CAGGATAAATCC
2503	Hcd788 left	0.25 GACAAACATGCAGGAAAAATTATCCCCTGGGGATTCTACA
2504	Hcd970 left	0.31 TTGTGGGT CAGCTGCCAGCTATCGGCTGGATTAGTGAAT
2505	Hcd148_HPR225left	0.26 AATTAATGACCAAAATGTCAGATGTGTCCACAGCTAATTA
2506	Hcd102 left	0.27 ACTGGAATTATGTTTATCTTAAGTCCACACTGGATCCTC
2507	Hcd246 right	0.29 TAAAGTGAGTTATGGAGGTTACTCTCCTGTGAGAGGAAAT
2508	HPR199 right	0.28 TACACCTAAGGCATGTACTGTATTAATGAACCAATAAAC
2509	HPR233 right	0.27 CATGATGGGTGGGTGAGATGGGGAGCGAAGACTATTAC
2510	Hcd383 left	0.28 GCCCGGGCATGCATTCTAGCACCATGTGTT CAGCT
2511	MPR224 right	0.29 TGAATTATTGCACAATAAATT CATGCCCTTGTGTCTTA
2512	HPR172 right	0.26 GTTTAACAGCCAGTGCAAACATTAGATCTGAGTCAAA
2513	MPR216 left	0.34 GATCCTAGTAGTGCCAAAGTGCTCATAGTG CAGGTAGTT
2514	mir-321N02	0.25 CAGGGATTGTGGGTTCGAGTCCCACCCGGGTAAAGAAAG
2515	Hcd586 right	0.28 GAAC TGTTGCTTGGATGGCTTGGCCTCATTGGCTGA

TABLE 95-continued

<u>Methotrexate microRNA biomarkers.</u>		
SEQ ID NO	Medianprobe	Corr Sequence
2516	Hcd587 right	0.3 AAATAATGACTGGCCATAAGATCAAGACAAGTGTCCAAAG
2517	Hcd249 right	0.39 CAGGTACATGTTGATCAGCAGGGCTGGAGGCAGCGCTC
2518	Hcd279 right	0.27 CTCACGGCGTTGCCATGGAGACAACCTCGGGCTGGGCTC
2519	HPR159 left	0.3 TCCGTCACTTGAACTGGCTGCCAGCGTACAGACAGCTG
2520	Hcd689 right	0.28 GTACATCTGGATGTAGTTGTGCTGCAGCTGCTCTGGTAG
2521	Hcd691 right	0.32 CGGCAAAAACCTCTGTCAGAACAAAAATTAGGTGATCTATC
2522	mir-019b-1-prec	0.32 TTCTGCTGTGCAAATCCATGCAAAACTGACTGTGGTAGTG
2523	Hcd413 right	0.26 CACAAAAAGGCATAAGCAGACATCTGCCCTTGGTTCT
2524	Hcd581 right	0.26 AGGAGATATGCCAAGATATATTCACAGCTTATATACACA
2525	Hcd536_HPR104 right	0.28 GCTGCTCTGCTGAGGGCTGGACTCTGTCCAGAACAGCACCA
2526	Hcd230 left	0.28 CATTCTCTACAAGCATATGGCCTTGGGACATTAAGATGGC
2527	HPR154 left	0.28 AACATCAAGATCTATTGACCTGAGAGGTAATATTGACCG
2528	Hcd270 right	0.31 AAATGTTGTTATAGTATCCCACCTACCCGTATCTTT
2529	Hcd649 right	0.26 GAACAGGCTTCAGGTTCTGGCAGGAATATTCCGTGTAG
2530	Hcd889 right	0.27 ATGCCTTGCTCTGTGCTAATTAGAACAGAACAGCCTGT
2531	Hcd938 left	0.36 CTTGTCGACTAGCCAGTTATGAACAGAGGAGATGTTCTC
2532	HPR266 right	0.32 GGAGATCCCTCAAGGTTAGTTAAATGAGTGCTCT
2533	mir-025-prec	0.39 ACGCTGCCCTGGCATTGCACCTGTCTCGGTCTGACAGTG
2534	Hcd355_HPR190 left	0.25 TTGTGCACTGCACAACCTAGTGGGCCATTCAATTATAG
2535	MPR162 left	0.26 CTCTCTTTCTGCTTGATTGCTTAATGGAAGCTGACA
2536	Hcd923 right	0.34 CTGGAGATAATGATTCTGCATTCTAATTAACTCCCAGGT
2537	MPR237 left	0.32 AGCACATCCCCTGATCACAGTAATGTTCTGGAGATGTA
2538	MPR174 left	0.32 GAGCCGGCTCTTTACATCTCAAATACCAGGTATTTAGGT
2539	mir-019a-prec	0.31 TGTAGTTGCAAATCTATGCCAAACTGATGGTGGCCTGC
2540	hsa_mir_490_Hcd20 right	0.25 ACCAACCTGGAGGACTCCATGCTGTTGAGCTGTTACAAG
2541	mir-380-5p	0.36 AGGTACCTGAAAAGATGGTGACCATAGAACATGCGCTAT
2542	mir-093-prec-7.1 = 093-1	0.38 CCAAAAGTGTGCTCGTCAGGTAGTGTGATTACCCAACCT
2543	mir-106-prec-X	0.45 CCTTGGCCATGTAAAAGTGTCTACAGTGCAGGTAGCTTT
2544	Hcd627 left	0.3 GCATTAGGGAGAATAGTTGATGGATTACAAATCTCTGCAT
2545	mir-142-prec	0.27 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2546	HPR169 right	0.29 GTTCTTCTCACGGTAACGGCAGCCTCGTTGGCTG
2547	mir-001b-2-prec	0.28 TAAGCTATGGAATGAAAGAAGTATGTTCTCAGGCCGGG
2548	mir-018-prec	0.4 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
2549	mir-020-prec	0.48 TAAAGTCCTATAGTCAGGTAGTGTGTTAGTTACTG
2550	Hcd404 left	0.29 TGCTGCTGTTAATGCCATTAGGATGACTATTTATATCACC

TABLE 95-continued

<u>Methotrexate microRNA biomarkers.</u>			
SEQ	ID	NO Medianprobe	Corr Sequence
		2551 mir-384	0.25 CATAAGTCATTCTAGAAATTGTTCATATGCCTGTAACA
		2552 mir-4323p	0.4 CCTTACGTGGGCCACTGGATGGCTCCTCCATGTCTGGAG

TABLE 96

<u>Bleomycin microRNA biomarkers.</u>			
SEQ	ID	NO Medianprobe	Corr Sequence
		2553 mir-376aN01	0.27 AATCATAGAGGAAAATCCACGTTTCAGTATCAAATGCTG
		2554 mir-155-prec	0.35 TTAATGCTAATCGTGTAGGGTTTGCCTCCAAGTGAC
		2555 mir-409-3p	0.28 GACGAATGTTGCTCGGTGAACCCCTTCGGTATCAAATT
		2556 mir-495N01	0.29 GTGACGAAACAAACATGGTGCACTTCTTTCGGTATCAA
		2557 Hcd498 right	0.28 CACGAAGAAGTTCAGCAACCAGGAGACCAGGTGGGGCCG
		2558 mir-199a-2-prec	0.41 TCGCCCCAGTGTTCAGACTACCTGTTAGGACAATGCCGT
		2559 mir-382	0.3 GGTACTTGAAGAGAAGTTGTTGTGGATTGCGTTTAC
		2560 HPR271 right	0.27 AATTGAGCAACAGTGCATTCTGTAAATTATGCCAGTG
		2561 mir-145-prec	0.31 CCTCACGGTCCAGTTCCAGGAATCCCTAGATGCTAA
		2562 mir-199a-1-prec	0.35 GCCAACCCAGTGTTCAGACTACCTGTTAGGAGGCTCTCA

TABLE 97

<u>Methyl-GAG (methyl glyoxal bis amidinohydrazone dihydrochloride) microRNA biomarkers.</u>			
SEQ	ID	NO Medianprobe	Corr Sequence
		2563 mir-092-prec-X = 092-2	0.32 GTTCTATATAAAAGTATTGCACTTGTCCGGCTGTGGAAG
		2564 mir-101-prec-9	0.3 GCTGTATATCTGAAAGGTACAGTACTGTGATAACTGAAGA
		2565 mir-144-precNo2	0.29 CCCTGGCTGGATATCATCATATACTGTAAGTTGCGATG
		2566 mir-519a-1/526c	0.29 TCAGGCTGTGACACTCTAGAGGAAGCGCTTCTGGTC
		2567 mir-519b	0.33 GAAAAGAAAGTCATCCTTTAGAGGTTACTGTTGAGG
		2568 mir-015b-precNo2	0.26 TGCTACAGTCAGATGCGAATCATTATTGCTGCTCTAGA
		2569 mir-106aN01	0.27 CCTTGGCCATGTAAAAGTGTACAGTGCAGGTAGCTTT
		2570 mir-16-1No1	0.26 GTCAGCAGTGCCTTAGCAGCACGTAATATTGGCGTTAAG
		2571 mir-181dNo1	0.27 GAGGTACAATCAACATTGTTGTCGGTGGTTGTGA
		2572 mir-017-precNo2	0.31 GTCAGAATAATGTCAAAGTGCTTACAGTGCAGGTAGTGAT
		2573 mir-019b-2-prec	0.32 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
		2574 mir-192No2	0.26 TGCCAATTCCATAGGTACAGGTATGTCGCCTCAATGCC

TABLE 97-continued

Methyl-GAG (methyl glyoxal bis amidinohydrazone dihydrochloride)
microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2575	mir-213-precNo1	0.25 AACATTCAATTGCTGTCGGTGGGTGAACTGTGTGGACAAG
2576	mir-215-precNo2	0.3 CATTTCCTTAGGCCAATATTCTGTATGACTGTGCTACTTC
2577	mir-107No1	0.28 GGCATGGAGTTCAAGCAGCATTGTACAGGGCTATCAAAGC
2578	mir-200bNo1	0.28 GTCTCTAATACTGCCTGGTAATGATGACGGCGGAGCCCTG
2579	mir-103-prec-5 = 103-1	0.3 TATGGATCAAGCAGCATTGTACAGGGCTATGAAGGCATTG
2580	mir-519a-1/526c	0.37 TCAGGGCTGTGACACTCTAGAGGGAAGCGCTTCTGTTGTC
2581	MPR216 left	0.28 GATCCTAGTAGTGCCAAAGTGCTCATAGTCAGGTAGTTT
2582	mir-019b-1-prec	0.31 TTCTGCTGTGCAAATCCATGCAAAACTGACTGTGGTAGTG
2583	mir-107-prec-10	0.29 GGCATGGAGTTCAAGCAGCATTGTACAGGGCTATCAAAGC
2584	mir-135-2-prec	0.39 CACTCTAGTGCTTTATGGCTTTTATTCCATGTGATAGT
2585	mir-103-2-prec	0.29 GTAGCATTTCAGGTCAAGCAACATTGTACAGGGCTATGAAA
2586	mir-519a-2No2	0.29 TCTCAGGCTGTGTCCTCTACAGGGAACGCGTTCTGTTG
2587	mir-025-prec	0.33 ACGCTGCCCTGGCATTGCACTTGTCTCGGTCTGACAGTG
2588	mir-16-2No1	0.33 GTTCCACTCTAGCAGCACGTAAATATTGGCGTAGTGAAT
2589	MPR95 left	0.28 TTGTTGGACACTCTTCCCTGTTGCACACTGTGGCCCTC
2590	mir-016b-chr3	0.29 GTTCCACTCTAGCAGCACGTAAATATTGGCGTAGTGAAT
2591	Hcd948 right	0.27 TGATATAATAGTCATCTTAATGGCATTAACAGCAGCACT
2592	mir-195-prec	0.35 TCTAGCAGCACAGAAATATTGGCACAGGGAACGAGTCTG
2593	mir-093-prec-7.1 = 093 - 1	0.38 CCAAAGTGCTGTCGTGAGGTAGTGTGATTACCCAACCT
2594	mir-106-prec-X	0.42 CCTTGGCCATGTAAAAGTGCTTACAGTGCAGGTAGCTTT
2595	mir-142-prec	0.37 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGTG
2596	mir-519c/526c	0.27 TCTCAGGCTGTGACCCTCTAGAGGGAACGCGTTCTGTTG
2597	mir-200a-prec	0.29 GTCTCTAATACTGCCTGGTAATGATGACGGCGGAGCCCTG
2598	mir-016a-chr13	0.29 CAATGTCAGCAGTGCCTTAGCAGCACGTAAATATTGGCGT
2599	mir-018-prec	0.41 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
2600	mir-020-prec	0.39 TAAAGTGCTTATAGTGCAGGTAGTGTAGTTATCTACTG

TABLE 98

pXD101 HDAC inhibitors microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2601	mir-092-prec-X = 092-2	0.42 GTTCTATATAAAAGTATTGCACTTGTCCGGCCTGTGGAAG
2602	mir-123-precNo2	0.31 TGTGACACTTCAAACCTCGTACCGTGAGTAATAATGCCCG
2603	mir-106aNo1	0.36 CCTTGGCCATGTAAAAGTGCTTACAGTGCAGGTAGCTTT

TABLE 98-continued

pXD101 HDAC inhibitors microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2604	mir-20bNo1	0.36 AGTACCAAAGTGCTCATAGTCAGGTAGTTGGCATGAC
2605	mir-017-precNo2	0.32 GTCAGAATAATGTCAAAGTGCTTACAGTGCAGGTAGTGAT
2606	mir-019b-2-prec	0.42 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
2607	mir-033-prec	0.3 GTGGTGCAATTGTAGTTGCATTGCATGTTCTGGTGGTACCC
2608	mir-092-prec-13 = 092-1No2	0.31 TCTGTATGGTATTGCACTTGTCCCCGGCCTGTTGAGTTGG
2609	mir-122a-prec	0.29 CCTTAGCAGAGCTGTGGAGTGTGACAATGGTGTGTC
2610	Hcd783 left	0.27 CAGGCTCACACCTCCCTCCCCAACTCTCTGGAATGTATA
2611	MPR216 left	0.29 GATCCTAGTAGTGCCAAAGTGCTCATAGTCAGGTAGTT
2612	mir-019b-1-prec	0.41 TTCTGCTGTGCAAATCCATGCAAAACTGACTGTGGTAGTG
2613	mir-135-2-prec	0.46 CACTCTAGTGCTTATGGCTTTTATTCCTATGTGATAAGT
2614	mir-128b-precNo1	0.39 TCACAGTGAACCGGTCTTTCCCTACTGTGTCACACTCC
2615	mir-025-prec	0.45 ACGCTGCCCTGGCATTGCACTTGTCCTCGGTGACAGTG
2616	Hcd511 right	0.26 TACCTCAGAACGCTCACTCAACCCTCTCCGCTGAGTC
2617	mir-093-prec-7.1 = 093-1	0.45 CCAAAGTGCTGTCGTGAGGTAGTGTGATTACCCAACCT
2618	mir-106-prec-X	0.5 CCTTGGCCATGTAAGAAGCACTACTAACAGCACTGGAGGGTG
2619	mir-142-prec	0.5 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2620	HPR169 right	0.26 GTTTCTTCTCACGGTAACTGGCAGCCTCGTTGTC
2621	mir-223-prec	0.26 GAGTGTCAAGTTGTCAAATACCCAAAGTGCGGCACATGCT
2622	mir-018-prec	0.48 TAAGGTGCATCTAGTGCAAGATAGTGAAGTAGATTAGCATC
2623	mir-020-prec	0.52 TAAAGTGCTTATAGTCAGGTAGTGTAGTTATCTACTG

TABLE 99

5-Fluorouracil microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2624	mir-096-prec-7No2	0.27 TGGCCGATTTGGCACTAGCACATTTGCTTGTCTCT
2625	mir-429No1	0.25 CTAATACTGTCTGGAAAACCGTCCATCCGCTGCCTGATC
2626	Hcd693 right	0.25 AGGCTTGTGCGCGCATTAAAGCTGCCGGACCCCGACC
2627	HPR214 right	0.27 AGCAAAAGCTATTATTCGCTTGATGAGCCAATCAGATG
2628	Hcd586 left	0.26 GTCCTGTCTAAAGGAAGAAGTTGTTACTGTAAACAGT
2629	Hcd249 right	0.26 CAGGTACATGTTGATCAGCAGGGCTGGAGGCCCGCTC
2630	Hcd689 right	0.27 GTACATCTGGATGTAGTTGTGCTGCAGCTGCTTGTGAG
2631	mir-194-2No1	0.25 TGGTCCCGCCCCCTGTAACAGCAACTCCATGTGGAAGTG
2632	Hcd581 right	0.26 AGGAGATATGCCAAGATATATTACAGCTTATACACA
2633	Hcd270 right	0.3 AAATGTTGTTATAGTATCCCACCTACCCGATGTATCTT

TABLE 99-continued

5-Fluorouracil microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2634	mir-025-prec	0.27 ACGCTGCCCTGGGCATTGCAC TTGTCTCGGTCTGACAGTG
2635	Hcd340 left	0.27 GGACAATTCAACAGTGGTGAGTCACTTCCCACTTTCAAG
2636	mir-007-1-prec	0.27 TGTTGGCCTAGTTCTGTGTTGGAAAGACTAGTGATTTGTTG
2637	mir-093-prec-7.1 = 093-1	0.25 CCAAAGTGTGTTCTGTGCAGGTAGTAGTGATTACCCAACCT
2638	mir-106-prec-X	0.26 CCTTGGCCATGTAAAAGTGCTTACAGTGCAGGTAGCTTT
2639	Hcd794 right	0.27 GGCCACCACAGACACCAACAAAGTTCAAGTCCGTTCTGCAG
2640	mir-020-prec	0.26 TAAAGTGCTTATAGTGCAAGGTAGTGTTAGTTATCTACTG
2641	mir-4323p	0.26 CCTTACGTGGGCCACTGGATGGCTCCTCCATGTCTGGAG

TABLE 100

Radiation microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2642	mir-136-precNo2	0.3 TGAGCCCTCGGAGGACTCCATTGTTTGATGATGGATTG
2643	Hcd570 right	0.26 GCCCCAACAGAACAACTTGTCTCCAGAGCCTGAGGTTTA
2644	Hcd873 left	0.26 TCTTCTGACAATGAAGGTAGGCGACAACGAGGAGATTGC
2645	Hcd282PO right	0.26 GAAGACGGACTTGGTCCGTTGACCAGCCAGAGCAGGGGG
2646	Hcd799 left	0.25 GTCCGGCGCGAGTGGAGCTGGTAAAAATGGCGGCCGAAG
2647	Hcd829 right	0.39 AAAATGGCGCGGGAAAAGCGAGCGCGAGAGCGAGGGAGG
2648	Hcd210_HPR205 right	0.32 CGAACACATTGCGGGTGCACTTCTTTCAGTATCCTATTG
2649	mir-219-prec	0.26 ATTGTCCAAACGCAATTCTCGAGTCTATGGCTCCGGCGA
2650	mir-202*	0.31 CCCCCCCCGCCGTTCTTTCTATGCATATACTCTTGA
2651	mir-429No2	0.42 CACCGCGGGCGATGGCGTCTTACAGACATGGTTAGAC
2652	Hcd693 right	0.32 AGGCTTTGTGCGCGCATTAAGCTGCCGGACCCCGACC
2653	mir-022-prec	0.34 TGTCTGACCCAGCTAAAGCTGCCAGTTGAAGAACTGTTG
2654	NPR88 right	0.32 CTTACCCCTGGTGCCTGGGGCCGCAGGGCTAACACCAAAAA
2655	mir-198-prec	0.39 TCATTGGTCCAGAGGGAGATAGGTTCTGTGATTTTCC
2656	mir-199b-precNol	0.29 GTCTGCACATTGGTAGGCTGGCTGGTTAGACCCCTCGG
2657	Hcd145 left	0.26 AAAATCCCAGCGGCCACCTTCCTCCCTGCCCATGGGG
2658	mir-124a-2-prec	0.34 TTAAGGCACCGCGGTGAATGCCAAGAGCGGAGCCTACGGCT
2659	mir-138-2-prec	0.39 AGCTGGTGTGAATCAGGCCAGAGCAGCGCATCCTC
2660	Hcd960 left	0.29 CTCAGTCTGCGGGCCCCGAGGAGGGTTGTGGGCCCTTTT
2661	Hcd869 left	0.31 CGAGAGGCACTTTGTACTTCTGCCAGGAGACCATATGATA
2662	Hcd384 left	0.41 TTACCCAGCCGGCCCAACACAGATCCTCTCCCT

TABLE 100-continued

Radiation microRNA biomarkers.

SEQ	ID	Medianprobe	Corr Sequence
2663	mir-027b-prec	0.31	CCGCTTGTTACAGTGGCTAAGTCTGCACCTGAAGAGA
2664	Hcd444 right	0.31	GTATATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
2665	mir-194-2No1	0.3	TGGTCCCGCCCCCTGTAACAGCAACTCCATGTGGAAGTG
2666	mir-197-prec	0.44	TAAGAGCTCTTCACCCTTCACCACCTCTCCACCCAGCAT
2667	Hcd913 right	0.39	CAAACATCATGTGACGTCTGTGGAGCGGCGGGCGCGG
2668	HPR163 left	0.39	GCTGCCCTCCCTTAGCAACGTGGCCCCGGCGTTCCAAA
2669	mir-138-1-prec	0.25	AGCTGGTGTGTGAATCAGGGCGTTGCCAATCAGAGAACG
2670	mir-010a-precNo1	0.25	GTCTGTCTCTGTATATACCTGTAGATCCGAATTGTGT
2671	mir-023b-prec	0.34	AATCACATTGCCAGGGATTACCACGCAACCACGACCTTGG
2672	mir-193bNo2	0.35	CTGTTGTCAGAACATGGGGTTTGAGGGCGAGATGAGTT
2673	Hcd654 left	0.43	AACGAGTAAAGCGTACATGGAGCGCGGGCGGCAGAG
2674	Hcd542 left	0.26	ATCTCAGTAGCCAATATTTCTCTGCTGGTATCAAATGA
2675	mir-199a-2-prec	0.28	TCGCCCGAGTGTTCAGACTACCTGTTCAGGACAATGCGT
2676	mir-214-prec	0.43	TGTACAGCAGGCACAGACAGGCAGTCACATGACAACCCAG
2677	Hcd608 right	0.31	CTTGTGTTTACAGCAGGCCACAGGGCCCTACATCCTTCCT
2678	Hcd684 right	0.28	AGAAGGCAGCTCCCTGCTAGCCGGCTGTCTAAATTATA
2679	mir-145-prec	0.4	CCTCACGGTCCAGTTTCCCAGGAATCCCTAGATGCTAA
2680	mir-023a-prec	0.37	TCCTGTACAAATCACATTGCCAGGGATTCCAACCGGACC
2681	mir-024-2-prec	0.32	AGTTGGTTGTGTACACTGGTCAGTTCAGCAGGAAACAGG
2682	mir-199a-1-prec	0.29	GCCAACCCAGTGTTCAGACTACCTGTTCAGGAGGCTCTCA

TABLE 101

5-Aza-2'-deoxycytidine (decitabine) microRNA biomarkers.		
SEQ	ID	NO Medianprobe Corr Sequence
2683	mir-096-prec-7No1	0.36 CTCCGCTCTGAGCAATCATGTGCAGTGCCAATATGGAAA
2684	Hcd605 right	0.25 GGTTAAAGACTCTAACAAACGAGTTGTGAATTGTAGCAATG
2685	mir-20bNo1	0.3 AGTACCAAAAGTGCATAGTCAGGTAGTTGGCATGAC
2686	miR-373*No1	0.26 GGGATACTCAAAATGGGGCGCTTCCTTTGTCTGTAC
2687	HUMTRAB	0.3 ATGGTAGAGCGCTCGCTTGCTGCGAGAGGTAGCGGGAT
2688	mir-019b-1-prec	0.25 TTCTGCTGTGCAAATCCATGCAAAACTGACTGTGGTAGTG
2689	HPR163 left	0.31 GCTGCCCTCCCTTAGCAACGTGGCCCCGGCGTTCCA
2690	mir-371No1	0.25 ACTTTCTGCTCTGGTGAAGTGCGGCCATCTTTGAGT
2691	mir-025-prec	0.29 ACGCTGCCCTGGCATTGCACTTGTCTCGGTCTGACAGTG
2692	mir-18bNo2	0.27 AGCAGCTTGAATCTACTGCCCTAAATGCCCTTCTGCA

TABLE 101-continued

<u>5-Aza-2'-deoxycytidine (decitabine) microRNA biomarkers.</u>			
SEQ	ID	NO Medianprobe	Corr Sequence
		2693 mir-093-prec-7.1 = 093-1	0.28 CCAAAGTGCCTCGTCAGTAGTGATTACCCAACCT
		2694 mir-106-prec-X	0.29 CCTTGGCCATGTAAAAGTGCTTACAGTGCAGGTAGCTTT
		2695 mir-142-prec	0.29 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
		2696 mir-020-prec	0.29 TAAAGTGCTTATAGTGCAAGTAGTGTGTTAGTTACTG

TABLE 102

<u>Idarubicin microRNA biomarkers.</u>			
SEQ	ID	NO Medianprobe	Corr Sequence
		2697 HUMTRF	0.33 GATCTAAAGGCCCTGGTCGATCCCCGGTTTCGGCACCA
		2698 mir-483No1	0.3 ATCACGCCCTCCTCACTCCCTCTCCCTCCGCTTCTCCTCTC
		2699 MPR74 left	0.27 CAAAGGTACAATTAACATTCAATTGTTGTCGGTGGTTGT
		2700 mir-122a-prec	0.27 CCTTAGCAGAGCTGTGGAGTGTGACAATGGGTGTTGTGTC
		2701 ath-MIR180aNo2	0.29 TGAGAATCTTGATGATGCTGCATCGGAATCAACGACTAT
		2702 mir-128b-precNo1	0.26 TCACAGTGAACCGGTCTCTTCCCTACTGTGTCACACTCC
		2703 Hcd923 left	0.25 TGGGAACCTTGTAAAAATGCAGATTCTGATTCTCAGGTCT
		2704 mir-106-prec-X	0.25 CCTTGGCCATGTAAAAGTGCTTACAGTGCAGGTAGCTTT
		2705 mir-342No1	0.36 GTCTCACACAGAAATCGCACCCGTACCTGGCCTACTTA
		2706 mir-142-prec	0.34 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
		2707 HPR169 right	0.25 GTTTCTTCTCACGGTAACTGGCAGCCTCGTTGTGGCTG
		2708 mir-223-prec	0.36 GAGTGTCAAGTTGTCAAATACCCAAGTGCAGGACATGCT
		2709 Hcd754 left	0.26 TCCTCCCTCCTCTTTCGTTCCGGCTCCCTGGCTGGCTCC
		2710 mir-020-prec	0.29 TAAAGTGCTTATAGTGCAAGTAGTGTGTTAGTTACTG

TABLE 103

<u>Melphalan microRNA biomarkers.</u>			
SEQ	ID	NO Medianprobe	Corr Sequence
		2711 mir-124a-3-prec	0.32 TTAAGGCACGCCGGTGAATGCCAAGAGAGGCCCTCCGCCG
		2712 mir-181a-precNo1	0.28 TCAGAGGACTCCAAGGAACATTCAACGCTGTGGTAGTT
		2713 Hcd773 left	0.26 CTTCCTCCCTGGGCATCTCTAGCACAGGGGATCCCCAAC
		2714 Hcd683 left	0.25 CTATGACAGAAGGTACTCTGTGGGAGGGAGATAATAG
		2715 Hcd796 left	0.29 GGTGGGATTACCCGGCTGCCGCTGTCGCCCTGGATGGTCTC
		2716 HUMTRF	0.44 GATCTAAAGGCCCTGGTCGATCCGGTTCCGGCACCA

TABLE 103-continued

<u>Melphalan microRNA biomarkers.</u>			
SEQ	ID	NO Medianprobe	Corr Sequence
		2717 HUMTRS	0.27 TCTAGCGACAGAGTGGTTCAATTCCACCTTCGGCGCCA
		2718 mir-181b-2No1	0.25 CTGATGGCTGCACTAACATTGCTGTCGGTGGTTT
		2719 Hcd294 left	0.26 TTATCATAAAATAATCACAGCCCTCAGGTGCTGTGAGGCA
		2720 mir-20bNo1	0.27 AGTACCAAAGTGCTCATAGTCAGGTAGTTGGCATGAC
		2721 mir-181dNo1	0.27 GAGGTACAAATCAACATTGATTGTCGGTGGTTGTGA
		2722 mir-213-precNo1	0.4 AACATTTCATTGCTGTCGGTGGTTGAACTGTGTGGACAAG
		2723 Hcd148_HPR225left	0.29 AATTAATGACCAAAATGTCAGATGTGTCCACAGCTAATT
		2724 mir-515-15p	0.34 GATCTCATGCAGTCATTCTCCAAAAGAACGACTTCTGT
		2725 mir-181b-precNo1	0.43 TGAGGTTGCTTCAGTGAACATTCAACGCTGTCGGTGAGTT
		2726 Hcd783 left	0.26 CAGGCTCACACCTCCCTCCCCAACTCTCTGGAATGTATA
		2727 HUMTRAB	0.29 ATGGTAGAGCGCTCGCTTGCTTGCGAGAGGTAGCGGGAT
		2728 HUMTRN	0.27 CAATCGTTAGCGCGTCTGGCTGTTAACGAAAGGTTGGT
		2729 mir-181b-1No1	0.31 TTTAAAGGTACAATCAACATTGCTGTCGGTGGGT
		2730 mir-124a-1-prec1	0.31 ATACAATTAAGGCACGCCGTGAATGCCAAGAATGGGCTG
		2731 mir-367No1	0.26 TCTGTTGAATATAATTGAAATTGCACTTAGCAATGGTG
		2732 mir-128b-precNo1	0.38 TCACAGTGAACCCGTCTTTCCCTACTGTGTACACTCC
		2733 Hcd43_8right	0.25 GTGTTTATTGAAATCTCACATCGCTCATAAGAATACACGC
		2734 mir-025-prec	0.3 ACGCTGCCCTGGCATTGCACCTTGCTCGGTCTGACAGTG
		2735 mir-216-precNo1	0.35 CTGGGATTATGCTAACAGAGCAATTCTAGCCCTCACG
		2736 Hcd731 left	0.26 AATTGTCACAATGAGTGGGGAGTTGTGATGATTATC
		2737 mir-093-prec-7.1 = 093-1	0.25 CCAAAGTGCCTGTCGTGCAGGTAGTGTGATTACCCAACCT
		2738 mir-106-prec-X	0.27 CCTTGGCCATGTAAGGTGCTTACAGTGCAGGTAGCTTT
		2739 mir-342No1	0.36 GTCTCACACAGAAATGCACCCGTACCTTGGCTACTTA
		2740 mir-142-prec	0.53 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
		2741 HSHELA01	0.32 GGCGCAGCAACCTCGGTCGTATCCGAGTCACGGCACCA
		2742 HUMTRV1A	0.25 ACGCGAAAGGTCCCCGGTTCGAAACCGGGCGGAAACACCA
		2743 mir-223-prec	0.46 GAGTGTCAAGTTGTCAAATACCCAAGTGCAGGCACATGCT
		2744 Hcd754 left	0.45 TCCTCCTCCTCCTTGTGTTCCGGCTCCCTGGCTGGCTCC
		2745 mir-020-prec	0.3 TAAAGTGCTTATAGTGCAGGTAGTGTGTTAGTTACTG

TABLE 104

<u>IL4-PR3B fusion protein microRNA biomarkers.</u>			
SEQ	ID	NO	Medianprobe Corr Sequence
		2746	Hcd829 right 0.28 AAAATGGCGCGGGAAAAGCGAGCGCGAGAGCGAGGAGG
		2747	mir-197-prec 0.28 TAAGAGCTCTTCACCCCTCACCAACCTCTCCACCCAGCAT
		2748	HPR163 left 0.28 GCTGCCCTCCCTAGAACGTGGCCCCGGCGTTCCAAA
		2749	mir-150-prec 0.47 CTCCCCATGCCCTGTCTCCAACCCTGTACCAGTGCTG

TABLE 105

<u>Valproic acid (VPA) microRNA biomarkers.</u>			
SEQ	ID	NO	Medianprobe Corr Sequence
		2750	mir-034precNo1 0.26 GAGTGTTCCTTGGCAGTGTCTTAGCTGGTTGGTGAGC
		2751	Hcd255 left 0.28 CTAGCTCCGTTCTGATCCGGGAGCCTGGTGCCAGCGAGA
		2752	Hcd712 right 0.27 GAAGATCGGTTGTATCTGGTCTGGTCAGCCGGCCCCGA
		2753	Hcd965 left 0.26 TGTTAAGTGGAAAGCCTCCAGGAACGTGGCAGAAAAAGG
		2754	Hcd891 right 0.29 GCAACGGCCTGATTACAACACCAGCTGCCAACACACC
		2755	Hcd210_HPR205 right 0.31 CGAAACATTGCGGTGACTTCTTTAGTATCTATT
		2756	mir-429No2 0.33 CACCGCCGGCCGATGGCGTCTTACAGACATGGTAGAC
		2757	Hcd753 left 0.27 GACCTGATTCCCCTTTGTATTGGCGACCACCCGACTG
		2758	Hcd693 right 0.38 AGGCTTGTGCGCGATTAAAGCTGCCGGACCCCCGACC
		2759	MPR203 left 0.25 CTATATTGGACCGCAGCGCTGAGAGCTTTGTGTTAATG
		2760	Hcd704 left 0.4 TCTGTATTAATTGGCTCAGCCGGAAAGATTTGGCTC
		2761	Hcd863PO right 0.3 TTGCAGAGCCTAACACAGGCCAGAGAGGCAGTGATCG
		2762	mir-122a-prec 0.29 CCTTAGCAGAGCTGGAGTGTGACAATGGTGTGTC
		2763	Hcd760 left 0.35 TGTGGTCACGTTCTCCCTCTGCTGGCCCCATCTGTC
		2764	Hcd338 left 0.35 CTTCTCCTCCTGTCGCCGCAGGCCCGTCCCAGTAGTC
		2765	HPR213 right 0.33 ACAACTTGTGCTGGTGCCTGGGAAGTTGTCTCAA
		2766	Hcd852 right 0.26 AAAAGTAAACAACAATTGCGCTGCCAGCCTCCCATTAG
		2767	Hcd366 left 0.28 ATACTAGATTAATTTCAGCCCCGGCCAATCTGTCAAAG
		2768	MPR103 right 0.27 GAGGTGTTGTGCTCCACTCGGCTCCCTGGTTACATAAC
		2769	Hcd669 right 0.27 ATGTTAACAGTCCAGGTTGTAGAATATGTGGTGGACC
		2770	mir-188-prec 0.27 TCACATCCCTTGCATGGTGGAGGGTGAGCTTCTGAAAAC

TABLE 106

All-trans retinoic acid (ATRA) microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2771	Hcd257 left	0.42 CTTCTTGATAAGCACTGTGCTAAAATTGCAGACACTAGG
2772	mir-148-prec	0.45 TGAGTATGATAGAAGTCAGTGCACATACAGAACTTTGTCTC
2773	Hcd512 left	0.28 CTGCGCTCTCGGAATGACTCGCTCCAATCCGCTTCGCG
2774	HPR227 right	0.25 CAGTGCAATGATATTGTCAAAGCATCTGGGACCAGCCTG
2775	Hcd421 right	0.37 AGTAAACAAATGTCGGCTTCCGCCTCTCCCCGTGCCATCC
2776	MPR203 left	0.39 CTATATTGGACCGCAGCGCTGAGAGCTTTGTGTTAATG
2777	mir-017-precNo1	0.26 GCATCTACTGCAGTGAAGGCACTTGTAGCATTATGGTGAC
2778	mir-219-2No1	0.26 CTCAGGGCTTCGCCACTGATTGTCCAACGCAATTCTTG
2779	mir-328No1	0.3 GAAAGTGCATACAGCCCTGGCCCTCTGCCCTCCGTC
2780	Hcd783 left	0.31 CAGGCTCACACCTCCCTCCCCAACTCTCTGGAATGTATA
2781	Hcd181 left	0.32 TTGGCGTCCTTGTCTCTCTCCCAGTGGCCTCC
2782	HPR213 right	0.3 AACAAACTTGTGCTGGTGCAGGGAAAGTTGTCTCCAA
2783	mir-191-prec	0.31 CAACGGAATCCAAAAGCAGCTGTTCTCCAGAGCATTC
2784	mir-375	0.31 TTTTGTTCGTTCGGCTCGCGTGAGGCAGGGCGGCCTCTC
2785	mir-212-precNo2	0.26 CGGACAGCGCGCCGGCACCTTGCTCTAGACTGCTTACTG
2786	Hcd913 right	0.34 CAAACATCATGTGACGTCTGTGGAGCGCGGGCGCGCG
2787	Hcd716 right	0.48 CAATAAATGTGCCATAAAGGCGCCGGCTCGGGCGCG
2788	MPR207 right	0.3 AACAAACTTGTGCTGGTGCAGGGAAAGTTGTCTCCTA
2789	HPR206 left	0.26 CTATATTGGACCGCAGCGCTGAGAGCTTTGTGTTAATG
2790	mir-016b-chr3	0.29 GTTCCACTCTAGCAGCACGTAAATATTGGCGTAGTGAAAT
2791	Hcd654 left	0.34 AACGAGTAAAGGCGTACATGGAGCGCGGGCGGAG
2792	mir-195-prec	0.3 TCTAGCAGCACAGAAATTGGCACAGGGAAAGCGAGTCTG
2793	Hcd425 left	0.25 GGTTCTACTCTTACCCCTCCCCACGTGGTTGTGCTG
2794	mir-148aNo1	0.35 TGAGTATGATAGAAGTCAGTGCACATACAGAACTTTGTCTC
2795	mir-142-prec	0.36 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGTG
2796	mir-016a-chr3	0.25 CAATGTCAGCAGTGCCCTAGCAGCACGTAAATATTGGCGT

TABLE 107

Cytoxin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2797	Hcd99 right	0.25 CAATCCTGGCTGCAGGCATATTGCAATTGGATGCTGTG
2798	mir-520c/526a	0.32 TCTCAGGCTGTCGCTCTAGAGGAAAGCACTTCTGTTG
2799	mir-191-prec	0.32 CAACGGAATCCAAAAGCAGCTGTTCTCCAGAGCATTC
2800	mir-205-prec	0.35 TCCTTCATTCCACCGGAGTCTGTCTCATACCAACCAGAT

TABLE 107-continued

Cytoxan microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2801	mir-375	0.33 TTTTGTTCGGCTCGCGTGAGGCAGGGGCGGCCTCTC
2802	mir-423No1	0.29 CAAAAGCTCGGTCTGAGGCCCTCAGTCTTGCTTCATAAC
2803	mir-449No1	0.39 TGTGATGAGCTGGCAGTGTATTGTTAGCTGGTTGAATATG
2804	mir-196-2-precNo2	0.26 GCTGATCTGTGGCTTAGGTAGTTCATGTTGGGATTG

TABLE 108

Topotecan (Hycamtin) microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2805	HUMTRF	0.26 GATCTAAAGTCCCTGGTCGATCCCCGGTTTCGGCACCA
2806	MPR74 left	0.29 CAAAGGTACAATTAACATTCAATTGTTGCTGGTGGGTGT
2807	mir-213-precNo1	0.28 AACATTCAATTGCTGCGTGGGTTGAACGTGTGGACAAG
2808	mir-155-prec	0.31 TTAATGCTAACATCGTGATAGGGGTTTGCCTCCAAGTGAC
2809	mir-181b-precNo1	0.31 TGAGGTTGCTTCAGTGAACATTCAACGCTGTCGGTGAGTT
2810	mir-342No1	0.33 GTCTCACACAGAAATCGCACCCGTCACCTTGGCCTACTTA
2811	mir-4323p	0.28 CCTTACGTGGGCCACTGGATGGCTCCTCATGTCCTGGAG

TABLE 109

Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza) microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2812	mir-092-prec-X = 092-2	0.38 GTTCTATATAAAGTATTGCACTTGTCCCGGCCTGTGGAAG
2813	mir-123-precNo1	0.31 GACGGGACATTATTACTTTGGTACGCGCTGTGACACTTC
2814	mir-514-1No2	0.29 TGTCTGTGGTACCCACTCTGGAGAGTGACAATCATGTAT
2815	mir-101-prec-9	0.25 GCTGTATATCTGAAAGGTACAGTACTGTGATAACTGAAGA
2816	mir-148-prec	0.36 TGAGTATGATGAGACTCAGTGCACACAGAACTTGTCTC
2817	mir-106aNo1	0.34 CCTTGGCCATGTAAAAGTGCCTACAGTGCAGGTAGCTTT
2818	mir-20bNo1	0.41 AGTACCAAAGTGCATAGTGCAGGTAGTTGGCATGAC
2819	Hcd781 right	0.32 AGTTCTTTAATTAATGAAAGTTTGGGCTGCTCCACTT
2820	mir-017-precNo2	0.29 GTCAGAATAATGTCAGGTCTACAGTGCAGGTAGTGAT
2821	mir-019b-2-prec	0.42 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
2822	mir-033-prec	0.27 GTGGTGCAATTGTTAGTCAGTGCATGTTCTGGTGGTACCC
2823	mir-092prec-13 = 092-1No2	0.28 TCTGTATGGTATTGCACTTGTCCCGGCCTGTTGAGTTGG
2824	mir-107No1	0.29 GGCATGGAGTTCAAGCAGCATTGTACAGGGCTATCAAAGC

TABLE 109-continued

<u>Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza) microRNA biomarkers.</u>		
SEQ ID	NO Medianprobe	Corr Sequence
	2825 mir-103-prec-5 = 103-1	0.32 TATGGATCAAGCAGCATTGTACAGGGCTATGAAGGCATTG
	2826 MPR216 left	0.29 GATCCTAGTAGTGCCAAAGTGCCTAGTCAGGTAGTTT
	2827 mir-29b-2 = 102prec7.1 = 7.2	0.27 AGTGATTGTCTAGCACCATTGAAATCAGTGTCTGGGG
	2828 mir-019b-1-prec	0.4 TTCTGCTGTGCAAATCCATGCAAAATGACTGTGGTAGTG
	2829 mir-107-prec-10	0.3 GGCATGGAGTTCAAGCAGCATTGTACAGGGCTATCAAAGC
	2830 mir-135-2-prec	0.37 CACTCTAGTGCTTATGGCTTTTATTCCCTATGTGATAGT
	2831 Hcd581 right	0.28 AGGAGATATGCCAAGATATATTCACAGCTTATATACACA
	2832 mir-103-2-prec	0.29 GTAGCATTTCAGGTCAAGCAACATTGTACAGGGCTATGAAA
	2833 Hcd230 left	0.27 CATTCTCTACAAGCATATGGCCTTGGGACATTAAGATGGC
	2834 mir-025-prec	0.4 ACGCTGCCCTGGGCATTGCACTTGTCTCGGTCTGACAGTG
	2835 mir-208-prec	0.31 ACCTGATGCTCACGTATAAGACGAGCAAAAGCTTGTGG
	2836 mir-18bNo2	0.31 AGCAGCTTAGAATCTACTGCCCTAAATGCCCTTCTGGCA
	2837 mir-093-prec-7.1 = 093-1	0.39 CCAAAGTGCTGTTCGTGCAGGTAGTGTGATTACCCAACCT
	2838 mir-106-prec-X	0.48 CCTTGGCCATGTAAAGTGCCTACAGTGCAGGTAGCTTTT
	2839 mir-142-prec	0.37 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGTG
	2840 HPR169 right	0.28 GTTCTTTCTCACGGTAACTGGCAGCCTCGTTGGGCTG
	2841 mir-018-prec	0.44 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
	2842 mir-020-prec	0.48 TAAAGTGCTTATAGTGCAGGTAGTGTAGTTATCTACTG

TABLE 110

<u>Depsipeptide (FR901228) microRNA biomarkers.</u>		
SEQ ID	NO Medianprobe	Corr Sequence
	2843 Hcd415 right	0.27 GATGTTGGAAACAATGGGAGTGAGAGAATGGGAGAGCT
	2844 mir-147-prec	0.27 GACTATGGAAGCCAGTGTGGAAATGCTTCTGCTAGATT
	2845 mir-033b-prec	0.34 GTGCATTGCTGTTGCATTGCACGTGTGAGGGCGGGTGCA
	2846 Hcd778 right	0.34 CAGAGGGGAGGCCAGAGGAGGGAAAGCTGGCAAAG
	2847 mir-127-prec	0.25 TC GGATCCGCTGAGCTTGGCTGGTCAAAGTCTCATCAT
	2848 mir-324N01	0.28 TGGAGACCCACTGCCAGGTGCTGCTGGGGTTGTAGTC
	2849 Hcd794 right	0.35 GGCCACCACAGACACCAACAAGTTCAAGTCCAGTCCGTTCTGCAG
	2850 Hcd634 left	0.27 CTGCTCCGCTCAGAGCCTTCCCTCCACTCCTGTTCA

TABLE 111

Bortezomib microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2851	MPR121 left	0.31 CACCTGGCTCTGAGAACTGAATTCCATAGGCTGTGAGCTC
2852	Hcd115 left	0.27 CTCTGTGGCCATTTCGGTTTCCAGTCGATGCCCTGA
2853	Hcd693 right	0.28 AGGCTTGTGCGCGATTAAAGCTGCCGGACCCCGACC
2854	Hcd704 left	0.25 TCTGTATTTAATTGGCTCAGCCGGAAAGATTTGGCTC
2855	HPR100 right	0.28 GGTGTTGTGCTCCACTCAGCTCCCTGGTTACATAACAG
2856	Hcd760 left	0.26 TGTGGTCACGTTCTCCCTCTGCTGGCCCCATCTGTC
2857	mir-147-prec	0.3 GACTATGGAAGCCAGTGTGAAATGCTCTGCTAGATT
2858	mir-033b-prec	0.29 GTGCATTGCTGTTGCATTGCACGTGTGAGGCGGGTGCA
2859	mir-146-prec	0.33 TGAGAACTGAATTCCATGGGTTGTCAAGTGTGAGACACTC
2860	Hcd142 right	0.3 TAAATGTGTAATTCTCCCTTGACGGCCCCGGCCGCTGG
2861	mir-501No2	0.33 ATGCAATGCACCCGGCAAGGATTCTGAGAGGGTGAGCCC
2862	Hcd716 right	0.26 CAATAATGTGCCTATAAGGCGCCGGCTCGGGCGCG
2863	MPR207 right	0.27 AACAACTTGTGCTGGTGCCGGGAAGTTGTGCTCCA
2864	Hcd777 left	0.26 CAGGTGGGTGCTGAGGCCGCGTTGCTTGAAGCTAGCC
2865	mir-204-precNo2	0.27 AGGCTGGAAGGCAAAGGGACGTTCAATTGTCATCACTGG
2866	mir-146bNo1	0.26 CACCTGGCACTGAGAACTGAATTCCATAGGCTGTGAGCTC
2867	Hcd511 right	0.29 TACCTCAGAACGCTCACTCAACCCCTCTCCGCTGAGTC
2868	Hcd397 left	0.28 GTGTGTATACTTATGTGTTGATGTGAGTGTGAATAT
2869	MPR130 right	0.33 CAATCACAGATAGCACCCCTCACCTGAGCCCATTTCAC
2870	Hcd782 left	0.28 GGAGCCCTGTCTGCAAAGAGTGGTGCCTGTGCTGTGA
2871	mir-324No2	0.28 CTGACTATGCCTCCCCCATCCCCTAGGGCATTGGTGTAA
2872	Hcd794 right	0.34 GGCCACCACAGACACCAACAAGTTCAAGTCCAGTGTGAG
2873	Hcd739 right	0.29 TATTAGCTGAGGGAGGGCTGGAGGCGCTGCATTCCGACT

TABLE 112

Leukeran microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2874	mir-092prec-X = 092-2	0.39 GTTCTATATAAGTATTGCACTTGTCCCCGCTGTGGAAG
2875	mir-096-prec-7No1	0.26 CTCCGCTCTGAGCAATCATGTGCAGTGCATAATGGGAAA
2876	mir-123-precNo2	0.32 TGTGACACTTCAAACCTCGTACCGTGAGTAATAATGCC
2877	MPR249 left	0.26 TCGGTTGGTTCAAGCTGGTATGCTTCCAGTATCTCATTC
2878	HPR232 right	0.28 TGAATTATTGACAATAATTCAAGTCCAGTGTGATAACTGAAGA
2879	mir-101-prec-9	0.4 GCTGTATCTGAAAGGTACAGTACTGTGATAACTGAAGA
2880	mir-106aNo1	0.31 CCTTGGCCATGTAAAAGTGCCTACAGTGCAGGTAGCTTT

TABLE 112-continued

<u>Leukeran microRNA biomarkers.</u>		
SEQ	ID	NO Medianprobe Corr Sequence
2881	mir-20bNo1	0.38 AGTACCAAAGTGCTCATAGTCAGGTAGTTGGCATGAC
2882	Hcd861 right	0.25 AAGGTCTGGATTGATCGTACTGCTTCTGAAAGGTAAAAA
2883	mir-017-precNo2	0.26 GTCAGAATAATGTCAAAGTGCTTACAGTCAGGTAGTGAT
2884	mir-019b-2-prec	0.33 GTGGCTGTGCAAATCCATGCAAAAGTGAATTGTGATAATGT
2885	mir-033-prec	0.3 GTGGTGCATTGTTAGTTGCATTGCATGTTCTGGTAGTACCC
2886	Hcd102 left	0.26 ACTGGAATTATGTTTATCTTAAGTCCACACTGGATCCTC
2887	MFR216 left	0.32 GATCCTAGTAGTGCCAAAGTGCTCATAGTCAGGTAGTT
2888	Hcd975 left	0.25 GGTTTGTGTTTGTAAACAGCAGAAGGTATTAGTCCAT
2889	mir-019b-1-prec	0.3 TTCTGCTGTGCAAATCCATGCAAAAGTGAATGTGGTAGTG
2890	mir-135-2-prec	0.38 CACTCTAGTGCTTTATGGCTTTTATTCTATGTGATAGT
2891	Hcd581 right	0.26 AGGAGATATGCCAAGATATATTCAAGCTTATACACA
2892	Hcd536_HPR104 right	0.25 GCTGCTCTGCTGAGGGCTGGACTCTGTCCAGAACGACCA
2893	mir-128b-precNo2	0.25 GGGGCCGATAACTGTACGAGAGTGAGTAGCAGGTCTCA
2894	HSTRNL	0.37 TCCGGATGGAGCGTGGGTCGAATCCCACCTCTGACACCA
2895	mir-025-prec	0.47 ACGCTGCCCTGGGCATTGCACCTCTCGGTCTGACAGTG
2896	mir-18bNo2	0.27 AGCAGCTTAAATCTACTGCCCTAAATGCCCTCTGGCA
2897	HPR262 left	0.26 TCAGTTGGTTTCAGCTGGTATGCTTCCAGTATCTCATTC
2898	Hcd923 right	0.33 CTGGAGATAATGATTCTGCATTCTAATTAACCCAGGT
2899	Hcd434 right	0.3 CACTTTCCCTTGTGAAATCCTGGGTGACATCACCTCC
2900	Hcd658 right	0.28 GACTGCAGAGCAAAAGACACGATGGGTGCTATTGTTTC
2901	HPR129 left	0.29 TTTCCCTGCTGATTGCTTAATGGAAGCTGACAGTGAAAG
2902	mir-380-5p	0.32 AGTACCTGAAAAGATGGTGACCATAGAACATGCCCTAT
2903	mir-093-prec-7.1 = 093-1	0.45 CCAAAGTGCTGTTCGTCAGGTAGTGTGATTACCCAACCT
2904	mir-106-prec-X	0.5 CCTTGGCCATGTAAAGTGCTTACAGTCAGGTAGCTTT
2905	Hcd627 left	0.31 GCATTAGGGAGAATAGTTGATGGATTACAAATCTCTGCAT
2906	mir-142-prec	0.33 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2907	mir-018-prec	0.46 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
2908	mir-020-prec	0.5 TAAAGTGCTTATAGTGCAGGTAGTGTGTTAGTTACTG

TABLE 113

Fludarabine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2909	Hcd773 left	0.26 CTTCCCTCCCTGGGCATCTCTAGCACAGGGGATCCCCAAAC
2910	Hcd248 right	0.33 CATTATGCACAAATGGTATGAGAGGAAAATTAGGCAATAAGG
2911	mir-181dNo1	0.34 GAGGTCACAATCAACATTCAATTGTTGTCGGTGGGTGTGA
2912	MPR74 left	0.3 CAAAGGTACAATTAAACATTCAATTGTTGTCGGTGGGTGT
2913	mir-213-precNo1	0.37 AACATTCAATTGCTGTCGGTGGTTGAACTGTGTGGACAAG
2914	mir-155-prec	0.32 TTAATGCTAACCGTGATAGGGTTTTGCCTCCAAGTGAC
2915	MPR197 right	0.29 TATTATTACAAGGCCTTCTTCCCCTAAACTTGTCC
2916	mir-181b-precNo1	0.26 TGAGGTTGCTTCAGTGAACATTCAACGCTGTCGGTGAGTT
2917	mir-29b-2 = 102prec7.1 = 7.2	0.32 AGTGATTGTCTAGCACCATTGAAATCAGTGTCTGGGG
2918	mir-029c-prec	0.33 TTTGTCTAGCACCATTTGAAATCGGTTATGATGTAGGGG
2919	Hcd318 right	0.32 CAAGTGGTTAATTGAGCCCACAAGTGACCTACTCAATCAG
2920	mir-128b-precNo1	0.25 TCACAGTGAACCGGTCTCTTCCACTGTGTCAACTCC
2921	mir-130a-precNo2	0.27 TGTCTGCACCTGTCACTAGCAGTGCAATGTTAAAGGGCA
2922	mir-140No2	0.26 TTCTACCACAGGGTAGAACACCACGGACAGGATAACGGGGCA
2923	mir-16-2No1	0.31 GTTCCACTCTAGCAGCACGTAAATATTGGCGTAGTGAAAT
2924	mir-526a-2No1	0.26 GATCTCGTGCTGTGACCCCTCTAGAGGGAAAGCACTTCTGT
2925	mir-016b-chr3	0.3 GTTCCACTCTAGCAGCACGTAAATATTGGCGTAGTGAAAT
2926	mir-195-prec	0.34 TCTAGCAGCACAGAAATATTGGCACAGGGAAAGCGAGTCTG
2927	mir-216-precNo1	0.25 CTGGGATTATGCTAACAGAGCAATTCTAGCCTCAGC
2928	mir-342No1	0.26 GTCTCACACAGAAATCGCACCGTCACCTGGCTACTTA
2929	mir-29b-1No1	0.34 AGTGATTGTCTAGCACCATTGAAATCAGTGTCTGGGG
2930	Hcd627 left	0.33 GCATTAGGGAGAATAGTTGATGGATTACAAATCTCTGCAT
2931	mir-102-prec-1	0.33 TCTTGATCTAGCACCATTGAAATCAGTGTCTGGAG
2932	mir-142-prec	0.32 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGTG
2933	mir-223-prec	0.34 GAGTGTCAAGTTGTCAAATACCCCAAGTGCAGGCACATGCT
2934	let-7f-2-prec2	0.26 TGAGGTAGTAGATTGTATAGTTAGGGTCATACCCCATC
2935	mir-016a-chr13	0.36 CAATGTCAGCAGTGCCCTAGCAGCACGTAAATATTGGCGT

TABLE 114

Vinblastine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2936	Hcd794 right	0.33 GGCCACCACAGACACCAACAGTCAGTCGGTTCTGCAG
2937	Hcd754 left	0.25 TCCTCCTCCCTCTTCGTTCCGGCTCCCTGGCTGGCTCC

TABLE 115

Busulfan microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2938	mir-096-prec-7No2	0.27 TGGCCGATTTGGCACTAGCACATTGGCTTGTCTCT
2939	mir-124a-3-prec	0.25 TTAAGGCACGCCGTGAATGCCAAGAGAGGCCCTCCGCCG
2940	mir-101-prec-9	0.25 GCTGTATATCTGAAAGGTACAGTACTGTGATAACTGAAGA
2941	Hcd712 right	0.27 GAAGATCGGTTGTCATCTGGTCTGGTCAGCCCCCCCCGA
2942	Hcd693 right	0.26 AGGCTTGTCGCGCATTAAAGCTGCCGGACCCCCGACC
2943	mir-219-2No1	0.25 CTCAGGGCTTCGCCACTGATTGTCCAACGCAATTCTTG
2944	Hcd145 left	0.29 AAAAATCCCAGCGGCCACCTTCCTCCCTGCCCTCCCCATTGGG
2945	mir-155-prec	0.29 TTAATGCTAACCGTGTGATAGGGTTTGCCCTCAAAGTAC
2946	HPR213 right	0.3 AACAACTTGTGCTGGTGCCGGGAAGTTGTCTCCAA
2947	mir-212-precNo2	0.34 CGGACAGCGGCCGGCACCTGGCTCTAGACTGCTTACTG
2948	Hcd913 right	0.33 CAAACATCATGTGACGTCTGGAGCGCGCGCGCGCG
2949	Hcd716 right	0.51 CAATAATGTGCCTATAAGCGCCGGCTCCGGGGCGCG
2950	MFR207 right	0.26 AACAACTTGTGCTGGTGCCGGGAAGTTGTCTCCTA
2951	Hcd559 right	0.33 TTCTTTGTCTATACTTTCTAGATTCTATGCAGTTGGG
2952	Hcd654 left	0.28 AACGAGTAAAAGCGTACATGGAGCGCGGGCGAGAG
2953	Hcd739 right	0.27 TATTAGCTGAGGGAGGGCTGGAGGCCTGCATTCCACT
2954	mir-142-prec	0.4 CCCATAAAGTAGAACGACTAACAGCACTGGAGGGTG

TABLE 116

Dacarbazine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2955	mir-092-prec-X = 092-2	0.25 GTTCTATATAAGTATTGCACTTGTCCCGCCTGTGGAAG
2956	mir-123-precNo2	0.28 TGTGACACTTCAAACACTCGTACCGTGAGTAATAATGCCCG
2957	mir-101-prec-9	0.29 GCTGTATATCTGAAAGGTACAGTACTGTGATAACTGAAGA
2958	Hcd517 right	0.3 GAGGGATTACAGATTAACCTCCACTTCTCCAGACTCAGAA
2959	Hcd796 left	0.37 GGTGGGATTACCCGGCTGCCGCTGCGCCTGGATGGTCTC
2960	Hcd749 right	0.28 CGAGGAGGGAGGTGACTGCTGGATGGTTATGAGACAGAC
2961	Hcd674 left	0.25 CTCCAGTGTGGTGTGCCCTGCCCTCCGTATTGCTGTG
2962	mir-019b-2-prec	0.27 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
2963	mir-033-prec	0.29 GTGGTCATTGTAGTTGCATTGCATGTTCTGGTGGTACCC
2964	mir092-prec-13 = 092-1No2	0.33 TCTGTATGGTATTGCACTTGTCCGGCTGTTGAGTTGG
2965	mir-124a-2-prec	0.29 TTAAGGCACGCCGTGAATGCCAAGAGCGGAGCCTACGGCT
2966	mir-143-prec	0.36 CTGGTCAGTGGAGTCTGAGATGAAGCACTGTAGCTCAG
2967	mir-516-43p	0.28 AAAGAAAAGAAAGTGCCTCTTCAGAGGGTACTCTTG

TABLE 116-continued

<u>Dacarbazine microRNA biomarkers.</u>		
SEQ ID NO	Medianprobe	Corr Sequence
2968	mir-216-precNo1	0.31 CTGGGATTATGCTAAACAGAGCAATTCCCTAGCCCTCACG
2969	Hcd731 left	0.26 AATTGTGACAAC TGAGTGGGAGGTTGTGATGATTATC
2970	mir-106-prec-X	0.26 CCTTGCCCATGTAAAAGTGC TTACAGTGCAGGTAGCTTT
2971	mir-142-prec	0.48 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2972	mir-223-prec	0.48 CAGTGTCA GTTTGTCAAATACCCAAGTGC GGGCACATGCT
2973	Hcd754 left	0.32 TCCTCCCTCCCTTTCGTTCCGGCTCCCTGGCTGGCTCC
2974	mir-018-prec	0.27 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC

TABLE 117

<u>Oxaliplatin microRNA biomarkers.</u>		
SEQ ID NO	Medianprobe	Corr Sequence
2975	mir-092-prec-X = 092-2	0.36 GTTCTATATAAAGTATTGCAC TTGTCCCCCCTGTGGAAG
2976	mir-148-prec	0.27 TGAGTATGATAGAAGTCAGTGC ACTACAGAAC TTTGTCTC
2977	mir-20bNo1	0.27 AGTACCAAAGTGC TCA TAGTGCAGGTAGTTGGCATGAC
2978	mir-007-2-precNo2	0.28 GGACCGGCTGGCCCATCTGGAAAGACTAGTGATTTGTTG
2979	mir-017-precNo2	0.28 GTCAGAATAATGTCAAAGTGC TTACAGTGCAGGTAGTGAT
2980	mir-019b-2-prec	0.32 GTGGCTGTGCAAATCCATGC AAAACTGATTGTGATAATGT
2981	Hcd760 left	0.27 TGTGGTCACGTTCTCCCTCTGCTGGCCCCATCTGTC
2982	Hcd783 left	0.36 CAGGCTCACACCTCCCTCCCCA ACTCTCTGGATGTATA
2983	MPR216 left	0.26 GATCCTAGTAGTGCCAAAGTGC TCA TAGTGCAGGTAGTT
2984	mir-375	0.33 TTTTGTTCGGCTCGCGTGAGGCAGGGCGGCCTCTC
2985	mir-019b-1-prec	0.36 TTCTGCTGTGCAAATCCATGC AAAACTGACTGTGGTAGTG
2986	mir-135-2-prec	0.32 CACTCTAGTGCTTTATGGCTTTTATTCTATGTGATAGT
2987	mir-150-prec	0.25 CTCCCCATGGCCCTGTCTCCCAACCCTGTACAGTGC TG
2988	mir-128b-precNo1	0.33 TCACAGTGAACCGGTCTTTCCCTACTGTGTCACACTCC
2989	mir-499No2	0.26 GTGAACATCACAGCAAGTCTGTGCTGCTCCGTCC TAC
2990	mir-025-prec	0.38 ACGCTGCCCTGGCATTGCAC TTGTCTCGGTCTGACAGTG
2991	mir-007-1-prec	0.32 TGTTGGCCTAGTTCTGTGAGGAAAGACTAGTGATTTGTTG
2992	mir-019a-prec	0.33 TGTAGTTGTGCAAATCTATGC AAAACTGATGGTGGCCTGC
2993	mir-093-prec-7.1 = 093-1	0.46 CCAAAAGTGC TGTTCGTGCAGGTAGTGATTACCCAACCT
2994	mir-106-prec-X	0.45 CCTTGGCCATGTAAAAGTGC TTACAGTGCAGGTAGCTTT
2995	mir-142-prec	0.41 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
2996	HPR169 right	0.34 GTTCTTCTCACGGTA CTGGCAGCCTCGTTGTGGCTG

TABLE 117-continued

Oxaliplatin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
2997	mir-018-prec	0.4 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
2998	mir-020-prec	0.44 TAAAGTGCTTATAGTGCAGGTAGTGTGTTAGTTATCTACTG
2999	mir-484	0.33 GTCAGGCTCAGTCCCCTCCGATAACCCCTAAATAGGGA

TABLE 118

Hydroxyurea microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3000	Hcd257 left	0.34 CTTCTTGATAAGCACTGTGCTAAAATTGCAGACACTAGG
3001	Hcd768 right	0.26 GCCCTGGCGGAACGCTGAGAAGACAGTCGAACTTGACTAT
3002	Hcd796 left	0.25 GGTGGGATTACCCGGCTGCCGCTGTCGCGCTGGATGGCTC
3003	HUMTRF	0.48 GATCTAAAGTCCCTGGTCGATCCCCGGTTTCGGCACCA
3004	HUMTRS	0.3 TCTAGCGACAGAGTGGTTCAATTCCACCTTCGGCGCCA
3005	MPR74 left	0.28 CAAAGGTACAATTACATTCAATTGTTGTCGGTGGTTGT
3006	mir-213-precNo1	0.29 AACATTCAATTGCTGCGTGGTTGAACGTGCTGGACAAG
3007	mir-155-prec	0.35 TTAATGCTAACATCGTGATAGGGGTTTGCCTCAAATGAC
3008	Hcd763 right	0.25 GGTGCACTCTAAATTCCGTCCCTGCGGAAGGCTGACTAA
3009	mir-181b-precNo1	0.28 TGAGGTTGCTTCAGTGAACATTCAACGCTGTCGGTGAGTT
3010	ath-MIR180aNo2	0.26 TGAGAAATCTTGATGATGCTGCATCGGCAATCAACGACTAT
3011	mir-216-precNo1	0.37 CTGGGATTATGCTAACAGAGCAATTCCCTAGCCCTCACG
3012	mir-342No1	0.31 GTCTCACACAGAAATCGCACCCGTCACCTGGCCTACTTA
3013	mir-142-prec	0.49 CCCATAAAAGTAGAAAGCACTACTAACAGCACTGGAGGTG
3014	HSHELA01	0.31 GGCGCGAGCAACCTCGGTTGTATCCGAGTCACGGCACCA
3015	HUMTRV1A	0.26 ACGCGAAAGTCCCCGGTTCGAAACCGGGCGGAAACACCA
3016	mir-223-prec	0.59 CAGTGTCAAGTTGTCAAATACCCAAGTGCAGCACATGCT
3017	Hcd754 left	0.46 TCCTCCCTCCTCTTTCGTTCCGGCTCCCTGGCTGGCTCC
3018	mir-020-prec	0.26 TAAAGTGCTTATAGTGCAGGTAGTGTGTTAGTTATCTACTG

TABLE 119

Tegafur microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3019	Hcd257 right	0.26 CTTGGTTTGCATAATGCTAGCAGAGTACACACAAGAA
3020	Hcd946 left	0.26 CACAGGATTCAGGGAGAACGGTGGATTTACAAGAG
3021	Hcd503 left	0.3 GAGATGAGGTAGCTGCCAGGTGCCATGGGGTATAGGTGA

TABLE 119-continued

Tegafur microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3022	mir-429N01	0.25 CTAATACTGTCTGGTAAAACCGTCATCCGCTGCCGTGATC
3023	Hcd693 right	0.32 AGGCTTTGTGCGCGCATTAAAGCTGCCGGACCCCCGACC
3024	miR-373*No1	0.33 GGGATACTCAAATGGGGCGCTTCCTTTGTCTGTAC
3025	Hcd738 left	0.28 GAAAAACTTAAGATTCCCTCTCGGCCCTCATTTTAGCTG
3026	mir-328N01	0.33 GAAAGTGCATACAGCCCCTGCCCTCTGCCCTCCGTC
3027	Hcd783 left	0.36 CAGGCTCACACCTCCCTCCCCAACTCTTGTGAATGTATA
3028	Hcd181 right	0.34 GCTCACTGGCAGGAGCCCTAACGGATTGACAGCTGAG
3029	Hcd631 left	0.38 CAGATATTCTCAGGCAATCTCAGCCACAGCCTCTTG
3030	Hcd279 left	0.25 CGGACTAACACTCCGCGGGTGTTCATGGAGACCGAGGC
3031	mir-194-2N01	0.3 TGGTCCCAGCCCCCTGTAAACAGCAACTCCATGTGGAAGTG
3032	mir-197-prec	0.38 TAAGAGCTTTCACCCTTCACCACCTCTCCACCCAGCAT
3033	HPR163 left	0.39 GCTGCCCTCCCTTAGCAACGTGGCCCCGGCGTCCAAA
3034	mir-150-prec	0.32 CTCCCCATGGCCCTGTCTCCAAACCTTGTACAGTGTGCTG
3035	Hcd323 left	0.26 GTTGTAGCATGTGGTTGTATTAATGAACGTTACAGGAGAG
3036	mir-103-2-prec	0.28 GTAGCATTCAAGCAACATTGTACAGGGCTATGAAA
3037	Hcd243 right	0.27 TATTATACATCATTTCCATCAATCGACGAACTAAAGCCT
3038	Hcd938 right	0.27 ATTCCCTGCATCACTCTCATGAAATGGCTGAGAAAGTGAG
3039	mir-025-prec	0.29 ACGCTGCCCTGGCATTGCACTTGTCTCGGTCTGACAGTG
3040	mir-007-1-prec	0.36 TGTTGGCTAGTTCTGTGTGAAAGACTAGTGATTTGTTG
3041	MPR243 left	0.26 GTATTTACCTAGTTGTAATGTGGTTGCCATGGTGTGTTG
3042	Hcd511 right	0.27 TACCTCAGAACGCCTCACTCAACCCTCTCCGCTGAGTCTC
3043	Hcd654 left	0.26 AACGAGTAAAGGCGTACATGGAGCGGGGGCGGCAGAG
3044	mir-199a-2-prec	0.3 TCGCCCCAGTGTTCAGACTACCTGTTCAGGACAATGCCGT
3045	mir-214-prec	0.27 TGTACAGCAGGCACAGACAGGCAGTCACATGACAACCCAG
3046	mir-093-prec-7.1 = 093-1	0.33 CCAAAGTGCCTGTCGTGCAGGTAGTGTGATTACCCAACCT
3047	mir-106-prec-X	0.27 CCTTGGCCATGTAAAAGTGCCTACAGTGCAGGTAGCTTT
3048	Hcd794 right	0.41 GGCCACACAGACACCAACAAGTTCAAGTCCGTTCTGCAG
3049	Hcd530 right	0.26 AAGGAAAATCAAACCCACAATGCTGAACACAACAATGACC
3050	HSHELA01	0.34 GGCGCAGCAACCTCGGTTGTATCCGAGTCACGGCACCA
3051	Hcd754 left	0.29 TCCTCCTCCCTCTTCGTTCCGGCTCCCTGGCTGGCTCC
3052	mir-020-prec	0.29 TAAAGTGCCTTATAGTGCAGGTAGTGTGTTAGTTACTG

TABLE 120

Daunorubicin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3053	Hcd768 right	0.25 GCCCTGGCGAACGCTGAGAAGACAGTCGAACTTGACTAT
3054	HUMTRF	0.34 GATCTAAAGGTCCCTGGTTCGATCCGGGTTCGGCACCA
3055	Hcd145 left	0.28 AAAATCCCAGCGGCCACCTTCCTCCCTGCCATTGGG
3056	Hcd923 right	0.27 CTGGAGATAATGATTCTGCATTCTAATTAACCTCCAGGT
3057	mir-216-precNo1	0.27 CTGGGATTATGCTAACAGAGCAATTCTAGCCCTCACG
3058	mir-093-prec-7.1 = 093-1	0.25 CCAAAGTGCCTTCGTGCAGGTAGTGTGATTACCCAACCT
3059	mir-342N01	0.33 GTCTCACACAGAAATCGCACCCGTACCTGGCCTACTTA
3060	Hcd794 right	0.28 GGCCACCACAGACACCAACAAGTTCACTGGAGGGTG
3061	mir-142-prec	0.48 CCCATAAAGTAGAAAAGCACTACTAACAGCACTGGAGGGTG
3062	HSHELA01	0.3 GGCGCAGCAACCTCGGTTCTGATCCGAGTCACGGCACCA
3063	mir-223-prec	0.33 GAGTGTCAAGTTGTCAAATACCCAAAGTGCAGCACATGCT
3064	Hcd754 left	0.32 TCCTCCTCCTCCTTTCGTTCCGGCTCCCTGGCTGGCTCC

TABLE 121

Bleomycin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3065	mir-125b-2-precNo2	0.29 ACCAGACTTTCCCTAGTCCCTGAGACCCCTAACCTGTGAGG
3066	mir-022-prec	0.26 TGTCTGACCCAGCTAAAGCTGCCAGTTGAAGAACTGTTG
3067	mir-125b-1	0.29 TCCCTGAGACCCCTAACCTGTGATGTTACCGTTAAATCC
3068	mir-155-prec	0.38 TTAATGCTAACCGTGTAGAGGGTTTGCGCTCCAAGTGAC
3069	mir-100N01	0.25 CCTGTTGCCACAAACCGTAGATCCGAATTGTGGTATTA
3070	mir-409-3p	0.27 GACGAATGTTGCTCGGTGAACCCCTTTCGGTATCAAATT
3071	mir-495N01	0.31 GTGACGAAACAAACATGGTGACTTCTTTTGGTATCAA
3072	mir-199a-2-prec	0.29 TCGCCCCAGTGTCAAGACTACCTGTCAAGGACAATGCCGT
3073	mir-382	0.28 GGTACTTGAAGAGAAGTTGTTCGTGGATTGCTTTAC
3074	mir-100-1/2-prec	0.26 TGAGGCCTGTTGCCACAAACCGTAGATCCGAATTGTGG

TABLE 122

Estramustine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3075	Hcd338 left	0.32 CTCTCCTCCTGTCGCCGCAGGGCCCCGTCCAGTAGTC
3076	mir-099b-prec-19N01	0.25 GCCTTCGCCGCACAAAGCTCGTGTCTGTGGTCCGTGTC
3077	mir-149-prec	0.34 CGAGCTCTGGCTCCGTCTTCACTCCGTGCTTGCTCGA

TABLE 123

<u>Chlorambucil microRNA biomarkers.</u>		
SEQ	ID	NO Medianprobe Corr Sequence
3078	mir-181a-precN01	0.26 TCAGAGGACTCCAAGGAACATTCAACGCTGTCGGTGAGTT
3079	mir-181c-precN01	0.25 TGCCAAGGGTTGGGGAACATTCAACCTGTCGGTGAGTT
3080	HUMTRF	0.35 GATCTAAAGGTCCCTGGTTCGATCCC GGTTTCGGCACCA
3081	mir-181dN01	0.26 GAGGTCACAATCAACATTCAATTGTTGTCGGTGGTTGTGA
3082	MPR74 left	0.28 CAAAGGTACAATTAAACATTCAATTGTTGTCGGTGGTTGT
3083	Hcd817 left	0.28 TAATGAGAATTATGTTGCACATTGAGGCAGGATAATCC
3084	mir-213-precN01	0.42 AACATTCAATTGCTGTCGGTGGTTGAACGTGTGGACAAG
3085	mir-155-prec	0.33 TTAATGCTAACCGTGTAGGGTTTTGCCTCCAAGTGAC
3086	Hcd148_HPR225left	0.29 AATTAATGACCAAAATGTCAGATGTGTCCACAGCTAATTA
3087	mir-515-15p	0.27 GATCTCATGCAGTCATTCTCCAAAAGAAAGCAGTTCTGT
3088	mir-181b-precN01	0.41 TGAGGTTGCTTCAGTGAACATTCAACGCTGTCGGTGAGTT
3089	HUMTRN	0.27 CAATCGGTTAGCGCGTTCGGCTGTTAACCGAAAGGTTGGT
3090	mir-128b-precN01	0.37 TCACAGTGAACCGGTCTTTCCCTACTGTGTCAACTCC
3091	mir-450-2N01	0.29 GAAAGATGCTAAACTATTTTGCGATGTGTTCTAAATATG
3092	mir-216-precN01	0.29 CTGGGATTATGCTAACAGAGCAATTCCCTAGCCCTCACG
3093	mir-342N01	0.35 GTCTCACACAGAAATCGCACCCGTCACCTTGGCTACTTA
3094	mir-142-prec	0.45 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3095	mir-223-prec	0.39 GAGTGTCAAGTTGTCAAATACCCCAAGTGCAGGCACATGCT
3096	Hcd754 left	0.37 TCCTCCTCCTCCTTTCGTTCCGGCTCCCTGGCTGGCTCC
3097	mir-020-prec	0.28 TAAAGTGCTTATAGTCAGGTAGTGTAGTTATCTACTG

TABLE 124

<u>Mechlorethamine microRNA biomarkers.</u>		
SEQ	ID	NO Medianprobe Corr Sequence
3098	mir-124a-3-prec	0.33 TTAAGGCACGCCGGTGAATGCCAAGAGAGGCCCTCCGCCG
3099	Hcd946 left	0.3 CACAGGATTCAGGGAGAACGGTGGATTTCAAGAG
3100	Hcd683 left	0.29 CTATGACAGAAGGTACTCTGTCGGAGGGAGGAGATAATAG
3101	HPR264 right	0.25 CAAATGGCGCATCAATGACTATCGCTTACAAGCTCTT

TABLE 124-continued

Mechlorethamine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3102	MPR185 right	0.3 CAGAACATGCAATGCAACTACAATGCACCAAGCTGCCCG
3103	HUMTRF	0.37 GATCTAAAGGTCCCTGGTTCGATCCGGGTTCGCACCA
3104	Hcd294 left	0.25 TTATCATAAAAATAATCACAGCCTCAGGTGCTGTGAGGCA
3105	Hcd503 left	0.27 GAGATGAGGTAGCTGCCAGGTGCCATGGGGTATAGGTGA
3106	mir-20bNo1	0.27 AGTACCAAAGTGCTCATAGTCAGGTAGTTGGCATGAC
3107	MPR74 left	0.25 CAAAGGTACAAATTAAACATTTCATTGTTGTCGGTGGTTGT
3108	MPR234 right	0.28 GCTGACGTCACGGGAGAATTGTCCTATTAGGGATCCCG
3109	Hcd447 right	0.26 CTCAGGCCATTAACCTCAGTTGGTCACTAATCCCTAGGAA
3110	Hcd817 right	0.3 GAATCTTGCCTTGGATGCATACTGTAATTCCATTAAAG
3111	Hcd148_HPR225left	0.32 AATTAATGACCAAAATGTCAGATGTCACAGCTAATTAA
3112	mir-515-15p	0.29 GATCTCATGCAGTCATTCTCCAAAAGAACGACTTCTGT
3113	Hcd383 right	0.25 CTGATAGTACACGGGGCCAAAATAGATGTTGCTTCTAAG
3114	mir-181b-precNo2	0.31 ACCATCGACCCTTGATTGTACCTATGGCTAACCATCATC
3115	Hcd783 left	0.33 CAGGCTCACACCTCCCTCCCCAACTCTGGATGTATA
3116	MPR224 left	0.34 TGAGGCCCTCTAGGCCGTGAATTATGTCATAACTCAC
3117	HPR172 right	0.28 GTTTAACAGCCAGTGCAACATTAGATCTGAGTCAAAA
3118	MPR216 left	0.32 GATCCTAGTAGTGCCAAAGTGCTCATAGTCAGGTAGTT
3119	HUMTRN	0.28 CAATCGGTTAGCGCGTTCGGCTGTTAACGAAAGGTTGGT
3120	mir-321No1	0.3 TTGGCCTCTAACGCCAGGGATTGTGGGTCGAGTCCCACC
3121	HFR159 left	0.25 TCCGTCACTGAAACTGGCTGCCAGCGTTCACAGACAGCTG
3122	MPR228 left	0.29 TTTTGCTCCAGTCAGTAGGAAGATTGTTCAAATCTGT
3123	ath-MIR180aNo2	0.31 TGAGAATCTTGATGATGCTGCATCGGCAATCAACGACTAT
3124	mir-197-prec	0.28 TAAGAGCTCTCACCCCTCACCAACCTTCTCCACCCAGCAT
3125	mir-124a-1-prec1	0.26 ATACAATTAAAGGCACGCCGTGAATGCCAAGAATGGGCTG
3126	mir-128b-precNo1	0.31 TCACAGTGAAACCGGTCTCTTCCACTGTGTCACACTCC
3127	Hcd28_HPR39left	0.28 CTGACTTCAGTCCTATTAAAATGTCGAATTGGGAGC
3128	Hcd889 right	0.25 ATGCCTTGCTCTGTCTAATTTCAGAAGATAAGCCTGT
3129	Hcd350 right	0.26 TAGCACTTAGCAGGTTGTATTATCATTGTCGTGTCTATG
3130	mir-025-prec	0.31 ACGCTGCCCTGGCATTGCACTTGTCTCGGTCTGACAGTG
3131	mir-208-prec	0.27 ACCTGATGCTCACGTATAAGACGAGCAAAAGCTGTTGG
3132	mir-450-2No1	0.25 GAAAGATGCTAAACTATTGGCGATGTGTTCTAAATATG
3133	Hcd923 right	0.29 CTGGAGATAATGATTCTGCATTCTAATTAACTCCCAGGT
3134	Hcd434 right	0.28 CACTTTCCCTTGTTGGAAATCCTGGGTGACATCACCTCC
3135	HPR129 left	0.27 TTTCCCTGCTTGATTGCTTAATGGAAGCTGACAGTGAAAG
3136	HPR220 left	0.27 GGAGACACTGTAACACATTAACTCCTGACTGATTACAT

TABLE 124-continued

Mechlorethamine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3137	mir-380-5p	0.3 AGGTACCTGAAAAGATGGTGACCATAAACATGCGCTAT
3138	mir-093-prec-7.1 = 093-1	0.29 CCAAAGTGCCTGTCGTGCAGGTAGTGTGATTACCCAACCT
3139	mir-106-prec-X	0.3 CCTTGGCCATGTAAAAGTGCCTACAGTGCAGGTAGCTTT
3140	mir-342N01	0.28 GTCTCACACAGAAATCGCACCCGTACACCTGGCTACTTA
3141	mir-142-prec	0.45 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3142	HSHELA01	0.29 GGCGCAGCAACCTCGGTCGTATCGAGTCACGGCACCA
3143	mir-223-prec	0.32 GAGTGTCAAGTGTCAAATACCCAAAGTGCAGGCACATGCT
3144	Hcd754 left	0.32 TCCTCCTCCTCTTCGTTCCGGCTCCCTGGCTGGCTCC
3145	mir-020-prec	0.37 TAAAGTGCCTATAGTGCAGGTAGTGTGTTAGTTACTG
3146	mir-4323p	0.26 CCTTACGTGGGCCACTGGATGGCTCCATGTCTGGAG

TABLE 125

Streptozocin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3147	mir-483N01	0.2 ATCACGCCCTCCTCACTCCTCTCCTCCCCGCTTCTCCTCTC
3148	Hcd631 right	0.21 AAAACCAAATGGCTGGCTACTCATGTACTGTTGAATGTCT
3149	mir-212-precN01	0.24 CCTCAGTAACAGTCTCAGTCACGGCCACCGACGCCTGGC
3150	Hcd938 right	0.21 ATTCCCTGCATCACTCTCATGAAATGGCTGAGAAAGTGAG
3151	MPR133 right	0.2 CTGTAGATACTTCTCCCTGAGCCCCTCTGCCCTCTGC
3152	Hcd794 right	0.21 GGCCACCCACAGACACCAACAAGTCAGTCCGTTCTGCAG
3153	Hcd438 left	0.24 GTTTATTGAAATGTGTGATGGGGAGGTCAAAATGAAC
3154	Hcd886 right	0.23 CTCCAGTTGGGGTGGGAGTTGGAACAGTGTGAATGGG

TABLE 126

Carmustine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3155	mir-092-prec-X = 092-2	0.33 GTTCTATATAAGTATTGCACTTGTCCCGGCTGTGGAAG
3156	Hcd517 right	0.33 GAGGGATTACAGATTAACCTCCACTTCTCCAGACTCAGAA
3157	Hcd796 left	0.28 GGTGGGATTACCCGGCTGCCGCTGTCCCTGGATGGCTC
3158	HUMTRF	0.33 GATCTAAAGTCCCTGGTCGATCCGGGTTCCGGCACCA
3159	mir-20bN01	0.29 AGTACCAAAGTGCCTAGTGCAGGTAGTTGGCATGAC
3160	mir-019b-2-prec	0.25 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
3161	mir-033-prec	0.27 GTGGTGCAATTGAGTTGCATTGCATGTTCTGGTGGTACCC

TABLE 126-continued

Carmustine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3162	mir-092-prec-13 = 092-1No2	0.33 TCTGTATGGTATTGCACCTGTCCCGGCTGTTGAGTTGG
3163	Hcd148_HPR225left	0.27 AATTAATGACCAAAATGTCAGATGTGTCACAGCTAATTA
3164	HUMTRAB	0.3 ATGGTAGAGCGCTCGCTTGCTTGCGAGAGGTAGCGGGAT
3165	Hcd975 left	0.26 GGTTTTGTGTTTGTAAACAGCAGAAGGTATTAGTCAT
3166	mir-135-2-prec	0.28 CACTCTAGTGCTTTATGGCTTTTATTCTATGTGATAGT
3167	mir-128b-precN01	0.27 TCACAGTGAACCGGTCTCTTCCCTACTGTGTCACACTCC
3168	mir-143-prec	0.32 CTGGTCAGTTGGAGTCTGAGATGAAGCACTGTAGCTCAG
3169	mir-025-prec	0.33 ACGCTGCCCTGGGCATTGCACTTGTCTCGGTCTGACAGTG
3170	mir-216-precN01	0.34 CTGGGATTATGCTAACAGAGCAATTCCCTAGCCCTCACG
3171	mir-093-prec-7.1 = 093-1	0.3 CCAAAGTGTGTTCGTCAGGTAGTGTGATTACCCAACCT
3172	mir-106-prec-X	0.33 CCTTGGCCATGTAAAAGTGCTTACAGTGCAGGTAGCTTT
3173	mir-142-prec	0.61 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3174	HSHELA01	0.26 GGCGCAGCAACCTCGGTTCGTATCCGAGTCACGGCACCA
3175	HUMTRV1A	0.26 AC CGAAAGTCCCCGGTCGAAACCGGGCGGAAACACCA
3176	mir-223-prec	0.52 GAGTGTCA GTTGTCAAATACCCCAAGTGC GGACATGCT
3177	Hcd754 left	0.46 TCCTCCCTCCCTTTCGTTCCGGCTCCCTGGCTGGCTCC
3178	mir-018-prec	0.34 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
3179	mir-020-prec	0.35 TAAAGTGTCTATAGTGCAGGTAGTGTAGTTATCTACTG

TABLE 127

Lornustine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3180	mir-101-prec-9	0.27 GCTGTATATCTGAAAGGTACAGTACTGTGATAACTGAAGA
3181	Hcd796 left	0.26 GGTGGGATTACCCGGCTGCCGCTGTCGCCTGGATGGCTC
3182	mir-20bN01	0.28 AGTACCAAAGTGCCTAGTGCAAGGTAGTTGGCATGAC
3183	HUMTRAB	0.35 ATGGTAGAGCGCTCGCTTGCTTGCGAGAGGTAGCGGGAT
3184	mir-135-2-prec	0.27 CACTCTAGTGCTTTATGGCTTTTATTCTATGTGATAGT
3185	mir-153-1-prec1	0.32 CAGTTGCATAGTCACAAAAGTGATCATTGGCAGGTGTGGC
3186	mir-025-prec	0.29 ACGCTGCCCTGGCATTGCACTTGTCTCGGTCTGACAGTG
3187	mir-093-prec-7.1 = 093-1	0.26 CCAAAGTGTGTTCGTCAGGTAGTGTGATTACCCAACCT
3188	mir-106-prec-X	0.31 CCTTGGCCATGTAAAAGTGCTTACAGTGCAGGTAGCTTT
3189	mir-142-prec	0.41 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3190	HUMTRV1A	0.28 AC CGAAAGTCCCCGGTCGAAACCGGGCGGAAACACCA

TABLE 127-continued

Lornustine microRNA biomarkers.

SEQ	ID	NO Medianprobe	Corr Sequence
		3191 Hcd754 left	0.35 TCCTCCTCCCTCTTCGTTCCGGCTCCCTGGCTGGCTCC
		3192 mir-018-prec	0.27 TAAGGTGCATCTAGTGCAGATAAGTAGATTAGCATC
		3193 mir-020-prec	0.28 TAAAGTGCTTATAGTGCAGGTAGTGTTCAGTTATCTACTG

TABLE 128

Mercaptopurine microRNA biomarkers.

SEQ	ID	NO Medianprobe	Corr Sequence
		3194 mir-092-prec-X = 092-2	0.39 GTTCTATATAAAGTATTGCACTTGTCCCCGGCTGTGGAAG
		3195 mir-096-prec-7No1	0.26 CTCCGCTCTGAGCAATCATGTGCAGTGCCAATATGGAAA
		3196 mir-123-precNo2	0.32 TGTGACACTTCAAACCGTACCGTGAGTAATAATGCGCCG
		3197 MPR249 left	0.26 TCGGTTGGTTCACTGGTATGCTTCCAGTATCTCATTG
		3198 HPR232 right	0.28 TGAATTATTGCACAATAAATTGATGCCCTGTTGTCTTA
		3199 mir-101-prec-9	0.4 GCTGTATATCTGAAAGGTACAGTACTGTGATAACTGAAGA
		3200 mir-106aNo1	0.31 CCTTGCCATGTAAAAGTGCTTACAGTGCAGGTAGCTTT
		3201 mir-20bNo1	0.38 AGTACCAAAGTGCTCATAGTGCAGGTAGTTGGCATGAC
		3202 Hcd861 right	0.25 AAGGTCTGGATTGATCGTACTGCTTCTGAAAGGTAAAAAA
		3203 mir-017-precNo2	0.26 GTCAGAATAATGTCAAAGTGCTTACAGTGCAGGTAGTGT
		3204 mir-019b-2-prec	0.33 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
		3205 mir-033-prec	0.3 GTGGTGCATTGTTAGTGCAATTGCATGTTCTGGTGGTACCC
		3206 Hcd102 left	0.26 ACTGGAATTATGTTTATCTTAAGTCCACACTGGATCCTC
		3207 MPR216 left	0.32 GATCCTAGTAGTGCCAAAGTGCTCATAGTGCAGGTAGTTT
		3208 Hcd975 left	0.25 GGTTTGTTGTTGTAAACAGCAGAAGGTATTAGTCCAT
		3209 mir-019b-1-prec	0.3 TTCTGCTGTGCAAATCCATGCAAAACTGACTGTGGTAGTG
		3210 mir-135-2-prec	0.38 CACTCTAGTGCTTATGGCTTTTATTCTATGTGATAGT
		3211 Hcd581 right	0.26 AGGAGATATGCCAAGATATATTACAGCTTATATACACA
		3212 Hcd536_HPR104 right	0.25 GCTGCTGTGCTGAGGGGCTGGACTCTGTCCAGAACGACCA
		3213 mir-128b-precNo2	0.25 GGGGCCGATACACTGTACGAGAGTGAGTAGCAGGTCTCA
		3214 HSTRNL	0.37 TCCGGATGGAGCGTGGGTCGAATCCCACCTCTGACACCA
		3215 mir-025-prec	0.47 ACGCTGCCCTGGCATTGCACTTGTCTCGGTCTGACAGTG
		3216 mir-18bNo2	0.27 AGCAGCTTAAATCTACTGCCCTAAATGCCCTCTGGCA
		3217 HPR262 left	0.26 TCAGTTGGTTCACTGGTATGCTTCCAGTATCTCATTG
		3218 Hcd923 right	0.33 CTGGAGATAATGATTCTGCATTCTAATTAACTCCAGGT
		3219 Hcd434 right	0.3 CACTTTTCCCTTGTTGAAATCCTGGGTGACATCACCTCC
		3220 Hcd658 right	0.28 GACTGCAGAGCAAAAGACACGATGGGTGTCTATTGTTTC

TABLE 128-continued

Mercaptopurine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3221	HPR129 left	0.29 TTTCTGCTTGATTGCTTAATGGAAGCTGACAGTGAAG
3222	mir-380-5p	0.32 AGGTACCTGAAAAGATGGTTGACCATAAACATGCGCTAT
3223	mir-093-prec-7.1 = 093-1	0.45 CAAAGTGCCTGTCGTGCAGGTAGTGTGATTACCCAAACCT
3224	mir-106-prec-X	0.5 CCTGGCCATGTAAAAAGTGCCTACAGTGCAGGTAGCTTT
3225	Hcd627 left	0.31 GCATTAGGGAGAATAGTTGATGGATTACAAATCTCTGCAT
3226	mir-142-prec	0.33 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3227	mir-018-prec	0.46 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTAGCATC
3228	mir-020-prec	0.5 TAAAGTGCCTATAGTGCAGGTAGTGTGTTAGTTACTG

TABLE 129

Teniposide microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3229	mir-124a-3-prec	0.25 TTAAGGCACGCCGTGAATGCCAAGAGAGGCCCTCCGCCG
3230	Hcd768 right	0.28 GCCCTGGCGAACGCTGAGAAGACAGTCGAATTGACTAT
3231	HUMTRF	0.28 GATCTAAAGTCCCTGGTCGATCCCCGGTTCCGGCACCA
3232	mir-213-precNo1	0.25 AACATTCTTGCTGTCGGTGGTTGAACGTGTGGACAAG
3233	mir-181b-precNo2	0.28 ACCATCGACCGTTGATTGTACCTATGGCTAACCATCATC
3234	Hcd783 left	0.28 CAGGCTCACACCTCCCCTCCCCAACTCTCTGGAAATGTATA
3235	mir-212-precNo2	0.32 CGGACAGCGCGCCGGCACCTGGCTCTAGACTGCTTACTG
3236	mir-124a-1-prec1	0.25 ATACAATTAAAGGCACGCCGTGAATGCCAAGAATGGGCTG
3237	mir-342No1	0.29 GTCTCACACAGAAATCGCACCGTCACCTGGCTACTTA
3238	mir-142-prec	0.49 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3239	HSHELA01	0.3 GGCGCAGAACCTCGGTCGTATCCGAGTCACGGCACCA
3240	mir-223-prec	0.27 GAGTGTCAAGTTGTCAAATACCCAAGTGCAGGCACATGCT
3241	Hcd754 left	0.29 TCCTCCTCCCTTTCGTCGGCTCCCTGGCTGGCTCC

TABLE 130

Dactinomycin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3242	mir-025-prec	0.27 ACGCTGCCCTGGCATTGCACTTGCTCGGTCTGACAGTG
3243	mir-007-1-prec	0.28 TGTTGGCCTAGTTCTGTGTGAAAGACTAGTGATTGTTG
3244	mir-093-prec-7.1 = 093-1	0.3 CAAAGTGCCTGTCGTGCAGGTAGTGTGATTACCCAAACCT

TABLE 130-continued

Dactinomycin microRNA biomarkers.

SEQ	ID	NO Medianprobe	Corr Sequence
		3245 Hcd794 right	0.33 GGCCACCAACAGACACCAACAAGTTCACTGCGTTCTGCAG
		3246 mir-142-prec	0.34 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG

TABLE 131

Tretinoin microRNA biomarkers.

SEQ	ID	NO Medianprobe	Corr Sequence
		3247 Hcd257 left	0.42 CTTCTTGTATAAGCAGTCAGTGTGCTAAAATTGCAGACACTAGG
		3248 mir-148-prec	0.45 TGAGTATGATAGAAGTCAGTGCAGTACAGAACTTTGTCTC
		3249 Hcd512 left	0.28 CTGCGCTCTCGGAAATGACTCGCTCAAATCCGCTTCGCG
		3250 HPR227 right	0.25 CAGTGCAATGATATTGTCAAAGCATCTGGGACCAGCCTTG
		3251 Hcd421 right	0.37 AGTAAACAAATGTCGGCTTCCGCCTCCCTGCCATCC
		3252 MPR203 left	0.39 CTATATTGGACCGCAGCGCTGAGAGCTTTGTGTTAATG
		3253 mir-017-precNo1	0.26 GCATCTACTGCAGTAAGGCAGTTGATCATTATGGTGAC
		3254 mir-219-2No1	0.26 CTCAGGGCTTCGCCACTGATTGTCCAACGCAATTCTTG
		3255 mir-328No1	0.3 GAAAGTGCATACAGCCCCCTGGCCCTCTGCCCTCCGTC
		3256 Hcd783 left	0.31 CAGGCTCACACCTCCCTCCCCAACTCTCTGGAATGTATA
		3257 Hcd181 left	0.32 TTGGCGTCCTTGTCTCTCTCCCCTGCCAGTGGCTCC
		3258 HPR213 right	0.3 AACAACTTGTGCTGGTGCGGGAAAGTTGTGTCCTCAA
		3259 mir-191-prec	0.31 CAACGGAACTCCAAAAGCAGCTGTTGTCAGAGCATTC
		3260 mir-375	0.31 TTTTGTTCGTCGGCTCGCGTGAGGCAGGGCGGCCTCTC
		3261 mir-212-precNo2	0.26 CGGACAGCGCGCCGGCACCTGGCTCTAGACTGCTACTG
		3262 Hcd913 right	0.34 CAAACATCATGTGACGTCTGTGGAGCGCGGGCGCGCG
		3263 Hcd716 right	0.48 CAATAAAATGTGCCTATAAGGCGCCGGCTCGGGCGCG
		3264 MPR207 right	0.3 AACAACTTGTGCTGGTGCGGGAAAGTTGTGTCCTCA
		3265 HPR206 left	0.26 CTATATTGGACCGCAGCGCTGAGAGCTTTGTGTTAATG
		3266 mir-016b-chr3	0.29 GTTCCACTCTAGCAGCACGTAATATTGGCGTAGTCAAAT
		3267 Hcd654 left	0.34 AACGAGTAAAAGGCAGTACATGGGAGCGCGGGCGCAGAG
		3268 mir-195-prec	0.3 TCTAGCAGCACAGAAATATTGGCACAGGGAAAGCGAGTCG
		3269 Hcd425 left	0.25 GGTTCTACTCTTACCCCTCCCCACGTGGTTGTGCTG
		3270 mir-148aNo1	0.35 TGAGTATGATAGAAGTCAGTGCAGTACAGAACTTTGTCTC
		3271 mir-142-prec	0.36 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
		3272 mir-016a-chr13	0.25 CAATGTCAGCAGTGCCTTAGCAGCACGTAATATTGGCGT

TABLE 132

Ifosfamide microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3273	mir-092-prec-X = 092-2	0.28 GTTCTATATAAAGTATTGCACTTGTCCCGGCCTGTGGAAG
3274	mir-181b-2No1	0.28 CTGATGGCTGCACACTAACATTGCTGTGGTGGGTTT
3275	Hcd417 right	0.28 GGATTTAATGAGAAATATTGAGCCCTTGTTCAAGGAAC
3276	Hcd440_HPR257 right	0.28 GCTCTGTTGATAAAATTGGCTGTGCTTCATTGGACT
3277	mir-019b-2-prec	0.25 GTGGCTGTGCAAATCCATGCAAAACTGATTGTGATAATGT
3278	mir-213-precNo1	0.39 AACATTCAATTGCTGTCCGGTGGTTGAACGTGTGGACAAG
3279	mir-033-prec	0.29 GTGGTGCAATTGTAGTTGCATTGCATGTTCTGGTGGTACCC
3280	mir-092-prec-13 = 092-1No2	0.3 TCTGTATGGTATTGCACTTGTCCCGGCCTGTTGAGTTGG
3281	mir-181b-precNo1	0.36 TGAGGTTGCTTCAGTGAACATTCAACGCTGTGCGGTGAGTT
3282	mir-128b-precNo1	0.46 TCACAGTGAACCGGTCTTTCCCTACTGTGTCACACTCC
3283	mir-526a-2No2	0.29 GAAAAGAACATGCATCCTTCAGAGGGTTACTCTTGAGA
3284	MPR95 left	0.25 TTGTTGACACTTTCCCTGTTGCACTACTGTGGCCTC
3285	HPR220 right	0.27 GAGCATCAGTATGAGTGCATCAGTCAGGAGAAAATGAG
3286	mir-133a-1	0.35 CCTCTTCAATGGATTTGGTCCCCTCAACCAGCTGTAGCT
3287	mir-148aNo1	0.3 TGAGTATGATAGAAGTCAGTGCACACAGAACTTGTCTC
3288	mir-142-prec	0.4 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3289	HPR169 right	0.26 GTTCTTCTCACGGTAACGGCAGCTCGTTGTTGGCTG
3290	mir-223-prec	0.38 GAGTGTCAAGTTGTCAAATACCCAAGTGCACATGCT
3291	mir-018-prec	0.27 TAAGGTGCATCTAGTGCAGATAGTGAAGTAGATTGCATC
3292	mir-020-prec	0.25 TAAAGTGCTTATAGTGCAGGTAGTGTAGTTATCTACTG
3293	mir-484	0.27 GTCAGGGCTCAGTCCCCCTCCGATAAACCCCTAAATAGGGA

TABLE 133

Tamoxifen microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3294	mir-092-prec-X = 092-2	0.31 GTTCTATATAAAGTATTGCACTTGTCCCGGCCTGTGGAAG
3295	Hcd547 left	0.27 AAAATCAGCTTAATTAATTGAGTGCAGCTGTGTAT
3296	Hcd257 left	0.27 CTTCTTGTATAAGCAGTGTGCTAAATTGCAGACACTAGG
3297	mir-148-prec	0.27 TGAGTATGATAGAAGTCAGTGCACACAGAACTTGTCTC
3298	HUMTRS	0.25 TCTAGCGACAGACTGGTCAATTCCACCTTCGGGCCCA
3299	mir-033-prec	0.27 GTGGTGCAATTGAGTGTGCAATTGCATGTTCTGGTGGTACCC
3300	mir-092-prec-13 = 092-1No2	0.25 TCTGTATGGTATTGCACTTGTCCCGGCCTGTTGAGTTGG
3301	mir-375	0.46 TTTTGTTCGGCTCGCGTGAGGCAGGGCGGCCTCTC
3302	mir-095-prec-4	0.28 CGTTACATTCAACGGGTATTGAGCACCCACTGTG

TABLE 133-continued

Tamoxifen microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3303	mir-025-prec	0.35 ACGCTGCCCTGGGCATTGCACTTGTCCTCGGTCTGACAGTG
3304	mir-202-prec	0.34 GATCTGGCCTAAAGAGGTATAAGGCATGGGAAGATGGAGC
3305	mir-007-1-prec	0.26 TGTTGGCCTAGTCTGTGTTGGAAGACTAGTGATTTGTTG
3306	mir-093-prec-7.1 = 093-1	0.44 CCAAAGTGCTGTTCGTCAGGTAGTGATTACCCAACCT
3307	mir-106-prec-X	0.31 CCTTGGCCATGTAAAAGTGCCTACAGTCAGGTAGCTTT
3308	mir-142-prec	0.25 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3309	mir-223-prec	0.25 GAGTGTCAAGTTGTCAAATACCCCAAGTGCAGCACATGCT
3310	mir-018-prec	0.26 TAAGGTGCATCTAGTGCAAGATAGTGAAAGTAGATTAGCATC

TABLE 134

Floxuridine microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3311	HUMTRF	0.27 GATCTAAAGTCCCTGGTCGATCCCCGGTTTCGGCACCA
3312	HUMTRN	0.27 CAATCGGTTAGCGCGTTCGGCTGTTAACGAAAGGTTGGT
3313	mir-124a-1-prec1	0.31 ATACAATTAAGGCACGCGGTGAATGCCAAGAATGGGCTG
3314	mir-150-prec	0.33 CTCCCCATGGCCCTGTCCTCCAACCCTGTACCAAGTGCCTG
3315	Hcd923 left	0.26 TGGGAAACCTTGTAAAATGCAGATTCTGATTCTCAGGTCT
3316	HPR181 left	0.28 GAAGAAACATCTCAAATCATGCTGACAGCATTTCACTAT
3317	Hcd569 right	0.26 TTATTGCTTGAATGAGTTTCAGGGTATTGGCCTTCATAAA
3318	mir-199a-2-prec	0.25 TCGCCCCAGTGTTCAGACTACCTGTTCAAGGACAATGCCGT
3319	Hcd754 left	0.28 TCCTCCCTCCTCCTTTCGTTCCGGCTCCCTGGCTGGCTCC
3320	mir-4323p	0.3 CCTTACGTGGGCCACTGGATGGCTCCTCCATGTCTTGGAG

TABLE 135

Irinotecan microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3321	HUMTRF	0.27 GATCTAAAGTCCCTGGTCGATCCCCGGTTTCGGCACCA
3322	mir-380-5p	0.27 AGGTACCTGAAAGATGGTTGACCATAGAACATGCGCTAT
3323	mir-342No1	0.25 GTCTCACACAGAAATCGCACCCGTCACCTGGCCTACTTA
3324	mir-142-prec	0.35 CCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTG
3325	Hcd200 right	0.25 CAATTAGCCAATTGTGGGTATAATTAGCTGCATGTAGAAT

TABLE 136

Satraplatin microRNA biomarkers.

SEQ ID NO	Medianprobe	Corr Sequence
3326	Hcd289 left	0.31 TTCCTCTCAGAGCATGTTGTATAGAAGTAAATGAAAAGG
3327	Hcd939 right	0.25 CTCTCCTGCACATAATGAGGTCTGATTACTGTGATCATT
3328	Hcd330 right	0.28 ATTAATGGTAATTATGTGCGTAAATCCCCATGCTCTCAAT
3329	HPR76 right	0.25 GAGCCGTTAACATTAGCGCTTGGGCTGCCCTGGAGCGAG
3330	Hcd111 left	0.29 GCAGGGGATTTGAGGGGTGGTTGTGATTTGTACAGCTG
3331	Hcd976 right	0.36 CTTCTCAGAGTTGGAGATGAAAGAAAGAGAAGGTGGCAC
3332	mir-15aNo1	0.29 CCTTGGAGTAAAGTAGCAGCACATAATGGTTGTGGATT
3333	mir-001b-1-prec1	0.26 AATGCTATGGAATGTAAGAAGTATGTATTTGGTAGGC
3334	mir-450-1	0.36 AACGATACTAAACTGTTTGCATGTGTTCTAATATGC
3335	mir-200bNo2	0.3 CCAGCTCGGCAGCCGTGGCATCTTACTGGGCAGCATTG
3336	Hcd578 right	0.3 AATGATTGTAGAGGGCGGGGCATGAAGAGTGCCGTTCTG
3337	mir-200a-prec	0.28 GTCTCTAATACTGCCTGGTAATGATGACGGCGGAGCCCTG

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (<http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20090023149A1>). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. A method of determining sensitivity of a cancer patient to a treatment for cancer comprising measuring a level of expression of at least one gene in a cell of said patient, said gene selected from the group consisting of ACTB, ACTN4, ADA, ADAM9, ADAMTS1, ADD1, AF1Q, AIF1, AKAP1, AKAP13, AKR1C1, AKT1, ALDH2, ALDOC, ALG5, ALMS1, ALOX15B, AMIGO2, AMPD2, AMPD3, ANAPC5, ANP32A, ANP32B, ANXA1, AP1G2, APOBEC3B, APRT, ARHE, ARHGAP15, ARHGAP25, ARHGDI, ARHGEF6, ARL7, ASA1, ASPH, ATF3, ATIC, ATP2A2, ATP2A3, ATP5D, ATP5G2, ATP6V1B2, BC008967, BCAT1, BCHE, BCL11B, BDNF, BHLHB2, BIN2, BLMH, BMI1, BNIP3, BRDT, BRRN1, BTN3A3, C11orf2, C14orf139, C15orf25, C18orf10, C1orf24, C1orf29, C1orf18, C1QR1, C22orf18, C6orf2, CACNA1G, CACNB3, CALM1, CALML4, CALU, CAP350, CASP2, CASP6, CASP7, CAST, CBLB, CCNA2, CCNB1IP1, CCND3, CCR7, CCR9, CD1A, CD1C, CD1D, CD1E, CD2, CD28, CD3D, CD3E, CD3G, CD3Z, CD44, CD47, CD59, CD6, CD63, CD8A, CD8B1, CD99, CDC10, CDCl4B, CDH11, CDH2, CDKL5, CDKN2A, CDW52, CECR1, CENPB, CENTB1, CENTG2, CEP1, CG018, CHRNA3,

CHS1, CIAPIN1, CKAP4, CKIP-1, CNP, COL4A1, COL5A2, COL6A1, CORO1C, CRABP1, CRK, CRY1, CSDA, CTBP1, CTSC, CTSL, CUGBP2, CUTC, CXCL1, CXCR4, CXorf9, CYFIP2, CYLD, CYR61, DATF1, DAZAP1, DBN1, DBT, DCTN1, DDX18, DDX5, DGKA, DIAPH1, DKC1, DKFZP434J154, DKFZP564C186, DKFZP564G2022, DKFZp564J157, DKFZP564K0822, DNAJC10, DNAJC7, DNAPTP6, DOCK10, DOCK2, DPAGT1, DPEP2, DPYSL3, DSIP1, DUSP1, DXS9879E, EEF1B2, EFNB2, EHD2, EIF5A, ELK3, ENO2, EPAS1, EPB41L4B, ERCC2, ERG, ERP70, EVER1, EVI2A, EVL, EXT1, EZH2, F2R, FABP5, FAD104, FAM46A, FAU, FCGR2A, FCGR2C, FER1L3, FHL1, FHOD1, FKBP1A, FKBP9, FLJ10350, FLJ10539, FLJ10774, FLJ12270, FLJ13373, FLJ20859, FLJ21159, FLJ22457, FLJ35036, FLJ46603, FLNC, FLOT1, FMNL1, FNBP1, FOLH1, FOXF2, FSCN1, FTL, FYB, FYN, GOS2, G6PD, GALIG, GALNT6, GATA2, GATA3, GFPT1, GIMAP5, GIT2, GJA1, GLRB, GLTSCR2, GLUL, GMDS, GNAQ, GNB2, GNB5, GOT2, GPR65, GPRASP1, GPSM3, GRP58, GSTM2, GTF3A, GTSE1, GZMA, GZMB, H1F0, H1FX, H2AFX, H3F3A, HA-1, HEXB, HIC, HIST1H4C, HK1, HLA-A, HLA-B, HLA-DRA, HMGA1, HMMR, HNRPA1,

HNRPD, HNRPM, HOXA9, HRMT1L1, HSA9761, HSPA5, HSU79274, HTATSF1, ICAM1, ICAM2, IER3, IFI16, IFI44, IFITM2, IFITM3, IFRG28, IGFBP2, IGSF4, IL13RA2, IL21R, IL2RG, IL4R, IL6, IL6R, IL6ST, IL8, IMPDH2, INPP5D, INSIG1, IQGAP1, IQGAP2, IRS2, ITGA5, ITM2A, JARID2, JUNB, K-ALPHA-1, KHDRBS1, KIAA0355, KIAA0802, KIAA0877, KIAA0922, KIAA1078, KIAA1128, KIAA1393, KIFC1, LAIR1, LAMB1, LAMB3, LAT, LBR, LCK, LCP1, LCP2, LEF1, LEPRE1, LGALS1, LGALS9, LHFPL2, LNK, LOC54103, LOC55831, LOC81558, LOC94105, LONP, LOX, LOXL2, LPHN2, LPXN, LRMP, LRP12, LRRK5, LRRN3, LST1, LTB, LUM, LY9, LY96, MAGEB2, MAL, MAP1B, MAP1LC3B, MAP4K1, MAPK1, MARCKS, MAZ, MCAM, MCL1, MCM5, MCM7, MDH2, MDN1, MEF2C, MFNG, MGC17330, MGC21654, MGC2744, MGC4083, MGC8721, MGC8902, MGLL, MLPH, MPHOSPH6, MPP1, MPZL1, MRP63, MRPS2, MT1E, MT1K, MUF1, MVP, MYB, MYL9, MYO1B, NAPI1, NAPI1L2, NARF, NASP, NCOR2, NDN, NDUFAB1, NDUFAS6, NFKBIA, NID2, NIPA2, NME4, NME7, NNMT, NOL5A, NOL8, NOMO2, NOTCH1, NPC1, NQO1, NR1D2, NUDC, NUP210, NUP88, NVL, NXF1, OBFC1, OCRL, OGT, OXA1L, P2RX5, P4HA1, PACAP, PAF53, PAFAH1B3, PALM2-AKAP2, PAX6, PCBP2, PCCB, PFDN5, PFN1, PFN2, PGAM1, PHEMX, PHLDA1, PIM2, PITPN1, PLAC8, PLAGL1, PLAUR, PLCB1, PLEK2, PLEKHC1, PLD2, PLSCR1, PNAS-4, PNMA2, POLR2F, PPAP2B, PRF1, PRG1, PRIM1, PRKCH, PRKCQ, PRKD2, PRNP, PRP19, PRPF8, PRSS23, PSCDBP, PSMB9, PSMC3, PSME2, PTGER4, PTGES2, PTOV1, PTP4A3, PTPN7, PTPNS1, PTRF, PURA, PWP1, PYGL, QKI, RAB3GAP, RAB7L1, RAB9P40, RAC2, RAFTLIN, RAG2, RAP1B, RASGRP2, RBPM, RCN1, RFC3, RFC5, RGC32, RGS3, RHOH, RIMS3, RIOK3, RIPK2, RIS1, RNASE6, RNF144, RPL10, RPL10A, RPL12, RPL13A, RPL17, RPL18, RPL36A, RPLP0, RPLP2, RPS15, RPS19, RPS2, RPS4X, RPS4Y1, RRAS, RRAS2, RRBPI, RRM2, RUNX1, RUNX3, S100A4, SART3, SATB1, SCAP1, SCARB1, SCN3A, SEC31L2, SEC61G, SELL, SELPLG, SEMA4G, SEPT10, SEPT6, SERPINA1, SERPINB1, SERPINB6, SFRS5, SFRS6, SFRS7, SH2D1A, SH3GL3, SH3TC1, SHD1, SHMT2, SIAT1, SKB1, SKP2, SLA, SLC1A4, SLC20A1, SLC25A15, SLC25A5, SLC39A14, SLC39A6, SLC43A3, SLC4A2, SLC7A11, SLC7A6, SMAD3, SMOX, SNRPA, SNRPB, SOD2, SOX4, SPI40, SPANXC, SPI1, SRF, SRM, SSA2, SSBP2, SSRP1, SSSCA1, STAG3, STAT1, STAT4, STAT5A, STC1, STC2, STOML2, T3JAM, TACC1, TACC3, TAF5, TAL1, TAP1, TARP, TBCA, TCF12, TCF4, TFDP2, TFPI, TIMM17A, TIMP1, TJP1, TK2, TM4SF1, TM4SF2, TM4SF8, TM6SF1, TMEM2, TMEM22, TMSB10, TMSNB, TNFAIP3, TNFAIP8, TNFRSF10B, TNFRSF1A, TNFRSF7, TNK, TNPO1, TOB1, TOMM20, TOX, TPK1, TPM2, TRA@, TRA1, TRAM2, TRB@, TRD@, TRIM, TRIM14, TRIM22, TRIM28, TRIP13, TRPV2, TUBGCP3, TUSC3, TXN, TXNDC5, UBASH3A, UBE2A, UBE2L6, UBE2S, UCHL1, UCK2, UCP2, UFD1L, UGDH, ULK2, UMPS, UNG, USP34, USP4, VASP, VAV1, VLDR, VWF, WASPIP, WBSCR20A, WBSCR20C, WHSC1, WNT5A, ZAP70, ZFP36L1, ZNF32, ZNF335, ZNF593, ZNFN1A1, and ZYX, wherein said level of expression of said gene indicates said cell is sensitive to said treatment.

2. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPS4X, S100A4, NDUFAS6, C14orf139, SLC25A5, RPL10, RPL12, EIF5A, RPL36A, BLMH, CTBP1, TBCA, MDH2, and DDX9879E, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of UBB, B2M, MAN1A1, and SUI1, or wherein the method further comprises measuring a level of at least one microRNA selected from the group consisting of Hcd892, Hcd678, hsa-mir-007-1-prec, MPR243, Hcd654, hsa-mir-487, Hcd794, Hcd739, and Hcd562, wherein said level of expression of said gene or said level of said microRNA indicates that said cell is sensitive to Vincristine.

3. The method of claim 1, wherein said at least one gene is selected from the group consisting of C1QR1, SLA, PTPN7, ZNFN1A1, CENTB1, IFI16, ARHGEF6, SEC31L2, CD3Z, GZMB, CD3D, MAP4K1, GPR65, PRF1, ARHGAP15, TM6SF1, and TCF4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of HCLS1, CD53, PTPRCAP, and PTPRC, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of HUMTRF, HPR187, hsa-mir-450-1, hsa-mir-155-prec, hsa-mir-515-15p, hsa-mir-181b-prec, hsa-mir-124a-1-prec1, hsa-mir-450-2, Hcd923, hsa-mir-342, hsa-mir-142-prec, hsa-mir-223-prec, Hcd754, and Hcd213_HPR182, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Cisplatin.

4. The method of claim 1, wherein said at least one gene is selected from the group consisting of SRM, SCARB1, SIAT1, CUGBP2, ICAM1, WASPIP, ITM2A, PALM2-AKAP2, PTPNS1, MPP1, LNK, FCGR2A, RUNX3, EVI2A, BTN3A3, LCP2, BCHE, LY96, LCP1, IFI16, MCAM, MEF2C, SLC1A4, FYN, C1orf38, CHS1, FCGR2C, TNK, AMPD2, SEPT6, RAFTLIN, SLC43A3, RAC2, LPXN, CKIP-1, FLJ10539, FLJ35036, DOCK10, TRPV2, IFRG28, LEF1, and ADAMTS1, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of MSN, SPARC, VIM, GAS7, ANPEP, EMP3, BTN3A2, FN1, and CAPN3, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of MPR121, HUMTRS, hsa-mir-2,3-prec, hsa-mir-155-prec, hsa-mir-147-prec, hsa-mir-100, hsa-mir-138-1-prec, hsa-mir-140, hsa-mir-146-prec, hsa-mir-509, hsa-mir-146b, Hcd514, Hcd397, Hcd731, hsa-mir-034-prec, and hsa-mir-100-1/2-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Azaguanine.

5. The method of claim 1, wherein said at least one gene is selected from the group consisting of CD99, INSIG1, PRG1, MUF1, SLA, SSBP2, GNB5, MFNG, PSMB9, EVI2A, PTPN7, PTGER4, CXorf9, ZNFN1A1, CENTB1, NAP1L1, HLA-DRA, IFI16, ARHGEF6, PSCDBP, SELPLG, LAT, SEC31L2, CD3Z, SH2D1A, GZMB, SCN3A, RAFTLIN, DOCK2, CD3D, RAC2, ZAP70, GPR65, PRF1, ARHGAP15, NOTCH1, and UBASH3A, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of LAPTM5, HCLS1, CD53, GMFG, PTPRCAP, PTPRC, CORO1A, and ITK, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd415, Hcd768,

HUMTRF, Hcd866, Hcd145, HUMTRAB, Hcd913, HPR163, Hcd697, Hcd755, Hcd716, MPR207, HSTRNL, HPR206, MPR243, Hcd654, MPR130, Hcd782, Hcd794, Hcd739, hsa-mir-142-prec, HSHELA01, HUMTRV1A, and Hcd754, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Etoposide.

6. The method of claim 1, wherein said at least one gene is selected from the group consisting of CD99, ALDOC, SLA, SSBP2, IL2RG, CXorf9, RHOH, ZNFN1A1, CENTB1, CD1C, MAP4K1, CD3G, CCR9, CXCR4, ARHGEF6, SEL-PLG, LAT, SEC31L2, CD3Z, SH2D1A, CD1A, LAIR1, TRB@, CD3D, WBSCR20C, ZAP70, IFI44, GPR65, AIF1, ARHGAP15, NARF, and PACAP, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of LAPT5, HCLS1, CD53, GMFG, PTPRCAP, TCF7, CD1B, PTPRC, CORO1A, HEM1, and ITK, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd768, hsa-mir-483, Hcd145, hsa-mir-197-prec, hsa-mir-2,2-prec, HPR163, Hcd654, hsa-mir-342, Hcd794, hsa-mir-142-prec, and Hcd754, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Adriamycin.

7. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPL12, RPLP2, MYB, ZNFN1A1, SCAP1, STAT4, SP140, AMPD3, TNFAIP8, DDX18, TAF5, RPS2, DOCK2, GPR65, HOXA9, FLJ12270, and HNRPD, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of RPL32, FBL, and PTPRC, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-096-prec-7, Hcd605, hsa-mir-007-2-prec, hsa-mir-019b-2-prec, MPR216, hsa-mir-019b-1-prec, hsa-mir-135-2-prec, HSTRNL, hsa-mir-025-prec, hsa-mir-007-1-prec, hsa-mir-019a-prec, hsa-mir-380-5p, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, hsa-mir-018-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Aclarubicin.

8. The method of claim 1, wherein said at least one gene is selected from the group consisting of PGAM1, DPYSL3, INSIG1, GJA1, BNIP3, PRG1, G6PD, PLOD2, LOXL2, SSBP2, C1orf29, TOX, STC1, TNFRSF1A, NCOR2, NAP1L1, LOC94105, ARHGEF6, GATA3, TPPI, LAT, CD3Z, AF1Q, MAP1B, TRIM22, CD3D, BCAT1, IFI44, CUTC, NAP1L2, NME7, FLJ21159, and COL5A2, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of BASP1, COL6A2, PTPRC, PRKCA, CCL2, and RAB31, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd768, HUMTRF, hsa-mir-2,3-prec, hsa-mir-181b-prec, MPR244, hsa-mir-409-3p, HSTRNL, hsa-mir-382, hsa-mir-342, hsa-mir-142-prec, and Hcd200, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Mitoxantrone.

9. The method of claim 1, wherein said at least one gene is selected from the group consisting of STC1, GPR65, DOCK10, COL5A2, FAM46A, and LOC54103, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting

of HUMTRF, Hcd148_HPR225left, Hcd938, MPR174, and hsa-mir-4323p, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Mitomycin.

10. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPL10, RPS4X, NUDC, DKC1, DKFZP564C186, PRP19, RAB9P40, HSA9761, GMDS, CEP1, IL13RA2, MAGEB2, HMGN2, ALMS1, GPR65, FLJ10774, NOL8, DAZAPI, SLC25A15, PAF53, DDX9879E, PITPN1, SPANXC, and KIAA1393, or wherein the method further comprises measuring a level of expression of RALY, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-096-prec-7, hsa-mir-101-prec-9, hsa-mir-20b, hsa-mir-019b-2-prec, hsa-mir-032-prec, MPR156, hsa-mir-019b-1-prec, hsa-mir-135-2-prec, hsa-mir-025-prec, hsa-mir-007-1-prec, hsa-mir-361, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-098-prec-X, hsa-mir-142-prec, HPR169, hsa-mir-018-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Paclitaxel.

11. The method of claim 1, wherein said at least one gene is selected from the group consisting of PFN1, PGAM1, K-ALPHA-1, CSDA, UCHL1, PWP1, PALM2-AKAP2, TNFRSF1A, ATP5G2, AF1Q, NME4, and FHOD1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-123-prec, Hcd257, hsa-mir-155-prec, ath-MIR180a, Hcd448, HSTRNL, MPR174, Hcd200, hsa-mir-4323p, and HPR244, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Gemcitabine.

12. The method of claim 1, wherein said at least one gene is selected from the group consisting of ANP32B, GTF3A, RRM2, TRIM14, SKP2, TRIP13, RFC3, CASP7, TXN, MCM5, PTGES2, OBFC1, EPB41L4B, and CALML4, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-096-prec-7, hsa-mir-095-prec-4, HSTRNL, and hsa-mir-007-1-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Taxotere.

13. The method of claim 1, wherein said at least one gene is selected from the group consisting of IFITM2, UBE2L6, USP4, ITM2A, IL2RG, GPRASP1, PTPN7, CXorf9, RHOH, GIT2, ZNFN1A1, CEP1, TNFRSF7, MAP4K1, CCR7, CD3G, ATP2A3, UCP2, GATA3, CDKN2A, TARP, LAIR1, SH2D1A, SEPT6, HA-1, ERCC2, CD3D, LST1, AIF1, ADA, DATF1, ARHGAP15, PLAC8, CECR1, LOC81558, and EHD2, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of LAPT5, ITGB2, ANPEP, CD53, CD37, ADORA2A, GNA15, PTPRC, CORO1A, HEM1, FLII, and CREB3L1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of MPR141, hsa-mir-424, Hcd690, Hcd783, hsa-mir-150-prec, Hcd266, hsa-mir-503, hsa-mir-128b-prec, Hcd397, and hsa-mir-484, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Dexamethasone.

14. The method of claim 1, wherein said at least one gene is selected from the group consisting of ITM2A, RHOH,

PRIM1, CENTB1, NAP1L1, ATP5G2, GATA3, PRKCQ, SH2D1A, SEPT6, NME4, CD3D, CD1E, ADA, and FHOD1, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of GNA15, PTPRC, and RPL13, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of HUMTRF, hsa-mir-155-prec, hsa-mir-515-15p, Hcd938, Hcd642, Hcd120, hsa-mir-380-5p, hsa-mir-342, hsa-mir-142-prec, hsa-mir-223-prec, and hsa-mir-4323p, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Ara-C.

15. The method of claim 1, wherein said at least one gene is selected from the group consisting of CD99, ARHGDI β , VWF, ITM2A, LGALS9, INPP5D, SATB1, TFPD2, SLA, IL2RG, MFNG, SELL, CDW52, LRMP, ICAM2, RIMS3, PTPN7, ARHGAP25, LCK, CXorf9, RHOH, GIT2, ZNFN1A1, CENTB1, LCP2, SPI1, GZMA, CEP1, CD8A, SCAP1, CD2, CD1C, TNFRSF7, VAV1, MAP4K1, CCR7, C6orf32, ALOX1SB, BRDT, CD3G, LTB, ATP2A3, NVL, RASGRP2, LCP1, CXCR4, PRKD2, GATA3, TRA@, KIAA0922, TARP, SEC31L2, PRKCQ, SH2D1A, CHRNA3, CD1A, LST1, LAIR1, CACNA1G, TRB@, SEPT6, HA-1, DOCK2, CD3D, TRD@, T3JAM, FNBP1, CD6, AIF1, FOLH1, CD1E, LY9, ADA, CDKL5, TRIM, EVL, DATF1, RGC32, PRKCH, ARHGAP15, NOTCH1, BIN2, SEMA4G, DPEP2, CECR1, BCL11B, STAG3, GALNT6, UBASH3A, PHEMX, FLJ13373, LEF1, IL21R, MGC17330, AKAP13, ZNF335, and GIMAP5, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of SRRM1, LAPTMS, ITGB2, CD53, CD37, GMFG, PTPRCAP, GNA15, BLM, PTPRC, CORO1A, PRKCB1, HEM1, and UGT2B17, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd544, hsa-mir-181c-prec, Hcd517, MPR151, hsa-mir-2,3-prec, hsa-mir-181b-prec, hsa-mir-150-prec, hsa-mir-153-1-prec, hsa-mir-128b-prec, Hcd812, hsa-mir-195-prec, hsa-mir-342, hsa-mir-370, hsa-mir-142-prec, hsa-mir-223-prec, and hsa-mir-484, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Methylprednisolone.

16. The method of claim 1, wherein said at least one gene is selected from the group consisting of PRPF8, RPL18, GOT2, RPL13A, RPS15, RPLP2, CSDA, KHDRBS1, SNRPA, IMPDH2, RPS19, NUP88, ATP5D, PCBP2, ZNF593, HSU79274, PRIM1, PFDN5, OXA1L, H3F3A, ATIC, CIAPIN1, RPS2, PCCB, SHMT2, RPLP0, HNRPA1, STOML2, SKB1, GLTSCR2, CCNB1IP1, MRPS2, FLJ20859, and FLJ12270, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of RNPS1, RPL32, EEF1G, PTMA, RPL13, FBL, RBMX, and RPS9, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-096-prec-7, hsa-mir-123-prec, Hcd250, hsa-mir-518e, HPR232, Hcd263, hsa-mir-516-33p, Hcd605, Hcd373, MPR254, MPR215, HUMTRF, hsa-mir-106a, hsa-mir-20b, Hcd361, Hcd412, Hcd781, hsa-mir-019b-2-prec, HPR214, Hcd807, Hcd817, Hcd788, Hcd970, Hcd148_HPR225left, Hcd102, Hcd246, HPR199, HPR233, Hcd383, MPR224, HPR172, MPR216, hsa-mir-321, Hcd586, Hcd587, Hcd249, Hcd279, HPR159, Hcd689, Hcd691, hsa-mir-019b-1-prec, Hcd413, Hcd581,

Hcd536_HPR104, Hcd230, HPR154, Hcd270, Hcd649, Hcd889, Hcd938, HPR266, hsa-mir-025-prec, Hcd355_HPR190, MPR162, Hcd923, MPR237, MPR174, hsa-mir-019a-prec, hsa_mir_490_Hcd20, hsa-mir-380-5p, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, Hcd627, hsa-mir-142-prec, HPR169, hsa-mir-001b-2-prec, hsa-mir-018-prec, hsa-mir-020-prec, Hcd404, hsa-mir-384, and hsa-mir-4323p, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Methotrexate.

17. The method of claim 1, wherein said at least one gene is selected from the group consisting of PFN1, HK1, MCL1, ZYX, RAPIB, GNB2, EPAS1, PGAM1, CKAP4, DUSP1, MYL9, K-ALPHA-1, LGALS1, CSDA, IFTM2, ITGA5, DPYSL3, JUNB, NFKBIA, LAMB1, FHL1, INSIG1, TIMP1, GJA1, PSME2, PRG1, EXT1, DKFZP434J154, MVP, VASP, ARL7, NNMT, TAP1, PLOD2, ATF3, PALM2-AKAP2, IL8, LOXL2, IL4R, DGKA, STC2, SEC61G, RGS3, F2R, TPM2, PSMB9, LOX, STC1, PTGER4, IL6, SMAD3, WNT5A, BDNF, TNFRSF1A, FLNC, DKFZP564K0822, FLOT1, PTRF, HLA-B, MGC4083, TNFRSF10B, PLAGL1, PNMA2, TFPI, LAT, GZMB, CYR61, PLAUR, FSCN1, ERP70, AF1Q, HIC, COL6 μ l, IFITM3, MAP1B, FLJ46603, RAFTLIN, RRAS, FTL, KIAA0877, MT1E, CDC10, DOCK2, TRIM22, RIS1, BCAT1, PRF1, DBN1, MT1K, TMSB10, FLJ10350, C1orf24, NME7, TMEM22, TPK1, COL5A2, ELK3, CYLD, ADAMTS1, EHD2, and ACTB, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of MSN, ACTR2, AKR1B1, VIM, ITGA3, OPTN, M6PRBP1, COL1A1, BASP1, ANPEP, TGFB1, NFIL3, NK4, CSPG2, PLAU, COL6A2, UBC, FGFR1, BAX, COL4A2, and RAB31, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-376a, hsa-mir-155-prec, hsa-mir-409-3p, hsa-mir-495, Hcd498, hsa-mir-199a-2-prec, hsa-mir-382, HPR271, hsa-mir-145-prec, and hsa-mir-199a-1-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Bleomycin.

18. The method of claim 1, wherein said at least one gene is selected from the group consisting of SSRP1, NUDC, CTSC, AP1G2, PSME2, LBR, EFNB2, SERPINAI, SSSCA1, EZH2, MYB, PRIM1, H2AFX, HMGA1, HMMR, TK2, WHSC1, DIAPH1, LAMB3, DPAGT1, UCK2, SERPINB1, MDN1, BRRN1, GOS2, RAC2, MGC21654, GTSE1, TACC3, PLEK2, PLAC8, HNRPD, and PNAS-4, or wherein the method further comprises measuring the level of expression of PTMA, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-101-prec-9, hsa-mir-144-prec, hsa-mir-519a-1, hsa-mir-519b, hsa-mir-015b-prec, hsa-mir-106a, hsa-mir-16-1, hsa-mir-181d, hsa-mir-017-prec, hsa-mir-019b-2-prec, hsa-mir-192, hsa-mir-2,3-prec, hsa-mir-2,5-prec, hsa-mir-107, hsa-mir-200b, hsa-mir-103-prec-5=103-1, hsa-mir-519a-1/526c, MPR216, hsa-mir-019b-1-prec, hsa-mir-107-prec-10, hsa-mir-135-2-prec, hsa-mir-103-2-prec, hsa-mir-519a-2, hsa-mir-025-prec, hsa-mir-16-2, MPR95, hsa-mir-016b-chr3, Hcd948, hsa-mir-195-prec, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, hsa-mir-519c/526c, hsa-mir-200a-prec, hsa-mir-016a-chr13, hsa-mir-018-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Methyl-GAG.

19. The method of claim 1, wherein said at least one gene is selected from the group consisting of ITGA5, TNFAIP3, WNT5A, FOXF2, LOC94105, IFI16, LRRN3, DOCK10, LEPRE1, COL5A2, and ADAMTS1, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of MSN, VIM, CSPG2, and FGFR1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd829, HUMTRF, HPR187, Hcd210_HPR205, hsa-mir-379, hsa-mir-2,3-prec, hsa-mir-4325p, hsa-mir-450-1, hsa-mir-155-prec, Hcd28_HPR39right, MPR244, hsa-mir-409-3p, hsa-mir-124a-1-prec1, hsa-mir-154-prec1, hsa-mir-495, hsa-mir-515-23p, Hcd438right, Hcd770, hsa-mir-382, hsa-mir-223-prec, Hcd754, and Hcd213_HPR182, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Carboplatin.

20. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPL18, RPL10A, ANAPC5, EEF1B2, RPL13A, RPS15, AKAP1, NDUFAB1, APRT, ZNF593, MRP63, IL6R, SART3, UCK2, RPL17, RPS2, PCCB, TOMM20, SHMT2, RPLP0, GTF3A, STOML2, DKFZp564J157, MRPS2, ALG5, and CALML4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of RNPS1, RPL13, RPS6, and RPL3, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-096-prec-7, hsa-mir-429, Hcd693, HPR214, Hcd586, Hcd249, Hcd689, hsa-mir-194-2, Hcd581, Hcd270, hsa-mir-025-prec, Hcd340, hsa-mir-007-1-prec, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, Hcd794, hsa-mir-020-prec, and hsa-mir-4323p, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to 5-FU (5-Fluorouracil).

21. The method of claim 1, wherein said at least one gene is selected from the group consisting of KIFC1, VLSDLR, RUNX1, PAFAH1B3, H1FX, RNF144, TMSNB, CRY1, MAZ, SLA, SRF, UMPS, CD3Z, PRKCQ, HNRPM, ZAP70, ADD1, RFC5, TM4SF2, PFN2, BMI1, TUBGCP3, ATP6V1B2, CD1D, ADA, CD99, CD2, CNP, ERG, CD3E, CD1A, PSMC3, RPS4Y1, AKT1, TAL1, UBE2A, TCF12, UBE2S, CCND3, PAX6, RAG2, GSTM2, SATB1, NASP, IGFBP2, CDH2, CRABP1, DBN1, AKR1C1, CACNB3, CASP2, CASP2, LCP2, CASP6, MYB, SFRS6, GLRB, NDN, GNAQ, TUSC3, GNAQ, JARID2, OCRL, FHL1, EZH2, SMOX, SLC4A2, UFD1L, ZNF32, HTATSF1, SHD1, PTOV1, NXF1, FYB, TRIM28, BC008967, TRB@, H1F0, CD3D, CD3G, CENPB, ALDH2, ANXA1, H2AFX, CD1E, DDX5, CCNA2, ENO2, SNRNP, GATA3, RRM2, GLUL, SOX4, MAL, UNG, ARHGDI, RUNX1, MPHOSPH6, DCTN1, SH3GL3, PLEKHC1, CD47, POLR2F, RHOH, and ADD1, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of ITK, RALY, PSMC5, MYL6, CD1B, STMN1, GNA15, MDK, CAPG, ACTN1, CTNNA1, FARSLA, E2F4, CPSF1, SEPW1, TFRC, ABL1, TCF7, FGFR1, NUCB2, SMA3, FAT, VIM, and ATP2A3, wherein said level of expression of said gene indicates that said cell is sensitive to Rituximab.

22. The method of claim 1, wherein said at least one gene is selected from the group consisting of TRA1, ACTN4, CALM1, CD63, FKBP1A, CALU, IQGAP1, MGC8721, STAT1, TACC1, TM4SF8, CD59, CKAP4, DUSP1, RCN1,

MGC8902, LGALS1, BHLHB2, RRBP1, PRNP, IER3, MARCKS, LUM, FER1L3, SLC20A1, HEXB, EXT1, TJP1, CTSI, SLC39A6, RIOK3, CRK, NNMT, TRAM2, ADAM9, DNAJC7, PLSCR1, PRSS23, PLOD2, NPC1, TOB1, GFPT1, IL8, PYGL, LOXL2, KIAA0355, UGDH, PURA, ULK2, CENTG2, NID2, CAP350, CXCL1, BTN3A3, IL6, WNT5A, FOXF2, LPHN2, CDH11, P4HA1, GRP58, DSPI, MAP1LC3B, GALIG, IGSF4, IRS2, ATP2A2, OGT, TNFRSF10B, KIAA1128, TM4SF1, RBPM, RIPK2, CBLB, NR1D2, SLC7A11, MPZL1, SSA2, NQO1, ASPH, ASAHI, MGLL, SERPINB6, HSPA5, ZFP36L1, COL4A1, CD44, SLC39A14, NIPA2, FKBP9, IL6ST, DKFZP564G2022, PPAP2B, MAP1B, MAPK1, MYO1B, CAST, RRAS2, QKI, LHFPL2, 38970, ARHE, KIAA1078, FTL, KIAA0877, PLCB1, KIAA0802, RAB3GAP, SERPINB1, TIMM17A, SOD2, HLA-A, NOMO2, LOC55831, PHLDA1, TMEM2, MLPH, FAD104, LRRC5, RAB7L1, FLJ35036, DOCK10, LRP12, TXNDC5, CDCl4B, HRMT1L1, CORO1C, DNAJC10, TNPO1, LONP, AMIGO2, DNAPTP6, and ADAMTS1, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of WARS, CD81, CTSB, PKM2, PPP2CB, CNN3, ANXA2, JAK1, EIF4G3, COL1A1, DYRK2, NFIL3, ACTN1, CAPN2, BTN3A2, IGFBP3, FN1, COL4A2, and KPNB1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-136-prec, Hcd570, Hcd873, Hcd282PO, Hcd799, Hcd829, Hcd210_HPR205, hsa-mir-2,9-prec, hsa-mir-202, hsa-mir-429, Hcd693, hsa-mir-022-prec, MPR88, hsa-mir-198-prec, hsa-mir-199b-prec, Hcd145, hsa-mir-124a-2-prec, hsa-mir-138-2-prec, Hcd960, Hcd869, Hcd384, hsa-mir-027b-prec, Hcd444, hsa-mir-194-2, hsa-mir-197-prec, Hcd913, HPR163, hsa-mir-138-1-prec, hsa-mir-010a-prec, hsa-mir-023b-prec, hsa-mir-193b, Hcd542, hsa-mir-199a-2-prec, hsa-mir-2,4-prec, Hcd608, Hcd684, hsa-mir-145-prec, hsa-mir-023a-prec, hsa-mir-024-2-prec, and hsa-mir-199a-1-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to radiation therapy.

23. The method of claim 1, wherein said at least one gene is selected from the group consisting of FAU, NOL5A, ANP32A, ARHGDI, LBR, FABP5, ITM2A, SFRS5, IQGAP2, SLC7A6, SLA, IL2RG, MFNG, GPSM3, PIM2, EVER1, LRMP, ICAM2, RIMS3, FMNL1, MYB, PTPN7, LCK, CXorf9, RHOH, ZNFN1A1, CENTB1, LCP2, DBT, CEP1, IL6R, VAV1, MAP4K1, CD28, PTP4A3, CD3G, LTB, USP34, NVL, CD8B1, SFRS6, LCP1, CXCR4, PSCDBP, SELPLG, CD3Z, PRKCQ, CD1A, GATA2, P2RX5, LAIR1, C1orf38, SH2D1A, TRB@, SEPT6, HA-1, DOCK2, WBSCR20C, CD3D, RNASE6, SFRS7, WBSCR20A, NUP210, CD6, HNRPA1, AIF1, CYFIP2, GLTSCR2, C11orf2, ARHGAP15, BIN2, SH3TC1, STAG3, TM6SF1, C15orf25, FLJ22457, PACAP, and MGC2744, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-123-prec, hsa-mir-106a, hsa-mir-20b, hsa-mir-0,7-prec, hsa-mir-019b-2-prec, hsa-mir-033-prec, hsa-mir-092-prec-13=092-1, hsa-mir-122a-prec, Hcd783, MPR216, hsa-mir-019b-1-prec, hsa-mir-135-2-prec, hsa-mir-128b-prec, hsa-mir-025-prec, Hcd511, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, HPR169, hsa-mir-223-prec, hsa-mir-018-prec, and

hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to PXD101 (belinostat).

24. The method of claim 1, wherein said at least one gene is selected from the group consisting of CD99, SNRPA, CUGBP2, STAT5A, SLA, IL2RG, GTSE1, MYB, PTPN7, CXorf9, RHOH, ZNFN1A1, CENTB1, LCP2, HIST1H4C, CCR7, APOBEC3B, MCM7, LCP1, SELPLG, CD3Z, PRKCQ, GZMB, SCN3A, LAIR1, SH2D1A, SEPT6, CG018, CD3D, C18orf10, PRF1, AIF1, MCM5, LPXN, C22orf18, ARHGAP15, and LEF1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-096-prec-7, Hcd605, hsa-mir-20b, hsa-miR-373*, HUMTRAB, hsa-mir-019b-1-prec, HPR163, hsa-mir-371, hsa-mir-025-prec, hsa-mir-18b, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to 5-Aza-2'-deoxycytidine (Decitabine).

25. The method of claim 1, wherein said at least one gene is selected from the group consisting of SLC9A3R1, RPS19, ITM2A, SSBP2, CXorf9, RHOH, ZNFN1A1, FXYD2, CCR9, NAP1L1, CXCR4, SH2D1A, CD1A, TRB@, SEPT6, RPS2, DOCK2, CD3D, CD6, ZAP70, AIF1, CD1E, CYFIP2, ADA, TRIM, GLTSCR2, FLJ10858, BCL11B, GIMAP6, STAG3, UBASH3A or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of MRPS24, TRIM22, TRIM41, LAT, CD1C, MRPS22, ADAM11, RPL13, RPS27, RPL13, RPS25, RPL18A, CORO1A, PTPRCAP, GMFG, ITK, CD1B, GMFG, PTPRCAP, CORO1A, ITGB2, HCLS1, and ATP2A3, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of HUMTRF, hsa-mir-483, MPR74, hsa-mir-122a-prec, ath-MIR180a, hsa-mir-128b-prec, Hcd923, hsa-mir-106-prec-X, hsa-mir-342, hsa-mir-142-prec, HPR169, hsa-mir-223-prec, Hcd754, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Idarubicin.

26. The method of claim 1, wherein said at least one gene is selected from the group consisting of CD99, HLA-DPB1, ARHGDIB, IFITM1, UBE2L6, ITM2A, SERPINA1, STAT5A, INPP5D, DGKA, SATB1, SEMA4D, TFDP2, SLA, IL2RG, CD48, MFNG, ALOX5AP, GPSM3, PSMB9, KIAA0711, SELL, ADA, EDG1, RIMS3, FMNL1, MYB, PTPN7, LCK, CXorf9, RHOH, ZNFN1A1, CENTB1, LCP2, FXYD2, CD1D, BATF, STAT4, VAV1, MAP4K1, CCR7, PDE4C, CD3G, CCR9, SP110, LCP1, IFI16, CXCR4, ARHGEF6, GATA3, SELPLG, SEC31L2, CD3Z, PRKCQ, SH2D1A, GZMB, CD1A, SCN3A, LAIR1, FYB, TRB@, SEPT6, HA-1, DOCK2, CG018, CD3D, T3JAM, FNBP1, CD6, ZAP70, LST1, GPR65, PRF1, AIF1, FLJ20331, RAG2, WDR45, CD1E, CYFIP2, TARP, TRIM, RPL10L, GLTSCR2, GIMAP5, ARHGAP15, NOTCH1, BIN2, C13orf18, CECR1, BCL11B, GIMAP6, STAG3, TM6SF1, HSD17B7, UBASH3A, MGC5566, FLJ22457, TPK1, PHF11, and DKFZP434B0335, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of FLJ10534, PTPRC, TRIM22, C18orf1, EVL, TRIM41, PSME2, LAT, CD1C, MYBBP1A, ICAM3, ADAM11, CD53, FARSLA, RPL13, RAC2, RPL13, GNA15, PGF, LAPTM5, RPL18A, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG,

GNA15, ITK, CD1B, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, GNA15, TCF7, ITGB2, PTPRC, HCLS1, ATP2A3, MYBL1, and FARSLA, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-124a-3-prec, hsa-mir-181a-prec, Hcd773, Hcd683, Hcd796, HUMTRF, HUMTRS, hsa-mir-181b-2, Hcd294, hsa-mir-20b, hsa-mir-181d, hsa-mir-2,3-prec, Hcd148_HPR225left, hsa-mir-515-15p, hsa-mir-181b-prec, Hcd783, HUMTRAB, HUMTRN, hsa-mir-181b-1, hsa-mir-124a-1-prec1, hsa-mir-367, hsa-mir-128b-prec, Hcd438right, hsa-mir-025-prec, hsa-mir-2,6-prec, Hcd731, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-342, hsa-mir-142-prec, HSHELA01, HUMTRV1A, hsa-mir-223-prec, Hcd754, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Melphalan.

27. The method of claim 1, wherein said at least one gene is selected from the group consisting of MCL1, DDX23, JUNB, ZFP36, IFITM1, CKS1B, SERPINA1, IL4R, CLDN3, ARL4A, HMMR, FLJ12671, ANKHD1, KIF2C, RPA3, MCC2, CDH17, LSM5, PRF1, ROD1, FLJ12666, SUV420H1, MUC13, C13orf18, and CDCA8, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of ETS2, AR1D1A, ID1, DDC, NID2, CCT3, ID2, NFIL3, and AREG, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd829, hsa-mir-197-prec, HPR163, and hsa-mir-150-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to IL4-PE38 fusion protein.

28. The method of claim 1, wherein said at least one gene is selected from the group consisting of MCL1, DDX23, JUNB, ZFP36, IFITM1, CKS1B, SERPINA1, IL13R, CLDN3, ARL4A, HMMR, FLJ12671, ANKHD1, KIF2C, RPA3, MCC2, CDH17, LSM5, PRF1, ROD1, FLJ12666, SUV420H1, MUC13, C13orf18, and CDCA8, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of ETS2, AR1D1A, ID1, DDC, NID2, CCT3, ID2, NFIL3, and AREG, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd829, hsa-mir-197-prec, HPR163, and hsa-mir-150-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to IL13-PE38QQR fusion protein (cintredekin besudotox).

29. The method of claim 1, wherein said at least one gene is selected from the group consisting of STOM, TNFAIP3, ASNS, GARS, CXCR4, EGLN3, LBH, and GDF15, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of STOML1 and KIAA0746, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-034-prec, Hcd255, Hcd712, Hcd965, Hcd891, Hcd210_HPR205, hsa-mir-429, Hcd753, Hcd693, MPR203, Hcd704, Hcd863PO, hsa-mir-122a-prec, Hcd760, Hcd338, HPR213, Hcd852, Hcd366, MPR103, Hcd669, and hsa-mir-188-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Valproic acid (VPA).

30. The method of claim 1, wherein said at least one gene is selected from the group consisting of PPIB, ZFP36L2, IFI30, USP7, SRM, SH3BP5, ALDOC, FADS2, GUSB,

PSCD1, IQGAP2, STS, MFNG, FLI1, PIM2, INPP4A, LRMP, ICAM2, EVI2A, MAL, BTN3A3, PTPN7, IL10RA, SPI1, TRAF1, ITGB7, ARHGAP6, MAP4K1, CD28, PTP4A3, LTB, C1orf38, WBSCR22, CD8B1, LCP1, FLJ13052, MEF2C, PSCDBP, IL16, SELPLG, MAGEA9, LAIR1, TNFRSF25, EVI2B, IGJ, PDCD4, RASA4, HA-1, PLCL2, RNASE6, WBSCR20C, NUP210, RPL10L, C11orf2, CABC1, ARHGEF3, TAPBPL, CHST12, FKBP11, FLJ35036, MYLIP, TXND5, PACAP, TOSO, PNAS-4, IL21R, and TCF4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of CLTB, BTN3A2, BCL2, SETBP1, ICAM3, BCL2, BCL2, BCL2, CD53, CCND2, CLTB, CLTB, BCL2L11, BTN3A2, CD37, MYCL2, CTSS, LAPTOM5, CD53, CORO1A, HEM1, CD53, CORO1A, HEM1, HCLS1, BCL2L11, MYCL1, MYC, and MAN1A1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd257, hsa-mir-148-prec, Hcd512, HPR227, Hcd421, MPR203, hsa-mir-0,7-prec, hsa-mir-219-2, hsa-mir-328, Hcd783, Hcd181, HPR213, hsa-mir-191-prec, hsa-mir-375, hsa-mir-2,2-prec, Hcd913, Hcd716, MPR207, HPR206, hsa-mir-016b-chr3, Hcd654, hsa-mir-195-prec, Hcd425, hsa-mir-148a, hsa-mir-142-prec, and hsa-mir-016a-chr13, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to All-trans retinoic acid (ATRA).

31. The method of claim 1, wherein said at least one gene is selected from the group consisting of C6orf29, TRIM31, CD69, LRRN3, GPR35, and CDW52, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd99, hsa-mir-520c/526a, hsa-mir-191-prec, hsa-mir-205-prec, hsa-mir-375, hsa-mir-423, hsa-mir-449, and hsa-mir-196-2-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Cytoxan.

32. The method of claim 1, wherein said at least one gene is selected from the group consisting of K-ALPHA-1, CSDA, UCHL1, NAP1L1, ATP5G2, HDGFRP3, and IFI44, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of HUMTRF, MPR74, hsa-mir-2,3-prec, hsa-mir-155-prec, hsa-mir-181b-prec, hsa-mir-342, and hsa-mir-4323p, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Topotecan (Hycamtin).

33. The method of claim 1, wherein said at least one gene is selected from the group consisting of NOL5A, STOM, SIAT1, CUGBP2, GUSB, ITM2A, JARID2, RUNX3, ICAM2, PTPN7, VAV1, PTP4A3, MCAM, MEF2C, IDH3B, RFP, SEPT6, SLC43A3, WBSCR20C, SHMT2, GLTSCR2, CABC1, FLJ20859, FLJ20010, MGC10993, and FKBP11, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of STOML1, EIF4A1, PDE3B, BCL11A, INPP4B, HLA-DMA, TRFP, EIF4A1, GAS7, MYCL2, HCLS1, MYCL1, and MYC, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-123-prec, hsa-mir-514-1, hsa-mir-101-prec-9, hsa-mir-148-prec, hsa-mir-106a, hsa-mir-20b, Hcd781, hsa-mir-0,7-prec, hsa-mir-019b-2-prec, hsa-mir-033-prec, hsa-mir-092-prec-13=092-1, hsa-mir-107, hsa-mir-103-prec-5=103-1, MPR216, hsa-mir-29b-2=102prec7.

1=7.2, hsa-mir-019b-1-prec, hsa-mir-107-prec-10, hsa-mir-135-2-prec, Hcd581, hsa-mir-103-2-prec, Hcd230, hsa-mir-025-prec, hsa-mir-208-prec, hsa-mir-18b, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, HPR169, hsa-mir-0,8-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Suberoylanilide hydroxamic acid (SAHA, vorinostat, Zolinza).

34. The method of claim 1, wherein said at least one gene is selected from the group consisting of ZFP36L2, TRIB2, LCP2, C6orf32, IL16, CACNA1G, SPDEF, HAB1, TOSO, and ARHGAP25, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of SGCD and CAPN3, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd415, hsa-mir-147-prec, hsa-mir-033b-prec, Hcd778, hsa-mir-127-prec, hsa-mir-324, Hcd794, and Hcd634, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Depsipeptide (FR901228).

35. The method of claim 1, wherein said at least one gene is selected from the group consisting of PLEKHB2, ARPC1B, MX1, CUGBP2, IFI16, TNFRSF14, SP110, ELF1, LPXN, IFRG28, LEF1, and PYCARD, or wherein the method further comprises measuring a level of expression of HMX1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of MPR121, Hcd115, Hcd693, Hcd704, HPR100, Hcd760, hsa-mir-147-prec, hsa-mir-033b-prec, hsa-mir-146-prec, Hcd142, hsa-mir-501, Hcd716, MPR207, Hcd777, hsa-mir-204-prec, hsa-mir-146b, Hcd511, Hcd397, MPR130, Hcd782, hsa-mir-324, Hcd794, and Hcd739, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Bortezomib.

36. The method of claim 1, wherein said at least one gene is selected from the group consisting of SSRP1, ALDOC, C1QR1, TTF1, PRIM1, USP34, TK2, GOLGIN-67, NPDO14, KIAA0220, SLC43A3, WBSCR20C, ICAM2, TEX10, CHD7, SAMSN1, and TPRT, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, CD53, RNPS1, H3F3A, NUDC, SMARCA4, RPL32, PTMA, CD53, PTPRCAP, PTPRC, RPL32, PTPRCAP, PTPRC, CD53, PTPRC, HCLS1, and SLC19A1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-096-prec-7, hsa-mir-123-prec, MPR249, HPR232, hsa-mir-101-prec-9, hsa-mir-106a, hsa-mir-20b, Hcd861, hsa-mir-017-prec, hsa-mir-019b-2-prec, hsa-mir-033-prec, Hcd102, MPR216, Hcd975, hsa-mir-019b-1-prec, hsa-mir-135-2-prec, Hcd581, Hcd536_HPR104, hsa-mir-128b-prec, HSTRNL, hsa-mir-025-prec, hsa-mir-18b, HPR262, Hcd923, Hcd434, Hcd658, HPR129, hsa-mir-380-5p, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, Hcd627, hsa-mir-142-prec, hsa-mir-018-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Leukieran.

37. The method of claim 1, wherein said at least one gene is selected from the group consisting of HLA-E, BAT3, ENO2, UBE2L6, CUGBP2, ITM2A, PALM2-AKAP2, JARID2, DGKA, SLC7A6, TFDP2, ADA, EDG1, ICAM2, PTPN7, CXorf9, RHOH, MX2, ZNFN1A1, COCH, LCP2,

CLGN, BNC1, FLNC, HLA-DRB3, UCP2, HLA-DRB1, GATA3, PRKCQ, SH2D1A, NFATC3, TRB@, FNBP1, SEPT6, NME4, DKFZP434C171, ZC3HAV1, SLC43A3, CD3D, AIF1, SPTAN1, CD1E, TRIM, DATF1, FHOD1, ARHGAP15, STAG3, SAP130, and CYLD, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, MX2004PA11424, TRIM22, TRIM41, CD1C, CHD8, ADAM11, ANPEP, RBMX2, RAC2, GNA15, LAPT M5, PTPRCAP, PTPRC, GNA15, CD1B, PTPRCAP, PTPRC, GNA15, PTPRC, and ATP2A3, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd773, Hcd248, hsa-mir-181d, MPR74, hsa-mir-2,3-prec, hsa-mir-155-prec, MPR197, hsa-mir-181b-prec, hsa-mir-29b-2=102prec7.1=7.2, hsa-mir-029c-prec, Hcd318, hsa-mir-128b-prec, hsa-mir-130a-prec, hsa-mir-140, hsa-mir-16-2, hsa-mir-526a-2, hsa-mir-016b-chr3, hsa-mir-195-prec, hsa-mir-2,6-prec, hsa-mir-342, hsa-mir-29b-1, Hcd627, hsa-mir-102-prec-1, hsa-mir-142-prec, hsa-mir-223-prec, hsa-let-7f-2-prec2, and hsa-mir-016a-chr13, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Fludarabine.

38. The method of claim 1, wherein said at least one gene is CD99, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd794 and Hcd754, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Vinblastine.

39. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPLP2, BTG1, CSDA, ARHGDIB, INSIG1, ALDOC, WASPIP, C1QR1, EDEM1, SLA, MFNG, GPSM3, ADA, LRMP, EVI2A, FMNL1, PTPN7, RHOH, ZNFN1A1, CENTB1, MAP4K1, CD28, SP110, NAP1L1, IFI16, ARHGEF6, SELPLG, CD3Z, SH2D1A, LAIR1, RAFTLIN, HA-1, DOCK2, CD3D, T3JAM, ZAP70, GPR65, CYFIP2, LPXN, RPL10L, GLTSCR2, ARHGAP15, BCL11B, TM6SF1, PACAP, and TCF4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, BCL2, LAT, ICAM3, BCL2, BCL2, BCL2, ADAM11, CD53, FARSLA, BCL2L11, RPL13, RAC2, RPL13, MYCL2, LAPT M5, RPL18A, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, PTPRC, HCLS1, BCL2L11, MYCL1, FARSLA, and MYC, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-096-prec-7, hsa-mir-124a-3-prec, hsa-mir-101-prec-9, Hcd712, Hcd693, hsa-mir-219-2, Hcd145, hsa-mir-155-prec, HPR213, hsa-mir-2,2-prec, Hcd913, Hcd716, MPR207, Hcd559, Hcd654, Hcd739, and hsa-mir-142-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Busulfan.

40. The method of claim 1, wherein said at least one gene is selected from the group consisting of ARHGDIB, ITM2A, SSBP2, PIM2, SELL, ICAM2, EVI2A, MAL, PTPN7, ZNFN1A1, LCP2, ARHGAP6, CD28, CD8B1, LCP1, NPDO14, CD69, NFATC3, TRB@, IGJ, SLC43A3, DOCK2, FHOD1, and PACAP, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of ICAM3, CD53, SMARCA4, CD37, LAPT M5, CD53, CORO1A, HEM1, GMFG, GMFG, CD53, CORO1A, HEM1, and HCLS1, or

wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-123-prec, hsa-mir-101-prec-9, Hcd517, Hcd796, Hcd749, Hcd674, hsa-mir-019b-2-prec, hsa-mir-033-prec, hsa-mir-092-prec-13=092-1, hsa-mir-124a-2-prec, hsa-mir-143-prec, hsa-mir-516-43p, hsa-mir-2,6-prec, Hcd731, hsa-mir-106-prec-X, hsa-mir-142-prec, hsa-mir-223-prec, Hcd754, and hsa-mir-018-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Dacarbazine.

41. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPL18, RPL10A, RPS3A, EEF1B2, GOT2, RPL13A, RPS15, NOL5A, RPLP2, SLC9A3R1, EIF3S3, MTHFD2, IMPDH2, ALDOC, FABP5, ITM2A, PCK2, MFNG, GCH1, PIM2, ADA, ICAM2, TTF1, MYB, PTPN7, RHOH, ZNFN1A1, PRIM1, FHIT, ASS, SYK, OXA1L, LCP1, DDX18, NOLA2, KIAA0922, PRKCQ, NFATC3, ANAPC5, TRB@, CXCR4, FNBP4, SEPT6, RPS2, MDN1, PCCB, RASA4, WBSCR20C, SFRS7, WBSCR20A, NUP210, SHMT2, RPLP0, MAP4K1, HNRPA1, CYFIP2, RPL10L, GLTSCR2, MRPL16, MRPS2, FLJ12270, CDK5RAP3, ARHGAP15, CUTC, FKBP11, ADPGK, FLJ22457, PUS3, PACAP, and CALML4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of MRPS24, DUSP2, EIF4A1, BRD2, BCL11A, RASSF2, MRPL37, MRPL30, RASSF1, MYBBP1A, LASS2, MRPS22, ADAM11, CD53, RPS6 KB1, RNPS1, BRD2, EIF4A1, FBL, BRD2, RPL36A, RPL13, RPL38, H3F3A, KIAA0182, RPS27, RPS6, EEF1G, RPL13, MYCL2, FBLN1, RPS25, RPL32, PTMA, RPL18A, RPL3, CD53, CORO1A, HEM1, GMFG, RPL32, GMFG, CD53, CORO1A, HEM1, HCLS1, ATP2A3, RASSF7, MYCL1, MYBL1, MYC, RPS15A, RASSF2, and LASS6, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-148-prec, hsa-mir-20b, hsa-mir-007-2-prec, hsa-mir-0,7-prec, hsa-mir-019b-2-prec, Hcd760, Hcd783, MPR216, hsa-mir-375, hsa-mir-019b-1-prec, hsa-mir-135-2-prec, hsa-mir-150-prec, hsa-mir-128b-prec, hsa-mir-499, hsa-mir-025-prec, hsa-mir-007-1-prec, hsa-mir-019a-prec, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, HPR169, hsa-mir-018-prec, hsa-mir-020-prec, and hsa-mir-484, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Oxaliplatin.

42. The method of claim 1, wherein said at least one gene is selected from the group consisting of CSDA, INSIG1, UBE2L6, PRG1, ITM2A, DGKA, SLA, PCBP2, IL2RG, ALOX5AP, PSMB9, LRMP, ICAM2, PTPN7, CXorf9, RHOH, ZNFN1A1, CENTB1, LCP2, STAT4, CCR7, CD3G, SP110, TNFAIP8, IFI16, CXCR4, ARHGEF6, SELPLG, CD3Z, PRKCQ, SH2D1A, CD1A, NFATC3, LAIR1, TRB@, SEPT6, RAFTLIN, DOCK2, CD3D, CD6, AIF1, CD1E, CYFIP2, TARP, ADA, ARHGAP15, GIMAP6, STAG3, FLJ22457, PACAP, and TCF4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, TRIM22, PSME2, LAT, CD1C, ICAM3, ADAM11, CD53, FARSLA, RPL13, RAC2, RPL13, NK4, LAPT M5, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, ITGB2, PTPRC, HCLS1, ATP2A3, and FARSLA, or wherein the method further comprises measuring a level of expression

of at least one microRNA selected from the group consisting of Hcd257, Hcd768, Hcd796, HUMTRF, HUMTRS, MPR74, hsa-mir-2,3-prec, hsa-mir-155-prec, Hcd763, hsa-mir-181b-prec, ath-MIR180a, hsa-mir-2,6-prec, hsa-mir-342, hsa-mir-142-prec, HSHELA01, HUMTRV1A, hsa-mir-223-prec, Hcd754, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Hydroxyurea.

43. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPL11, RPL17, ANAPC5, RPL13A, STOM, TUFM, SCARB1, FABP5, KIAA0711, IL6R, WBSCR22, UCK2, GZMB, C1orf38, PCBP2, GPR65, GLTSCR2, and FKBP11, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of STOML1, MRPL37, MRPL30, RPL36A, RPL38, HSPD1, MIF, RPL32, RPL3, and RPL32, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd257, Hcd946, Hcd503, hsa-mir-429, Hcd693, hsa-mir-373*, Hcd738, hsa-mir-328, Hcd783, Hcd181, Hcd631, Hcd279, hsa-mir-194-2, hsa-mir-197-prec, HPR163, hsa-mir-150-prec, Hcd323, hsa-mir-103-2-prec, Hcd243, Hcd938, hsa-mir-025-prec, hsa-mir-007-1-prec, MPR243, Hcd511, Hcd654, hsa-mir-199a-2-prec, hsa-mir-2,4-prec, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, Hcd794, Hcd530, HSHELA01, Hcd754, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Tegafur.

44. The method of claim 1, wherein said at least one gene is selected from the group consisting of ALDOC, ITM2A, SLA, SSBP2, IL2RG, MFNG, SELL, STC1, LRMP, MYB, PTPN7, CXorf9, RHOH, ZNFN1A1, CENTB1, MAP4K1, CCR7, CD3G, CCR9, CBFA2T3, CXCR4, ARHGEF6, SELPLG, SEC31L2, CD3Z, SH2D1A, CD1A, SCN3A, LAIR1, TRB@, DOCK2, WBSCR20C, CD3D, T3JAM, CD6, ZAP70, GPR65, AIF1, WDR45, CD1E, CYFIP2, TARP, TRIM, ARHGAP15, NOTCH1, STAG3, UBASH3A, MGC5566, and PACAP, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, TRIM22, TRIM41, LAT, CD1C, MYBBP1A, CD53, FARSLA, PPP2CA, LAPTM5, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG, ITK, CD1B, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, TCF7, PTPRC, HCLS1, ATP2A3, MYBL1, and FARSLA, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd768, HUMTRF, Hcd145, Hcd923, hsa-mir-2,6-prec, hsa-mir-093-prec-7.1=093-1, hsa-mir-342, Hcd794, hsa-mir-142-prec, HSHELA01, hsa-mir-223-prec, and Hcd754, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Daunorubicin.

45. The method of claim 1, wherein said at least one gene is selected from the group consisting of PFN1, CALU, ZYX, PSMD2, RAP1B, EPAS1, PGAM1, STAT1, CKAP4, DUSP1, RCN1, UCHL1, ITGA5, NFKBIA, LAMB1, TGFBI, FHL1, GJA1, PRG1, EXT1, MVP, NNMT, TAP1, CRIM1, PLOD2, RPS19, AXL, PALM2-AKAP2, IL8, LOXL2, PAPSS2, CAV1, F2R, PSMB9, LOX, C1orf29, STC1, LIF, KCNJ8, SMAD3, HPCAL1, WNT5A, BDNF, TNFRSF1A, NCOR2, FLNC, HMGA2, HLA-B, FLOT1, PTRF, IFI16, MGC4083, TNFRSF10B, PNMA2, TFPI, CLECSF2, SP110, PLAUR, ASPH, FSCN1, HIC, HLA-C,

COL6A1, IL6ST, IFITM3, MAP1B, FLJ46603, RAFTLIN, FTL, KIAA0877, MT1E, CDC10, ZNF258, BCAT1, IFI44, SOD2, TMSB10, FLJ10350, C1orf24, EFHD2, RPS27L, TNFRSF12A, FAD104, RAB7L1, NME7, TMEM22, TPK1, ELK3, CYLD, AMIGO2, ADAMTS1, and ACTB, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of ACLY, MPZL1, STC2, BAX, RAB31, RAB31, (UBC12, LOXL1, EMP3, FGFR1OP, IL6, TRIM22, OPTN, CYR61, METAP1, SHC1, FN1, EMP3, RAB31, LOXL1, BAX, BAX, RAB31, FN1, CD44, ANXA1, COL5A2, LGALS1, FGFR1, PLAU, TFP12, TFP12, VCAM1, SHC1, CSF2RA, EMP3, COL1A1, TGFB1, COL6A2, FGFR1, ITGA3, AKR1B1, MSN, EMP3, VIM, EMP3, COL6A2, MSN, PSMC5, UBC, FGFR1, BASP1, ANXA11, CSPG2, M6PRBP1, PRKCA, OPTN, OPTN, SPARC, CCL2, and ITGA3, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-125b-2-prec, hsa-mir-022-prec, hsa-mir-125b-1, hsa-mir-155-prec, hsa-mir-100, hsa-mir-409-3p, hsa-mir-495, hsa-mir-199a-2-prec, hsa-mir-382, and hsa-mir-100-1/2-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Bleomycin.

46. The method of claim 1, wherein said at least one gene is selected from the group consisting of HSPCB, LDHA, and TM4SF7, or wherein the method further comprises measuring a level of expression of LY6E, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd338, hsa-mir-099b-prec-19, and hsa-mir-149-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Estramustine.

47. The method of claim 1, wherein said at least one gene is selected from the group consisting of CSDA, INSIG1, UBE2L6, PRG1, ITM2A, DGKA, TFDP2, SLA, IL2RG, ALOX5AP, GPSM3, PSMB9, SELL, ADA, EDG1, FMNL1, PTPN7, CXorf9, RHOH, ZNFN1A1, CENTB1, LCP2, CD1D, STAT4, VAV1, MAP4K1, CCR7, PDE4C, CD3G, CCR9, SP110, TNFAIP8, LCP1, IFI16, CXCR4, ARHGEF6, SELPLG, SEC31L2, CD3Z, PRKCQ, SH2D1A, GZMB, CD1A, LAIR1, AF1Q, TRB@, SEPT6, DOCK2, RPS19, CD3D, T3JAM, FNBP1, CD6, ZAP70, LST1, BCAT1, PRF1, AIF1, RAG2, CD1E, CYFIP2, TARP, TRIM, GLTSCR2, GIMAP5, ARHGAP15, NOTCH1, BCL11B, GIMAP6, STAG3, TM6SF1, UBASH3A, MGC5566, FLJ22457, and TPK1, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, TRIM22, EVL, TRIM41, PSME2, LAT, CD1C, ADAM11, CD53, FARSLA, RPL13, RAC2, RPL13, GNA15, LAPTM5, RPL18A, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG, GNA15, ITK, CD1B, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, GNA15, ITGB2, PTPRC, HCLS1, ATP2A3, and FARSLA, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-181a-prec, hsa-mir-181c-prec, HUMTRF, hsa-mir-181d, MPR74, Hcd817, hsa-mir-2,3-prec, hsa-mir-155-prec, Hcd148_HPR225left, hsa-mir-515-15p, hsa-mir-181b-prec, HUMTRN, hsa-mir-128b-prec, hsa-mir-450-2, hsa-mir-2,6-prec, hsa-mir-342, hsa-mir-142-prec, hsa-mir-223-prec, Hcd754, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Chlorambucil.

48. The method of claim 1, wherein said at least one gene is selected from the group consisting of PRG1, SLC2A3, RPS19, PSMB10, ITM2A, DGKA, SEMA4D, SLA, IL2RG, MFNG, ALOX5AP, GPSM3, PSMB9, SELL, ADA, FMNL1, MYB, PTPN7, CXorf9, RHOH, ZNFN1A1, CENTB1, FXYD2, CD1D, STAT4, MAP4K1, CCR7, PDE4C, CD3G, CCR9, SP110, TK2, TNFAIP8, NAP1L1, SELPLG, SEC31L2, CD3Z, PRKCQ, SH2D1A, GZMB, CD1A, LAIR1, TRB@, SEPT6, DOCK2, CG018, WBSCR20C, CD3D, CD6, LST1, GPR65, PRF1, ALMS1, AIF1, CD1E, CYFIP2, TARP, GLTSCR2, FLJ12270, ARHGAP15, NAP1L2, CECR1, GIMAP6, STAG3, TM6SF1, C15orf25, MGC5566, FLJ22457, ET, TPK1, and PHF11, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of ETS2, PTPRC, PETER, SETBP1, LAT, MYBBP1A, ETV5, METAP1, ETS1, ADAM11, CD53, FARSLA, RPL13, ARMET, TETRAN, BET1, RPL13, MET, LAPT5, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG, CD1B, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, ETV4, ITGB2, PTPRC, HCLS1, MYBL1, FARSLA, and METAP2, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-124a-3-prec, Hcd946, Hcd683, HPR264, MPR185, HUMTRF, Hcd294, Hcd503, hsa-mir-20b, MPR74, MPR234, Hcd447, Hcd817, Hcd148_HPR225left, hsa-mir-515-15p, Hcd383, hsa-mir-181b-prec, Hcd783, MPR224, HPR172, MPR216, HUMTRN, hsa-mir-321, HPR159, MPR228, ath-MIR180a, hsa-mir-197-prec, hsa-mir-124a-1-prec1, hsa-mir-128b-prec, Hcd28_HPR39left, Hcd889, Hcd350, hsa-mir-025-prec, hsa-mir-208-prec, hsa-mir-450-2, Hcd923, Hcd434, HPR129, HPR220, hsa-mir-380-5p, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-342, hsa-mir-142-prec, HSHELA01, hsa-mir-223-prec, Hcd754, hsa-mir-020-prec, and hsa-mir-4323p, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Mechlorethamine.

49. The method of claim 1, wherein said at least one gene is selected from the group consisting of PGK1, SCD, INSIG1, IGBP1, TNFAIP3, TNFSF10, ABCA1, AGA, ABCA8, DBC1, PTGER2, UGT1A3, C10orf10, TM4SF13, CGI-90, LXN, DNAJC12, HIPK2, and C9orf95, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of FGFR1OP, PLXNA1, PSCD2L, TUBB, FGFR1, TUBB2, PAGA, TUBB2, UBB, TUBB2, FGFR1, FGFR1, and TUBB-PARALOG, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-483, Hcd631, hsa-mir-2,2-prec, Hcd938, MPR133, Hcd794, Hcd438, and Hcd886, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Streptozocin.

50. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPLP2, CD99, IFITM1, INSIG1, ALDOC, ITM2A, SERPINA1, C1QR1, STAT5A, INPP5D, SATB1, VPS16, SLA, IL2RG, MFNG, SELL, LRMP, ICAM2, MYB, PTPN7, ARHGAP25, LCK, CXorf9, RHOH, ZNFN1A1, CENTB1, ADD2, LCP2, SPI1, DBT, GZMA, CD2, BATF, HIST1H4C, ARHGAP6, VAV1, MAP4K1, CCR7, PDE4C, CD3G, CCR9, SP140, TK2, LCP1, IFI16, CXCR4, ARHGEF6, PSCDBP, SELPLG, SEC31L2, CD3Z, PRKCQ, SH2D1A, GZMB, CD1A, GATA2, LY9, LAIR1, TRB@, SEPT6, HA-1, SLC43A3,

DOCK2, CG018, MLC1, CD3D, T3JAM, CD6, ZAP70, DOK2, LST1, GPR65, PRF1, ALMS1, AIF1, PRDX2, FLJ12151, FBXW12, CD1E, CYFIP2, TARP, TRIM, RPL10L, GLTSCR2, CKIP-1, NRN1, ARHGAP15, NOTCH1, PSCD4, C13orf18, BCL11B, GIMAP6, STAG3, NARF, TM6SF1, C15orf25, FLJ11795, SAMS1, UBASH3A, PACAP, LEF1, IL21R, TCF4, and DKFZP434B0335, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of FLJ10534, PTPRC, CD27BP, TRIM22, TRIM41, PSCD2L, CD1C, MYBBP1A, ICAM3, CD53, FARSLA, GAS7, ABCD2, CD24, CD29, RAC2, CD37, GNA15, PGF, LAPT5, RPL18A, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG, GNA15, ITK, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, GNA15, TCF7, ITGB2, PTPRC, HCLS1, PRKCB1, ATP2A3, PRKCB1, MYBL1, and FARSLA, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, Hcd517, Hcd796, HUMTRF, hsa-mir-20b, hsa-mir-019b-2-prec, hsa-mir-033-prec, hsa-mir-092-prec-13=092-1, Hcd148_HPR225left, HUMTRAB, Hcd975, hsa-mir-135-2-prec, hsa-mir-128b-prec, hsa-mir-143-prec, hsa-mir-025-prec, hsa-mir-2,6-prec, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, HSHELA01, HUMTRV1A, hsa-mir-223-prec, Hcd754, hsa-mir-018-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Carmustine.

51. The method of claim 1, wherein said at least one gene is selected from the group consisting of RPS15, INSIG1, ALDOC, ITM2A, C1QR1, STAT5A, INPP5D, VPS16, SLA, USP20, IL2RG, MFNG, LRMP, EVI2A, PTPN7, ARHGAP25, RHOH, ZNFN1A1, CENTB1, LCP2, SPI1, ARHGAP6, MAP4K1, CCR7, LY96, C6orf32, MAGEA1, SP140, LCP1, IFI16, ARHGEF6, PSCDBP, SELPLG, CD3Z, PRKCQ, GZMB, LAIR1, SH2D1A, TRB@, RFP, SEPT6, HA-1, SLC43A3, CD3D, T3JAM, GPR65, PRF1, AIF1, LPXN, RPL10L, SITPEC, ARHGAP15, C13orf18, NARF, TM6SF1, PACAP, and TCF4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, ICAM3, TRFP, CD53, FARSLA, RAC2, MAGEA11, LAPT5, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, PTPRC, HCLS1, SLC19A1, FARSLA, and RPS15A, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-101-prec-9, Hcd796, hsa-mir-20b, HUMTRAB, hsa-mir-135-2-prec, hsa-mir-153-1-prec1, hsa-mir-025-prec, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, HUMTRV1A, Hcd754, hsa-mir-018-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Lomustine.

52. The method of claim 1, wherein said at least one gene is selected from the group consisting of SSRP1, ALDOC, C1QR1, TTF1, PRIM1, USP34, TK2, GOLGIN-67, NPDO14, KIAA0220, SLC43A3, WBSCR20C, ICAM2, TEX10, CHD7, SAMS1, and TPRT, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, CD53, RNPS1, H3F3A, NUDC, SMARCA4, RPL32, PTMA, CD53, PTPRCAP, PTPRC, RPL32, PTPRCAP,

PTPRC, CD53, PTPRC, HCLS1, and SLC19A1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-096-prec-7, hsa-mir-123-prec, MPR249, HPR232, hsa-mir-101-prec-9, hsa-mir-106a, hsa-mir-20b, Hcd861, hsa-mir-0,7-prec, hsa-mir-019b-2-prec, hsa-mir-033-prec, Hcd102, MPR216, Hcd975, hsa-mir-019b-1-prec, hsa-mir-135-2-prec, Hcd581, Hcd536_HPR104, hsa-mir-128b-prec, HSTRNL, hsa-mir-025-prec, hsa-mir-18b, HPR262, Hcd923, Hcd434, Hcd658, HPR129, hsa-mir-380-5p, hsa-mir-093-prec-7.1=093-1, hsa-mir-106-prec-X, Hcd627, hsa-mir-142-prec, hsa-mir-018-prec, and hsa-mir-020-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Mercaptopurine.

53. The method of claim 1, wherein said at least one gene is selected from the group consisting of CD99, INSIG1, PRG1, ALDOC, ITM2A, SLA, SSBP2, IL2RG, MFNG, ALOX5AP, C1orf29, SELL, STC1, LRMP, MYB, PTPN7, CXorf9, RHOH, ZNFN1A1, CENTB1, ADD2, CD1D, BATF, MAP4K1, CCR7, PDE4C, CD3G, CCR9, SP110, TNFAIP8, NAP1L1, CXCR4, ARHGEF6, GATA3, SELPLG, SEC31L2, CD3Z, SH2D1A, GZMB, CD1A, SCN3A, LAIR1, AF1Q, TRB@, DOCK2, MLC1, CD3D, T3JAM, CD6, ZAP70, IFI44, GPR65, PRF1, AIF1, WDR45, CD1E, CYFIP2, TARP, TRIM, ARHGEF15, NOTCH1, STAG3, NARF, TM6SF1, UBASH3A, and MGC5566, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of FLJ10534, PTPRC, TRIM22, C18orf1, TRIM41, LAT, CD1C, MYBBP1A, CD53, FARSLA, PPP2CA, COL5A2, LAPTOM5, CD53, CORO1A, PTPRCAP, PTPRC, HEM1, GMFG, ITK, CD1B, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, HEM1, TCF7, PTPRC, HCLS1, ATP2A3, MYBL1, and FARSLA, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-124a-3-prec, Hcd768, HUMTRF, hsa-mir-2,3-prec, hsa-mir-181b-prec, Hcd783, hsa-mir-2,2-prec, hsa-mir-124a-1-prec1, hsa-mir-342, hsa-mir-142-prec, HSHELA01, hsa-mir-223-prec, and Hcd754, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Teniposide.

54. The method of claim 1, wherein said at least one gene is selected from the group consisting of ALDOC, C1QR1, SLA, WBSCR20A, MFNG, SELL, MYB, RHOH, ZNFN1A1, LCP2, MAP4K1, CBFA2T3, LCP1, SELPLG, CD3Z, LAIR1, WBSCR20C, CD3D, GPR65, ARHGEF15, FLJ10178, NARF, and PUS3, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, MYBBP1A, ICAM3, CD53, FARSLA, CD53, PTPRCAP, PTPRC, HEM1, GMFG, GMFG, PTPRCAP, PTPRC, CD53, HEM1, PTPRC, HCLS1, PRKCB1, PRKCB1, MYBL1, and FARSLA, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-025-prec, hsa-mir-007-1-prec, hsa-mir-093-prec-7.1=093-1, Hcd794, and hsa-mir-142-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Dactinomycin.

55. The method of claim 1, wherein said at least one gene is selected from the group consisting of PPIB, ZFP36L2, IFI30, USP7, SRM, SH3BP5, ALDOC, FADS2, GUSB,

PSCD1, IQGAP2, STS, MFNG, FLI1, PIM2, INPP4A, LRMP, ICAM2, EVI2A, MAL, BTN3A3, PTPN7, IL10RA, SPI1, TRAF1, ITGB7, ARHGEF6, MAP4K1, CD28, PTP4A3, LTB, C10orf38, WBSCR22, CD8B1, LCP1, FLJ13052, MEF2C, PSCDBP, IL16, SELPLG, MAGEA9, LAIR1, TNFRSF25, EVI2B, IGJ, PDCD4, RASA4, HA-1, PLCL2, RNASE6, WBSCR20C, NUP210, RPL10L, C11orf2, CABC1, ARHGEF3, TAPBPL, CHST12, FKBP11, FLJ35036, MYLIP, TXNDC5, PACAP, TOSO, PNAS-4, IL21R, and TCF4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of CLTB, BTN3A2, BCL2, SETBP1, ICAM3, BCL2, BCL2, BCL2, CD53, CCND2, CLTB, CLTB, BCL2L11, BTN3A2, CD37, MYCL2, CTSS, LAPTOM5, CD53, CORO1A, HEM1, CD53, CORO1A, HEM1, HCLS1, BCL2L11, MYCL1, MYC, and MAN1A1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd257, hsa-mir-148-prec, Hcd512, HPR227, Hcd421, MPR203, hsa-mir-0,7-prec, hsa-mir-219-2, hsa-mir-328, Hcd783, Hcd181, HPR213, hsa-mir-191-prec, hsa-mir-375, hsa-mir-2,2-prec, Hcd913, Hcd716, MPR207, HPR206, hsa-mir-016b-chr3, Hcd654, hsa-mir-195-prec, Hcd425, hsa-mir-148a, hsa-mir-142-prec, and hsa-mir-016a-chr13, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Tretinoin.

56. The method of claim 1, wherein said at least one gene is selected from the group consisting of PDGFRB, KDR, KIT, and FLT3, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of FLT1, FLT4, PDGFRA, and CSF1R, wherein said level of expression of said gene indicates that said cell is sensitive to sunitinib.

57. The method of claim 1, wherein said at least one gene is BCL2, wherein said level of expression of said gene indicates that said cell is sensitive to SPC2996.

58. The method of claim 1, wherein said at least one gene is selected from the group consisting of ARHGDIB, ZFP36L2, ITM2A, LGALS9, INPP5D, SATB1, TFDP2, IL2RG, CD48, SELL, ADA, LRMP, RIMS3, LCK, CXorf9, RHOH, ZNFN1A1, LCP2, CD1D, CD2, ZNF91, MAP4K1, CCR7, IGLL1, CD3G, ZNF430, CCR9, CXCR4, KIAA0922, TARP, FYN, SH2D1A, CD1A, LST1, LAIR1, TRB@, SEPT6, CD3D, CD6, AIF1, CD1E, TRIM, GLTSCR2, ARHGEF15, BIN2, SH3TC1, CECR1, BCL11B, GIMAP6, STAG3, GALNT6, MGC5566, PACAP, and LEF1, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of CD27BP, TRIM22, TRA@, C18orf1, EVL, PRKCH, TRIM41, PSCD2L, CD1C, ADAM11, ABCD2, CD24, CD29, CD37, GNA15, LAPTOM5, CORO1A, HEM1, GMFG, GNA15, CD1B, GMFG, CORO1A, HEM1, GNA15, ITGB2, PRKCB1, ATP2A3, and PRKCB1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, hsa-mir-181b-2, Hcd417, Hcd440_HPR257, hsa-mir-019b-2-prec, hsa-mir-2,3-prec, hsa-mir-033-prec, hsa-mir-092-prec-13=092-1, hsa-mir-181b-prec, hsa-mir-128b-prec, hsa-mir-526a-2, MPR95, HPR220, hsa-mir-133a-1, hsa-mir-148a, hsa-mir-142-prec, HPR169, hsa-mir-223-prec, hsa-mir-018-prec, hsa-mir-020-prec, and hsa-mir-484, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Ifosfamide.

59. The method of claim 1, wherein said at least one gene is selected from the group consisting of MLP, GLUL, SLC9A3R1, ZFP36L2, INSIG1, TBL1X, NDUFAB1, EBP, TRIM14, SRPK2, PMM2, CLDN3, GCH1, ID11, TTF1, MYB, RASGRP1, HIST1H3H, CBFA2T3, SRRM2, ANAPC5, MBD4, GATA3, H1ST1H2BG, RAB14, PIK3R1, MGC50853, ELF1, ZRF1, ZNF394, S100A14, SLC6A14, GALNT6, SPDEF, TPRT, and CALML4, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of EIF4A1, TFF1, TFF1, MYBBP1A, AKAP1, DGKZ, EIF4A1, KIAA0182, SLC19A1, ATP2A3, MYBL1, EIF4EBP2, G1P2, and MAN1A1, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of hsa-mir-092-prec-X=092-2, Hcd547, Hcd257, hsa-mir-148-prec, HUMTRS, hsa-mir-033-prec, hsa-mir-092-prec-13=092-1, hsa-mir-375, hsa-mir-095-prec-4, hsa-mir-025-prec, hsa-mir-202-prec, hsa-mir-007-1-prec, hsa-mir-093-prec-7, 1=093-1, hsa-mir-106-prec-X, hsa-mir-142-prec, hsa-mir-223-prec, and hsa-mir-018-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Tamoxifen.

60. The method of claim 1, wherein said at least one gene is selected from the group consisting of CSDA, F8A1, KYNU, PHF14, SERPINB2, OPHN1, HRMT1L2, TNFRSF1A, PPP4C, CES1, TP53AP1, TM4SF4, RPL5, BC008967, TLK2, COL4A6, PAK3, RECK, LOC51321, MST4, DERP6, SCD4, and FLJ22800, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of STC2, BAX, CDKN1A, DDB2, RGS2, BAX, BAX, RPL13, RPL13, CDKN1A, and GABPB2, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of HUMTRF, HUMTRN, hsa-mir-124a-1-prec1, hsa-mir-150-prec, Hcd923, HPR181, Hcd569, hsa-mir-199a-2-prec, Hcd754, and hsa-mir-4323p, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Flouxuridine.

61. The method of claim 1, wherein said at least one gene is selected from the group consisting of CSDA, UBE2L6, TAP1, RPS19, SERPINA1, C1QR1, SLA, GPSM3, PSMB9, EDG1, FMNL1, PTPN7, ZNFN1A1, CENTB1, BATF, MAP4K1, PDE4C, SP110, HLA-DRA, IFI16, HLA-DRB1, ARHGEF6, SELPLG, SEC31L2, CD3Z, PRKCQ, SH2D1A, GZMB, TRB@, HLA-DPA1, AIM1, DOCK2, CD3D, IFITM1, ZAP70, PRF1, C1orf24, ARHGAP15, C13orf18, and TM6SF1, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of PTPRC, TRIM22, PSME2, LAT, METAP1, CD53, FARSLA, RPL13, RAC2, RPL13, PTMA, CD53, CORO1A, PTPRCAP, PTPRC, GMFG, ITK, GMFG, PTPRCAP, PTPRC, CD53, CORO1A, ITGB2, PTPRC, HCLS1, and FARSLA, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of HUMTRF, hsa-mir-380-5p, hsa-mir-342, hsa-mir-142-prec, and Hcd200, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Irinotecan.

62. The method of claim 1, wherein said at least one gene is selected from the group consisting of STAT1, HSBP1, IFI30, RIOK3, TNFSF10, ALOX5AP, ADFP, IRS2, EFEMP2, RIPK2, DKFZp56411922, MT1K, RNASET2,

EFHD2, TRIB3, ACSL5, IFIH1, and DNAPTP6, or wherein the method further comprises measuring a level of expression of at least one gene selected from the group consisting of IFI27, OPTN, C20orf18, FN1, LOC51123, FN1, OPTN, and OPTN, or wherein the method further comprises measuring a level of expression of at least one microRNA selected from the group consisting of Hcd289, Hcd939, Hcd330, HPR76, Hcd111, Hcd976, hsa-mir-15a, hsa-mir-001b-1-prec1, hsa-mir-450-1, hsa-mir-200b, Hcd578, and hsa-mir-200a-prec, wherein said level of expression of said gene or microRNA indicates that said cell is sensitive to Satraplatin.

63. The method of claim 1, wherein said level of expression of said gene is determined by detecting the level of mRNA transcribed from said gene.

64. The method of claim 1, wherein said level of expression of said gene is determined by detecting the level of a protein product of said gene.

65. The method of claim 1, wherein said level of expression of said gene is determined by detecting the level of the biological activity of a protein product of said gene.

66. The method of claim 1, wherein an increase in the level of expression of said gene or microRNA indicates increased sensitivity of said cell to said treatment.

67. The method of claim 1, wherein said cell is a cancer cell.

68. The method of claim 1, wherein a decrease in the level of expression of said gene or microRNA indicates increased sensitivity of said cell to said treatment.

69. The method of claim 1, wherein said level of expression of said gene or microRNA is measured using a quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

70. A method for determining the development of resistance of a cell in a patient to a treatment to which said cell in said patient has previously been sensitive, said method comprising measuring a level of expression of at least one gene or microRNA of claim 1 of said cell, wherein a decrease in said level of expression of said gene or microRNA in said cell relative to the level of expression of said gene or microRNA in a control cell sensitive to said treatment indicates resistance or a propensity to develop resistance to the treatment by said patient.

71. A method for determining the development of resistance of a cell in a patient to a treatment to which said cell in said patient has previously been sensitive, said method comprising measuring a level of expression of at least one gene or microRNA of claim 1 in said cell, wherein an increase in said level of expression of said gene or microRNA in said cell relative to the level of expression of said gene or microRNA in a control cell sensitive to said treatment indicates resistance or a propensity to develop resistance to the treatment by said patient.

72. A method of determining sensitivity of a cancer patient to a treatment for cancer comprising measuring a level of expression of at least one microRNA in a cell of said patient, said microRNA selected from the group consisting of ath-MIR180aNo2, Hcd102 left, Hcd111 left, Hcd115 left, Hcd120 left, Hcd142 right, Hcd145 left, Hcd148_HPR225 left, Hcd181 left, Hcd181 right, Hcd210_HPR205 right, Hcd213_HPR182 left, Hcd230 left, Hcd243 right, Hcd246 right, Hcd248 right, Hcd249 right, Hcd250 left, Hcd255 left, Hcd257 left, Hcd257 right, Hcd263 left, Hcd266 left, Hcd270 right, Hcd279 left, Hcd279 right, Hcd28_HPR39left, Hcd28_HPR39right, Hcd282PO right, Hcd289 left, Hcd294 left, Hcd318 right, Hcd323 left, Hcd330 right, Hcd338 left,

Hcd340 left, Hcd350 right, Hcd355_HPR190 left, Hcd361 right, Hcd366 left, Hcd373 right, Hcd383 left, Hcd383 right, Hcd384 left, Hcd397 left, Hcd404 left, Hcd412 left, Hcd413 right, Hcd415 right, Hcd417 right, Hcd421 right, Hcd425 left, Hcd438right, Hcd434 right, Hcd438 left, Hcd440_HPR257 right, Hcd444 right, Hcd447 right, Hcd448 left, Hcd498 right, Hcd503 left, Hcd511 right, Hcd512 left, Hcd514 right, Hcd517 left, Hcd517 right, Hcd530 right, Hcd536_HPR104 right, Hcd542 left, Hcd544 left, Hcd547 left, Hcd559 right, Hcd562 right, Hcd569 right, Hcd570 right, Hcd578 right, Hcd581 right, Hcd586 left, Hcd586 right, Hcd587 right, Hcd605 left, Hcd605 left, Hcd605 right, Hcd608 right, Hcd627 left, Hcd631 left, Hcd631 right, Hcd634 left, Hcd642 right, Hcd649 right, Hcd654 left, Hcd658 right, Hcd669 right, Hcd674 left, Hcd678 right, Hcd683 left, Hcd684 right, Hcd689 right, Hcd690 right, Hcd691 right, Hcd693 right, Hcd697 right, Hcd704 left, Hcd704 left, Hcd712 right, Hcd716 right, Hcd731 left, Hcd738 left, Hcd739 right, Hcd739 right, Hcd749 right, Hcd753 left, Hcd754 left, Hcd755 left, Hcd760 left, Hcd763 right, Hcd768 left, Hcd768 right, Hcd770 left, Hcd773 left, Hcd777 left, Hcd778 right, Hcd781 left, Hcd781 right, Hcd782 left, Hcd783 left, Hcd788 left, Hcd794 right, Hcd796 left, Hcd799 left, Hcd807 right, Hcd812 left, Hcd817 left, Hcd817 right, Hcd829 right, Hcd852 right, Hcd861 right, Hcd863PO right, Hcd866 right, Hcd869 left, Hcd873 left, Hcd886 right, Hcd889 right, Hcd891 right, Hcd892 left, Hcd913 right, Hcd923 left, Hcd923 right, Hcd938 left, Hcd938 right, Hcd939 right, Hcd946 left, Hcd948 right, Hcd960 left, Hcd965 left, Hcd970 left, Hcd975 left, Hcd976 right, Hcd99 right, HPR100 right, HPR129 left, HPR154 left, HPR159 left, HPR163 left, HPR169 right, HPR172 right, HPR181 left, HPR187 left, HPR199 right, HPR206 left, HPR213 right, HPR214 right, HPR220 left, HPR220 right, HPR227 right, HPR232 right, HPR233 right, HPR244 right, HPR262 left, HPR264 right, HPR266 right, HPR271 right, HPR76 right, hsa_mir_490_Hcd20 right, HSHELA01, HSTRNL, HUMTRAB, HUMTRF, HUMTRN, HUMTRS, HUMTRV1A, let-7f-2-prec2, mir-001b-1-prec1, mir-001b-2-prec, mir-007-1-prec, mir-007-2-precNo2, mir-010a-precNo1, mir-015b-precNo2, mir-016a-chr13, mir-016b-chr3, mir-017-precNo1, mir-017-precNo2, mir-018-prec, mir-019a-prec, mir-019b-1-prec, mir-019b-2-prec, mir-020-prec, mir-022-prec, mir-023a-prec, mir-023b-prec, mir-024-2-prec, mir-025-prec, mir-027b-prec, mir-029c-prec, mir-032-precNo2, mir-033b-prec, mir-033-prec, mir-034-precNo1, mir-034-precNo2, mir-092-prec-13=092-1No2, mir-092-prec-X=092-2, mir-093-prec-7.1=093-1, mir-095-prec-4, mir-096-prec-7No1, mir-096-prec-7No2, mir-098-prec-X, mir-099b-prec-19No1, mir-100-1/2-prec, mir-100No1, mir-101-prec-9, mir-102-prec-1, mir-103-2-prec, mir-103-prec-5=103-1, mir-106aNo1, mir-106-prec-X, mir-107No1, mir-107-prec-10, mir-122a-prec, mir-123-precNo1, mir-123-precNo2, mir-124a-1-prec1, mir-124a-2-prec, mir-124a-3-prec, mir-125b-1, mir-125b-2-precNo2, mir-127-prec, mir-128b-precNo1, mir-128b-precNo2, mir-133a-1, mir-135-2-prec, mir-136-precNo2, mir-138-1-prec, mir-140No2, mir-142-prec, mir-143-prec, mir-144-precNo2, mir-145-prec, mir-146bNo1, mir-146-prec, mir-147-prec, mir-148aNo1, mir-148-prec, mir-149-prec, mir-150-prec, mir-153-1-prec1, mir-154-prec1No1, mir-155-prec, mir-15aNo1, mir-16-1No1, mir-16-2No1, mir-181a-precNo1, mir-181b-1No1, mir-181b-2No1, mir-181b-precNo1, mir-181b-precNo2, mir-181c-precNo1, mir-181dNo1, mir-188-prec, mir-18bNo2, mir-191-prec, mir-192No2, mir-

193bNo2, mir-194-2No1, mir-195-prec, mir-196-2-precNo2, mir-197-prec, mir-198-prec, mir-199a-1-prec, mir-199a-2-prec, mir-199b-precNo1, mir-200a-prec, mir-200bNo1, mir-200bNo2, mir-202*, mir-202-prec, mir-204-precNo2, mir-205-prec, mir-208-prec, mir-20bNo1, mir-2,2-precNo1, mir-2,2-precNo2, mir-2,3-precNo1, mir-2,4-prec, mir-2,5-precNo2, mir-2,6-precNo1, mir-219-2No1, mir-2,9-prec, mir-223-prec, mir-29b-1No1, mir-29b-2=102prec7.1=7.2, mir-321No1, mir-321No2, mir-324No1, mir-324No2, mir-328No1, mir-342No1, mir-361No1, mir-367No1, mir-370No1, mir-371No1, miR-373*No1, mir-375, mir-376aNo1, mir-379No1, mir-380-5p, mir-382, mir-384, mir-409-3p, mir-423No1, mir-424No2, mir-429No1, mir-429No2, mir-4323p, mir-4325p, mir-449No1, mir-450-1, mir-450-2No1, mir-483No1, mir-484, mir-487No1, mir-495No1, mir-499No2, mir-501No2, mir-503No1, mir-509No1, mir-514-1No2, mir-515-15p, mir-515-23p, mir-516-33p, mir-516-43p, mir-518e/526c, mir-519a-1/52, mir-519a-2No2, mir-519b, mir-519c/52, mir-520c/52, mir-526a-2No1, mir-526a-2No2, MPR103 right, MPR121 left, MPR121 left, MPR130 left, MPR130 right, MPR133 right, MPR141 left, MPR151 left, MPR156 left, MPR162 left, MPR174 left, MPR174 right, MPR185 right, MPR197 right, MPR203 left, MPR207 right, MPR215 left, MPR216 left, MPR224 left, MPR224 right, MPR228 left, MPR234 right, MPR237 left, MPR243 left, MPR244 right, MPR249 left, MPR254 right, MPR74 left, MPR88 right, and MPR95 left, wherein said level of expression of said microRNA indicates said cell is sensitive to said treatment.

73-136. (canceled)

137. A kit comprising a single-stranded nucleic acid molecule that is substantially complementary to or substantially identical to at least 5 consecutive nucleotides of at least one gene selected from the group consisting of ACTB, ACTN4, ADA, ADAM9, ADAMTS1, ADD1, AF1Q, AIF1, AKAP1, AKAP13, AKR1C1, AKT1, ALDH2, ALDOC, ALG5, ALMS1, ALOX15B, AMIGO2, AMPD2, AMPD3, ANAPC5, ANP32A, ANP32B, ANXA1, AP1G2, APOBEC3B, APRT, ARHE, ARHGAP15, ARHGAP25, ARHGDIIB, ARHGEF6, ARL7, ASAHI, ASPH, ATF3, ATIC, ATP2A2, ATP2A3, ATP5D, ATP5G2, ATP6V1B2, BC008967, BCAT1, BCHE, BCL11B, BDNF, BHLHB2, BIN2, BLMH, BMI1, BNIP3, BRDT, BRRN1, BTN3A3, C11orf2, C14orf139, C15orf25, C18orf10, C1orf24, C1orf29, C1orf38, C1QR1, C22orf18, C6orf32, CACNA1G, CACNB3, CALM1, CALML4, CALU, CAP350, CASP2, CASP6, CASP7, CAST, CBLB, CCNA2, CCNB1IP1, CCND3, CCR7, CCR9, CD1A, CD1C, CD1D, CD1E, CD2, CD28, CD3D, CD3E, CD3G, CD3Z, CD44, CD47, CD59, CD6, CD63, CD8A, CD8B1, CD99, CDC10, CDCl4B, CDH11, CDH2, CDKL5, CDKN2A, CDW52, CECR1, CENPB, CENTB1, CENTG2, CEP1, CG018, CHRNA3, CHS1, CIAPIN1, CKAP4, CKIP-1, CNP, COL4A1, COL5A2, COL6A1, CORO1C, CRABP1, CRK, CRY1, CSDA, CTBP1, CTSC, CTSI, CUGBP2, CUTC, CXCL1, CXCR4, CXorf9, CYFIP2, CYLD, CYR61, DATF1, DAZAP1, DBN1, DBT, DCTN1, DDX18, DDX5, DGKA, DIAPH1, DKC1, DKFZP434J154, DKFZP564C186, DKFZP564G2022, DKFZp564J157, DKFZP564K0822, DNAJC10, DNAJC7, DNAPTP6, DOCK10, DOCK2, DPAGT1, DPEP2, DPYSL3, DSIFI, DUSP1, DXS9879E, EEF1B2, EFNB2, EHD2, EIF5A, ELK3, ENO2, EPAS1, EPB41L4B, ERCC2, ERG, ERP70, EVER1, EVI2A, EVL, EXT1, EZH2, F2R, FABP5, FAD104, FAM46A, FAU,

FCGR2A, FCGR2C, FER1L3, FHL1, FHOD1, FKBP1A, FKBP9, FLJ10350, FLJ10539, FLJ10774, FLJ12270, FLJ13373, FLJ20859, FLJ21159, FLJ22457, FLJ35036, FLJ46603, FLNC, FLOT1, FMNL1, FNBP1, FOLH1, FOXF2, FSCN1, FTL, FYB, FYN, GOS2, G6PD, GALIG, GALNT6, GATA2, GATA3, GFPT1, GIMAP5, GIT2, GJA1, GLRB, GLTSCR2, GLUL, GMDS, GNAQ, GNB2, GNB5, GOT2, GPR65, GPRASP1, GPSM3, GRP58, GSTM2, GTF3A, GTSE1, GZMA, GZMB, H1F0, H1FX, H2AFX, H3F3A, HA-1, HEXB, HIC, HIST1H4C, HK1, HLA-A, HLA-B, HLA-DRA, HMGA1, HMGN2, HMMR, HNRPA1, HNRPD, HNRPM, HOXA9, HRMT1L1, HSA9761, HSPA5, HSU79274, HTATSF1, ICAM1, ICAM2, IER3, IFI16, IFI44, IFITM2, IFITM3, IFRG28, IGFBP2, IGSF4, IL13RA2, IL21R, IL2RG, IL4R, IL6, IL6R, IL6ST, IL8, IMPDH2, INPP5D, INSIG1, IQGAP1, IQGAP2, IRS2, ITGA5, ITM2A, JARID2, JUNB, K-ALPHA-1, KHDRBS1, KIAA0355, KIAA0802, KIAA0877, KIAA0922, KIAA1078, KIAA1128, KIAA1393, KIFC1, LAIR1, LAMB1, LAMB3, LAT, LBR, LCK, LCP1, LCP2, LEF1, LEPRE1, LGALS1, LGALS9, LHFPL2, LNK, LOC54103, LOC55831, LOC81558, LOC94105, LONP, LOX, LOXL2, LPHN2, LPXN, LRMP, LRP12, LRRK5, LRRN3, LST1, LTB, LUM, LY9, LY96, MAGEB2, MAL, MAP1B, MAP1LC3B, MAP4K1, MAPK1, MARCKS, MAZ, MCAM, MCL1, MCM5, MCM7, MDH2, MDN1, MEF2C, MFNG, MGC17330, MGC21654, MGC2744, MGC4083, MGC8721, MGC8902, MGLL, MLPH, MPHOSPH6, MPPI, MPZL1, MRP63, MRPS2, MT1E, MT1K, MUF1, MVP, MYB, MYL9, MYO1B, NAP1L1, NAP1L2, NARF, NASP, NCOR2, NDN, NDUFAB1, NDUFAS6, NFKBIA, NID2, NIPA2, NME4, NME7, NNMT, NOL5A, NOL8, NOMO2, NOTCH1, NPC1, NQO1, NR1D2, NUDC, NUP210, NUP88, NVL, NXF1, OBFC1, OCRL, OGT, OXA1L, P2RX5, P4HA1, PACAP, PAF53, PAAH1B3, PALM2-AKAP2, PAX6, PCBP2, PCCB, PFDN5, PFN1, PFN2, PGAM1, PHEMX, PHlda1, PIM2, PTPN1C, PLAC8, PLAGL1, PLAUR, PLCB1, PLEK2, PLEKHC1, PLOD2, PLSCR1, PNAS-4, PNMA2, POLR2F, PPAP2B, PRF1, PRG1, PRIM1, PRKCH, PRKCQ, PRKD2, PRNP, PRP19, PRPF8, PRSS23, PSCDBP, PSMB9, PSMC3, PSME2, PTGER4, PTGES2, PTOV1, PTP4A3, PTPN7, PTPNS1, PTRF, PURA, PWP1, PYGL, QKI, RAB3GAP, RAB7L1, RAB9P40, RAC2, RAFTLIN, RAG2, RAP1B, RASGRP2, RBPM5, RCN1, RFC3, RFC5, RGC32, RGS3, RHOH, RIMS3, RIOK3, RIPK2, RIS1, RNASE6, RNF144, RPL10, RPL10A, RPL12, RPL13A, RPL17, RPL18, RPL36A, RPLP0, RPLP2, RPS15, RPS19, RPS2, RPS4X, RPS4Y1, RRAS, RRAS2, RRBPI, RRM2, RUNX1, RUNX3, S100A4, SART3, SATB1, SCAP1, SCARB1, SCN3A, SEC31L2, SEC61G, SELL, SELPLG, SEMA4G, SEPT10, SEPT6, SERPINA1, SERPINB1, SERPINB6, SFRS5, SFRS6, SFRS7, SH2D1A, SH3GL3, SH3TC1, SHD1, SHMT2, SIAT1, SKB1, SKP2, SLA, SLC1A4, SLC20A1, SLC25A15, SLC25A5, SLC39A14, SLC39A6, SLC43A3, SLC4A2, SLC7A11, SLC7A6, SMAD3, SMOX, SNRPA, SNRPB, SOD2, SOX4, SP140, SPANXC, SPI1, SRF, SRM, SSA2, SSBP2, SSRP1, SSSCA1, STAG3, STAT1, STAT4, STAT5A, STC1, STC2, STOML2, T3JAM, TACC1, TACC3, TAF5, TAL1, TAP1, TARP, TBKA, TCF12, TCF4, TFDP2, TFPI, TIMM17A, TIMP1, TJP1, TK2, TM4SF1, TM4SF2, TM4SF8, TM6SF1, TMEM2, TMEM22, TMSB10, TMSNB, TNFAIP3, TNFAIP8, TNFRSF10B, TNFRSF1A, TNFRSF7, TNIK, TNPO1,

TOB1, TOMM20, TOX, TPK1, TPM2, TRA@, TRA1, TRAM2, TRB@, TRD@, TRIM, TRIM14, TRIM22, TRIM28, TRIP13, TRPV2, TUBGCP3, TUSC3, TXN, TXNDC5, UBASH3A, UBE2A, UBE2L6, UBE2S, UCHL1, UCK2, UCP2, UFD1L, UGDH, ULK2, UMPS, UNG, USP34, USP4, VASP, VAV1, VLDDL, VWF, WASPIP, WBSCR20A, WBSCR20C, WHSC1, WNT5A, ZAP70, ZFP36L1, ZNF32, ZNF335, ZNF593, ZNFN1A1, and ZYX, wherein said single-stranded nucleic acid molecule allows detection of a level of expression of said gene when said single-stranded nucleic acid molecule is contacted with a nucleic acid molecule expressed from said gene, or its complement, under conditions allowing hybridization to occur between said single-stranded nucleic acid molecule and said nucleic acid molecule expressed from said gene, said kit further comprising instructions for applying nucleic acids collected from a sample from a cancer patient, instructions for measuring the level of expression of said gene, and instructions for determining said cell's sensitivity to a treatment for cancer.

138-204. (canceled)

205. A kit comprising a single-stranded nucleic acid molecule that is substantially complementary to or substantially identical to at least 5 consecutive nucleotides of at least one microRNA selected from the group consisting of ath-MIR180aNo2, Hcd102 left, Hcd111 left, Hcd115 left, Hcd120 left, Hcd142 right, Hcd145 left, Hcd148_HPR225 left, Hcd181 left, Hcd181 right, Hcd210_HPR205 right, Hcd213_HPR182 left, Hcd230 left, Hcd243 right, Hcd246 right, Hcd248 right, Hcd249 right, Hcd250 left, Hcd255 left, Hcd257 left, Hcd257 right, Hcd263 left, Hcd266 left, Hcd270 right, Hcd279 left, Hcd279 right, Hcd28_HPR39left, Hcd28_HPR39right, Hcd282PO right, Hcd289 left, Hcd294 left, Hcd318 right, Hcd323 left, Hcd330 right, Hcd338 left, Hcd340 left, Hcd350 right, Hcd355_HPR190 left, Hcd361 right, Hcd366 left, Hcd373 right, Hcd383 left, Hcd383 right, Hcd384 left, Hcd397 left, Hcd404 left, Hcd412 left, Hcd413 right, Hcd415 right, Hcd417 right, Hcd421 right, Hcd425 left, Hcd438right, Hcd434 right, Hcd438 left, Hcd440_HPR257 right, Hcd444 right, Hcd447 right, Hcd448 left, Hcd498 right, Hcd503 left, Hcd511 right, Hcd512 left, Hcd514 right, Hcd517 left, Hcd517 right, Hcd530 right, Hcd536_HPR104 right, Hcd542 left, Hcd544 left, Hcd547 left, Hcd559 right, Hcd562 right, Hcd569 right, Hcd570 right, Hcd578 right, Hcd581 right, Hcd586 left, Hcd586 right, Hcd587 right, Hcd605 left, Hcd605 left, Hcd605 right, Hcd608 right, Hcd627left, Hcd631 left, Hcd631 right, Hcd634 left, Hcd642 right, Hcd649 right, Hcd654 left, Hcd658 right, Hcd669 right, Hcd674 left, Hcd678 right, Hcd683 left, Hcd684 right, Hcd689 right, Hcd690 right, Hcd691 right, Hcd693 right, Hcd697 right, Hcd704 left, Hcd704 left, Hcd712 right, Hcd716 right, Hcd731 left, Hcd738 left, Hcd739 right, Hcd739 right, Hcd749 right, Hcd753 left, Hcd754 left, Hcd755 left, Hcd760 left, Hcd763 right, Hcd768 left, Hcd768 right, Hcd770 left, Hcd773 left, Hcd777 left, Hcd778 right, Hcd781 left, Hcd781 right, Hcd782 left, Hcd783 left, Hcd788 left, Hcd794 right, Hcd796 left, Hcd799 left, Hcd807 right, Hcd812 left, Hcd817 left, Hcd817 right, Hcd829 right, Hcd852 right, Hcd861 right, Hcd863PO right, Hcd866 right, Hcd869 left, Hcd873 left, Hcd886 right, Hcd889 right, Hcd891 right, Hcd892 left, Hcd913 right, Hcd923 left, Hcd923 right, Hcd938 left, Hcd938 right, Hcd939 right, Hcd946left, Hcd948 right, Hcd960 left, Hcd965 left, Hcd970 left, Hcd975 left, Hcd976 right, Hcd99 right, HPR100 right,

HPR129 left, HPR154 left, HPR159 left, HPR163 left, HPR169 right, HPR172 right, HPR181 left, HPR187 left, HPR199 right, HPR206 left, HPR213 right, HPR214 right, HPR220 left, HPR220 right, HPR227 right, HPR232 right, HPR233 right, HPR244 right, HPR262 left, HPR264 right, HPR266 right, HPR271 right, HPR76 right, hsa_mir_490_Hcd20 right, HSHELA01, HSTRNL, HUMTRAB, HUMTRF, HUMTRN, HUMTRS, HUMTRV1A, let-7f-2-prec2, mir-001b-1-prec1, mir-001b-2-prec, mir-007-1-prec, mir-007-2-precNo2, mir-010a-precNo1, mir-015b-precNo2, mir-016a-chr13, mir-016b-chr3, mir-017-precNo1, mir-017-precNo2, mir-018-prec, mir-019a-prec, mir-019b-1-prec, mir-019b-2-prec, mir-020-prec, mir-022-prec, mir-023a-prec, mir-023b-prec, mir-024-2-prec, mir-025-prec, mir-027b-prec, mir-029c-prec, mir-032-precNo2, mir-033b-prec, mir-033-prec, mir-034-precNo1, mir-034-precNo2, mir-092-prec-13=092-1No2, mir-092-prec-X=092-2, mir-093-prec-7.1=093-1, mir-095-prec-4, mir-096-prec-7No1, mir-096-prec-7No2, mir-098-prec-X, mir-099b-prec-19No1, mir-100-1/2-prec, mir-100No1, mir-101-prec-9, mir-102-prec-1, mir-103-2-prec, mir-103-prec-5=103-1, mir-106aNo1, mir-106-prec-X, mir-107No1, mir-107-prec-10, mir-122a-prec, mir-123-precNo1, mir-123-precNo2, mir-124a-1-prec1, mir-124a-2-prec, mir-124a-3-prec, mir-125b-1, mir-125b-2-precNo2, mir-127-prec, mir-128b-precNo1, mir-128b-precNo2, mir-133a-1, mir-135-2-prec, mir-136-precNo2, mir-138-1-prec, mir-140No2, mir-142-prec, mir-143-prec, mir-144-precNo2, mir-145-prec, mir-146bNo1, mir-146-prec, mir-147-prec, mir-148aNo1, mir-148-prec, mir-149-prec, mir-150-prec, mir-153-1-prec1, mir-154-prec1No1, mir-155-prec, mir-15aNo1, mir-16-1No1, mir-16-2No1, mir-181a-precNo1, mir-181b-1No1, mir-181b-2No1, mir-181b-precNo1, mir-181b-precNo2, mir-181c-precNo1, mir-181dNo1, mir-188-prec, mir-18bNo2, mir-191-prec, mir-192No2, mir-193bNo2, mir-194-2No1, mir-195-prec, mir-196-2-precNo2, mir-197-prec, mir-198-prec, mir-199a-1-prec, mir-199a-2-prec, mir-199b-precNo1, mir-200a-prec, mir-200bNo1, mir-200bNo2, mir-202*, mir-202-prec, mir-204-precNo2, mir-205-prec, mir-208-prec, mir-20bNo1, mir-2,2-precNo1, mir-2,2-precNo2, mir-2,3-precNo1, mir-2,4-prec, mir-2,5-precNo2, mir-2,6-precNo1, mir-219-2No1, mir-2,9-prec, mir-223-prec, mir-29b-1No1, mir-29b-2=102prec7.1=7.2, mir-321No1, mir-321No2, mir-324No1, mir-324No2, mir-328No1, mir-342No1, mir-361No1, mir-367No1, mir-370No1, mir-371No1, miR-373*No1, mir-375, mir-376aNo1, mir-379No1, mir-380-5p, mir-382, mir-384, mir-409-3p, mir-423No1, mir-424No2, mir-429No1, mir-429No2, mir-4323p, mir-4325p, mir-449No1, mir-450-1, mir-450-2No1, mir-483No1, mir-484, mir-487No1, mir-495No1, mir-499No2, mir-501No2, mir-503No1, mir-509No1, mir-514-1No2, mir-515-15p, mir-515-23p, mir-516-33p, mir-516-43p, mir-518e/526c, mir-519a-1/52, mir-519a-2No2, mir-519b, mir-519c/52, mir-520c/52, mir-526a-2No1, mir-526a-2No2, MPR103 right, MPR121 left,

MPR121 left, MPR130 left, MPR130 right, MPR133 right, MPR141 left, MPR151 left, MPR156 left, MPR162 left, MPR174 left, MPR174 right, MPR185 right, MPR197 right, MPR203 left, MPR207 right, MPR215 left, MPR216 left, MPR224 left, MPR224 right, MPR228 left, MPR234 right, MPR237 left, MPR243 left, MPR244 right, MPR249 left, MPR254 right, MPR74 left, MPR88 right, and MPR95 left, wherein said single-stranded nucleic acid molecule allows detection of a level of expression of said microRNA when said single-stranded nucleic acid molecule is contacted with said microRNA, or its complement, under conditions allowing hybridization to occur between said single-stranded nucleic acid molecule and said microRNA, said kit further comprising instructions for applying nucleic acids collected from a sample from a cancer patient, instructions for measuring the level of expression of said microRNA, and instructions for determining said cell's sensitivity to a treatment for cancer.

206-269. (canceled)

270. A method of identifying biomarkers useful for the determination of sensitivity of a cancer patient to a treatment for cancer comprising:

- a. obtaining pluralities of measurements of the level of expression of a gene or microRNA in different cell types and measurements of the growth of said cell types in the presence of a treatment for cancer relative to the growth of said cell types in the absence of said treatment for cancer;
- b. correlating each plurality of measurements of the level of expression of said gene or microRNA in said cells with the growth of said cells to obtain a correlation coefficient;
- c. selecting the median correlation coefficient calculated for said gene or microRNA; and
- d. identifying said gene or microRNA as a biomarker for use in determining the sensitivity of a cancer patient to said treatment for cancer if said median correlation coefficient exceeds 0.3.

271-280. (canceled)

281. A method of determining sensitivity of a cancer patient to a treatment for cancer comprising:

- a. obtaining a measurement of the level of expression of a gene or microRNA in a sample from said cancer patient;
- b. applying a model predictive of sensitivity to a treatment for cancer to said measurement, wherein said model is developed using an algorithm selected from the group consisting of linear sums, nearest neighbor, nearest centroid, linear discriminant analysis, support vector machines, and neural networks; and
- c. predicting whether or not said cancer patient will be responsive to said treatment for cancer.

282-301. (canceled)

* * * * *

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
1 December 2005 (01.12.2005)

PCT

(10) International Publication Number
WO 2005/114187 A2

- (51) International Patent Classification⁷: **G01N 33/53**
- (21) International Application Number:
PCT/US2005/013554
- (22) International Filing Date: 21 April 2005 (21.04.2005)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/564,588 23 April 2004 (23.04.2004) US
60/634,255 9 December 2004 (09.12.2004) US
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/114187 A2

(54) Title: METHODS AND COMPOSITIONS FOR DIAGNOSING AIDS AND OTHER DISEASES AND CONDITIONS INVOLVING IMMUNE SYSTEM ACTIVATION

(57) Abstract: This invention relates to methods and compositions suitable for monitoring the progression of AIDS, and other diseases whose progression involves immune system activation. In particular, the invention relates to the use of the TNF-Related Apoptosis-Inducing Ligand (TRAIL) and TRAIL Compounds to monitor the progression of AIDS, and other immune system diseases.

Title of the Invention:

Methods and Compositions For Diagnosing AIDS And Other Diseases And Conditions Involving Immune System Activation

5 Field of the Invention:

This invention relates to methods and compositions suitable for monitoring the progression of AIDS, and other diseases whose progression involves immune system activation. In particular, the invention relates to the use of the TNF-Related Apoptosis-Inducing Ligand (TRAIL) and TRAIL Compounds to monitor the 10 progression of AIDS, and such other diseases.

Cross-Reference to Related Applications:

This application claims priority to United States Patent Application Serial No. 60/564,588, filed April 23, 2004 and United States Patent Application Serial No. 60/634,255, filed December 9, 2004, which applications are herein 15 incorporated by reference in their entirety.

Statement of Governmental Interest:

This invention was funded by the National Cancer Institute at the National Institutes of Health. The United States Government has certain rights to this invention.

20 Background of the Invention:

Diseases and Conditions Involving Immune System Activation

A number of diseases and conditions are characterized by a progression that involves immune system activation. These diseases include Acquired 5 Immunodeficiency Syndrome (AIDS), allergic asthma, Alzheimer's disease, 15 autoimmune disorders, Crohn's disease, Grave's disease, leukemia, atherosclerosis, lupus, multiple sclerosis, Parkinson's disease, transplant rejection,

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etc. (Ringheim, G.E. *et al.* (2004) "NEURODEGENERATIVE DISEASE AND THE NEUROIMMUNE AXIS (ALZHEIMER'S AND PARKINSON'S DISEASE, AND VIRAL INFECTIONS)," *J. Neuroimmunol.* 147(1-2):43-49; Corry, D.B. *et al.* (2002) "BIOLOGY AND THERAPEUTIC POTENTIAL OF THE INTERLEUKIN-4/INTERLEUKIN-13 SIGNALING PATHWAY IN ASTHMA," *Am. J. Respir. Med.* 1(3):185-193; Fischer, A. (2004) "HUMAN PRIMARY IMMUNODEFICIENCY DISEASES: A PERSPECTIVE," *Nat Immunol.* 5(1):23-30; Franchini, G. *et al.* (2003) "T-CELL CONTROL BY HUMAN T-CELL LEUKEMIA/LYMPHOMA VIRUS TYPE 1," *Int J Hematol.* 78(4):280-296; Sandborn, W.J. *et al.* (2003) "NOVEL APPROACHES TO TREATING INFLAMMATORY BOWEL DISEASE: TARGETING ALPHA-4 INTEGRIN," *Am J Gastroenterol.* 98(11):2372-2382; Chitnis, T. *et al.* (2003) "ROLE OF COSTIMULATORY PATHWAYS IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS AND EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS," *J. Allergy Clin. Immunol.* 112(5):837-850; Aw, M.M. (2003) "TRANSPLANT IMMUNOLOGY," *J. Pediatr. Surg.* 38(9):1275-80; Zhou, X. (2003) "CD4+ T CELLS IN ATHEROSCLEROSIS," *Biomed Pharmacother.* 57(7):287-291).

The causal agent of AIDS has been found to be the retrovirus: Human Immunodeficiency Virus (HIV) (Wood, R.W. *et al.* (1987) "ACQUIRED IMMUNODEFICIENCY SYNDROME," *Infect Dis Clin North Am.* (1): 145-63; Gallo, R.C. *et al.* (2003) "THE DISCOVERY OF HIV AS THE CAUSE OF AIDS," *N Engl J Med.* 349(24): 2283-5; Holtgrave, D.R. (2004) "ESTIMATION OF ANNUAL HIV TRANSMISSION RATES IN THE UNITED STATES, 1978-2000," *J Acquir Immune Defic Syndr.* 35(1):89-92; Ebbesen, P.(1986) "THE GLOBAL EPIDEMIC OF AIDS," *AIDS Res. (2 Suppl)* 1: S23-8; Gallo, R.C.(1987) "THE AIDS VIRUS," *Sci Am.* 256(1):46-56; Morrow, C.D. *et al.* (1994) "VIRAL GENE PRODUCTS AND REPLICATION OF THE HUMAN IMMUNODEFICIENCY TYPE 1 VIRUS," *Am J Physiol.* 266(5 Pt 1): C1135-56).

Infection with HIV is followed by a latency period, during which a progressive and slow destruction of the immune system occurs (Hessol, N.A. *et al.* (1990) "RELATIONSHIP BETWEEN AIDS LATENCY PERIOD AND AIDS SURVIVAL

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IMMUNODEFICIENCY VIRUS-INFECTED ASYMPTOMATIC INDIVIDUALS" J Exp Med 175, 331-40; Ameisen, J. C. *et al.* (1991) "CELL DYSFUNCTION AND DEPLETION IN AIDS: THE PROGRAMMED CELL DEATH HYPOTHESIS," Immunol Today 12, 102-5). However, both CD4⁺ and CD8⁺ T cells are activated by HIV-1 infection, and none of the current models adequately accounts for the preferential loss of CD4⁺ T cells (Groux, H. *et al.* (1992) "ACTIVATION-INDUCED DEATH BY APOPTOSIS IN CD4⁺ T CELLS FROM HUMAN IMMUNODEFICIENCY VIRUS-INFECTED ASYMPTOMATIC INDIVIDUALS," J Exp Med 175, 331-40; Grossman, Z. *et al.* (2002) "CD4⁺ T-CELL DEPLETION IN HIV INFECTION: ARE WE CLOSER TO UNDERSTANDING THE CAUSE?" 10 Nat Med 8, 319-23).

AICD initiated by a variety of stimuli has been proposed as a potential mechanism for "bystander" cell killing. Among these stimuli, noninfectious viruses, which represent more than 95% of HIV-1 particles in plasma of patients, were shown to induce apoptosis of uninfected CD4⁺ and CD8⁺ T cells (Dimitrov, D. S. *et al.* (1993) "QUANTITATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION KINETICS," J Virol 67, 2182-90; Piatak, M., Jr. *et al.* (1993) "HIGH LEVELS OF HIV-1 IN PLASMA DURING ALL STAGES OF INFECTION DETERMINED BY COMPETITIVE PCR," Science 259, 1749-54; Esser, M.T. *et al.* (2001) "PARTIAL ACTIVATION AND INDUCTION OF APOPTOSIS IN CD4(+) AND CD8(+) T 15 LYMPHOCYTES BY CONFORMATIONALLY AUTHENTIC NONINFECTIOUS HUMAN IMMUNODEFICIENCY VIRUS TYPE 1," J Virol 75, 1152-64; Esser, M.T. *et al.* (2001) "DIFFERENTIAL INCORPORATION OF CD45, CD80 (B7-1), CD86 (B7-2), AND 20 MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I AND II MOLECULES INTO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 VIRIONS AND MICROVESICLES: IMPLICATIONS FOR VIRAL PATHOGENESIS AND IMMUNE REGULATION," J. VIROL. 75(13):6173- 25 6182).

Unfortunately, the complexity of AIDS has encumbered efforts to monitor the progression of the disease. The timing of the onset of clinical AIDS is difficult to predict because the clinical manifestations of HIV infection are variable (Stein, H. *et al.* (1991) "LYMPHOID TISSUES AND AIDS: ROLE OF LYMPHOCYTES AND 30

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TIME IN HOMOSEXUAL AND BISEXUAL MEN," J Acquir Immune Defic Syndr. 3(11):1078-85; Berkelman, R.L. *et al.* (1989) "EPIDEMIOLOGY OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION AND ACQUIRED IMMUNODEFICIENCY SYNDROME," Am J Med. 86(6 Pt 2):761-70). The latency period is characterized

- 5 by a decrease in the number of helper T cells, by qualitative defects in T cell function and by a relative decrease in the rate of virus replication.

The most characteristic feature of AIDS is the selective depletion of CD4⁺ cells. These immune system cells are the targets of HIV infection, and are destroyed by the HIV virus (Lang, W.H. *et al.* (1989) "PATTERNS OF T-

- 10 LYMPHOCYTES CHANGES WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION FROM SEROCONVERSION TO THE DEVELOPMENT OF AIDS," J. AIDS 2:63-69; Janeway CA, *et al.* (2001) "IMMUNOBIOLOGY," Ch.11, 5th ed. New York and London: Garland Publishing; Ghani, A.C. *et al.* (2001) "SURROGATE MARKERS FOR DISEASE PROGRESSION IN TREATED HIV INFECTION," J AIDS 28(2):226-231).

- 15 Significantly, the pathogenic mechanisms responsible for the extensive depletion of CD4⁺ T cells, that is a characteristic of AIDS, are not well understood. Although HIV-1-infected cells die, the frequency of infected cells is too low to account for the depletion of CD4⁺ T cells (Schnittman, S. M. *et al.* (1990) "INCREASING VIRAL BURDEN IN CD4+ T CELLS FROM PATIENTS WITH HUMAN
- 20 IMMUNODEFICIENCY VIRUS (HIV) INFECTION REFLECTS RAPIDLY PROGRESSIVE IMMUNOSUPPRESSION AND CLINICAL DISEASE," Ann Intern Med 113, 438-43; Grivel, J. C. *et al.* (2000) "HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INDUCES APOPTOSIS IN CD4(+) BUT NOT IN CD8(+) T CELLS IN EX VIVO-INFECTED HUMAN LYMPHOID TISSUE," J Virol 74, 8077-84). In order to address this disparity, 25 indirect mechanisms involving the death of uninfected CD4⁺ T cells have been proposed (Badley, A. D. *et al.* (2000) "MECHANISMS OF HIV-ASSOCIATED LYMPHOCYTE APOPTOSIS," Blood 96, 2951-64). Several reports have suggested that the Activation-Induced Cell Death ("AICD") of T lymphocytes may be a major contributor to CD4⁺ T cell depletion (Groux, H. *et al.* (1992) "ACTIVATION-30 INDUCED DEATH BY APOPTOSIS IN CD4+ T CELLS FROM HUMAN

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FOLLICULAR DENDRITIC CELLS (FDC)" Verh Dtsch Ges Pathol. 75:4-19; Caetano, J.A. (1991) "IMMUNOLOGIC ASPECTS OF HIV INFECTION," Acta Med Port. 4 Suppl 1:52S-58S; Reyes-Teran, G. *et al.* (1994) "PATHOGENESIS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION," Rev Invest Clin. 1994 46(2):113-47).

5 Additionally, since there are no clinically detectable characteristics of the disease (i.e., disease "markers") that can be universally employed to monitor the progression of the infection, disease progression must be monitored using "surrogate" markers that rely on characteristics that only generally correlate with the disease (see, Tsoukas, C.M. *et al.* (1994) "MARKERS PREDICTING PROGRESSION
10 OF HUMAN IMMUNODEFICIENCY VIRUS-RELATED DISEASE," Clin Microbiol Rev 7(1):14-28). A surrogate marker should, ideally, identify patients at highest risk of disease progression, provide information on how an individual was infected, help clinicians accurately determine the stage of the HIV infection, predict development of opportunistic infections associated with the infection, and monitor the efficacy
15 of antiviral treatments. In addition, surrogate markers should be easily quantifiable, reliable, chemically available, and affordable. With respect to HIV, surrogate markers are measurable traits that correlate with disease progression from symptomless HIV infection to full blown clinical AIDS (Tsoukas, C.M. *et al.* (1994) "MARKERS PREDICTING PROGRESSION OF HUMAN IMMUNODEFICIENCY
20 VIRUS-RELATED DISEASE," Clin Microbiol Rev 7(1):14-28).

Prognostic or surrogate markers of HIV-1 disease progression that have been described include viral load, CD4⁺ T cell number, soluble immune system activation markers and T helper cell functional profiles (Ho, D. D. (1996) "VIRAL COUNTS COUNT IN HIV INFECTION," Science 272:1124-1125; Mellors, J.W. *et al.* (1996) "PROGNOSIS IN HIV-1 INFECTION PREDICTED BY THE QUANTITY OF VIRUS
25 IN PLASMA," Science 272 1167-1170). The number of HIV-1 particles in infected patient blood has been reported to be the best available surrogate marker of HIV-1 disease progression (Ho, D. D. (1996) "VIRAL COUNTS COUNT IN HIV INFECTION," Science 272:1124-1125; Mellors, J.W. *et al.* (1996) "PROGNOSIS IN HIV-1
30 INFECTION PREDICTED BY THE QUANTITY OF VIRUS IN PLASMA," Science 272:

- 6 -

1167-1170). Additional prognostic surrogate markers of HIV-1 disease progression would be valuable, particularly those that investigate pathogenic mechanisms which result in CD4⁺ T cell depletion.

- At present, only two surrogate markers are recognized by the United States
- 5 Food & Drug Administration as clinically relevant to HIV progression: (1) the amount of viral RNA (i.e., the HIV-1 viral load) and (2) the absolute number of peripheral CD4⁺ T cells (i.e., the CD4⁺ T cell count) (see, Tsoukas, C.M. *et al.* (1994) "MARKERS PREDICTING PROGRESSION OF HUMAN IMMUNODEFICIENCY VIRUS-RELATED DISEASE," Clin Microbiol Rev 7(1):14-28; De Milito, A. *et al.*
- 10 (2003) "SURROGATE MARKERS AS A GUIDE TO EVALUATE RESPONSE TO ANTIRETROVIRAL THERAPY," Curr. Med. Chem. 10(5):349-365; Sondras, D. (1997) "IT'S TIME TO APPROVE MORE SURROGATE MARKERS," AIDS Treatment News: Issue # 267; Ghani, A.C. *et al.* (2001) "SURROGATE MARKERS FOR DISEASE PROGRESSION IN TREATED HIV INFECTION," JAIDS 28(2):226-231; Mellors, J.W.
- 15 *et al.* (1997) "PLASMA VIRAL LOAD AND CD4⁺ LYMPHOCYTES AS PROGNOSTIC MARKERS OF HIV-1 INFECTION," Ann Intern Med. 126(12):946-54).

- The total amount of HIV virus present in a patient at a given time, i.e., the "viral load," is a particularly widely employed HIV surrogate marker (Mellors, J. *et al.* (1996) "PROGNOSIS IN HIV-1 INFECTION PREDICTED BY THE QUANTITY OF
- 20 VIRUS IN PLASMA," Science 272: 1167-1170; Baker, R. (1996) "HIV VIRAL LOAD SUPERCEDES CD4 COUNT AS BEST MARKER FOR PREDICTING RISK OF AIDS AND DEATH," Bulletin of Experimental Treatments for AIDS; Pereira, R.S. (1997) "VIRAL LOAD TESTING: WHICH TEST FOR WHAT? BHIVA GUIDELINES COORDINATING COMMITTEE. BRITISH HIV ASSOCIATION GUIDELINES FOR ANTI-
- 25 RETROVIRAL TREATMENT OF HIV-SEROPOSITIVE INDIVIDUALS." Lancet 349: 1086-1092). Thus, to confirm an acute HIV infection in symptomatic individuals with potential HIV risk factors, current United States guidelines recommend use of HIV RNA (viral load) tests (U.S. Public Health Service, (August 13, 2001) "GUIDELINES FOR THE USE OF ANTIRETROVIRAL AGENTS IN HIV-INFECTED
- 30 ADULTS AND ADOLESCENTS").

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Unfortunately, however, present methods for assessing viral load, typically involve the use of the polymerase chain reaction (PCR) to amplify HIV-specific gene sequences, and are in some ways unsatisfactory (Craddock, M. (1995) "HIV: SCIENCE BY PRESS CONFERENCE," Reappraising AIDS 3(5): 1-4; Peter, J.B. *et al.* 5 (2004) "MOLECULAR-BASED METHODS FOR QUANTIFYING HIV VIRAL LOAD," AIDS Patient Care STDS. 18(2):75-9; Johnson, C. "VIRAL LOAD AND THE PCR-WHY THEY CAN'T BE USED TO PROVE HIV INFECTION," Continuum vol. 6 (3), <http://www.virusmyth.net/aids/continuum/article8.htm>). In particular, PCR amplification is highly susceptible to cross-contamination; a drawback that has 10 limited the clinical use of PCR in monitoring HIV infection (Schnittman, S.M. *et al* (1991) "FREQUENT DETECTION OF HIV-1-SPECIFIC mRNAs IN INFECTED INDIVIDUALS SUGGESTS ONGOING ACTIVE VIRAL EXPRESSION IN ALL STAGES OF DISEASE," AIDS Res Hum Retroviruses 7(4):361-7; Blattner, W.A. (1989) "Retroviruses," pp 545-592. In Viral Infections in Humans, third edition, edited by 15 A Evans. Plenum Medical Book Company, New York). Additionally, viral culture methods are complicated and susceptible to contamination (Bootman, J.S. *et al.* (1992) "AN INTERNATIONAL COLLABORATIVE STUDY TO ASSESS A SET OF REFERENCE REAGENTS FOR HIV-1 PCR," J. Vir. Meth. 37:23; Teo, I.A. *et al.* (1995) "PCR IN SITU: ASPECTS WHICH REDUCE AMPLIFICATION AND GENERATE 20 FALSE-POSITIVE RESULTS," Histochem. J. 27:660). While such methods quantitate overall viral production in a patient, they do not enumerate the number of patient cells infected (Eleopulos, E. *et al.* (1994) "IS HIV REALLY HIDING IN THE LYMPH NODES?" Letter to Nature; Sloand, E. *et al.* (1991) "HIV TESTING: STATE OF THE ART," JAMA 266:2861).

25 Viral load testing has helped to define the natural history and prognosis of cohorts of people with HIV infection (Rhame, F.S. *et al.* (1989) "THE CASE FOR WIDER USE OF TESTING FOR HIV INFECTION," N Engl J Med; 320:1248-1254; Carpenter, C.C. *et al.* (1997) "ANTIRETROVIRAL THERAPY FOR HIV INFECTION IN 1997: UPDATED RECOMMENDATION OF THE INTERNATIONAL AIDS SOCIETY - USA 30 PANEL," JAMA; 278:1962-1969; Panel on Clinical Practices for Treatment of HIV Infection. (November 5, 1997) "GUIDELINES FOR THE USE OF ANTIRETROVIRAL

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AGENTS IN HIV-INFECTED ADULTS AND ADOLESCENTS," DHHS and Henry J. Kaiser Family Foundation; Hammer, S.M. *et al.* (1997) "A CONTROLLED TRIAL OF TWO NUCLEOSIDE ANALOGUES PLUS INDINAVIR IN PERSONS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION AND CD4 CELL COUNTS OF 200 PER CUBIC MILLILITER OR LESS," *N Engl J Med* 337:724-733). If viral load testing is not available, current treatment guidelines recommend testing for p24 antigen, a viral protein. In either case, the diagnosis is to be confirmed by antibody testing.

Testing serum for antibodies to HIV with a standard ELISA is currently the most common, cost-effective, and accurate method of screening for HIV infection (Centers for Disease Control (1988) "UPDATE: SEROLOGIC TESTING FOR ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS," *MMWR* 36:833-840; Schwartz, J.S. *et al.* (1988) "HUMAN IMMUNODEFICIENCY VIRUS TEST EVALUATION, PERFORMANCE AND USE," *JAMA* 259:2574-2579; Burke, D.S. *et al.* (1988) "MEASUREMENT OF THE FALSE POSITIVE RATE IN A SCREENING PROGRAM FOR HUMAN IMMUNODEFICIENCY VIRUS INFECTIONS," *N Engl J Med* 319:961-964). Rapid serum HIV antibody tests, saliva- and urine- based antibody tests, and home HIV antibody testing kits have been approved by the Food and Drug Administration (FDA) and are being marketed (Bayer, R. *et al.* (1995) "SOUNDING BOARD: TESTING FOR HIV INFECTION AT HOME," *N Engl J Med* 332:1296-1299; Phillips, K.A. *et al.* (1995) "OCCASIONAL NOTES: POTENTIAL USE OF HOME HIV TESTING," *N Engl J Med* 332:1308-1310; Sax, P.E. *et al.* "NOVEL APPROACHES TO HIV ANTIBODY TESTING," *AIDS Clinical Care* 9:1-6). A variation of the standard antibody test, presently approved in the U.S. only for research, is the sensitive/less sensitive or "detuned" ELISA (Mirken, B. (2001) "HIV TESTING 101," AIDS Treatment News Issue #375 (Part 2 of 2)). The detuned test takes advantage of the fact that antibody levels rise in a predictable pattern during roughly the first four to six months after infection, eventually reaching a plateau that often stays roughly constant for many years.

In light of the problems attending to the use of viral load and CD4⁺ T cell count to diagnose AIDS, other HIV surrogate markers have been proposed. For

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- example, cellular markers like HLA-DR⁺, IL-2R⁺, and T cells; as well as soluble markers like beta-2 microglobulin (β_2 -M), neopterin, soluble Interleukin receptor (sIL-2R), soluble CD4 (sCD4), and soluble CD8 (sCD8) have been proposed (Tsoukas, C.M. *et al.* (1994) "MARKERS PREDICTING PROGRESSION OF HUMAN IMMUNODEFICIENCY VIRUS-RELATED DISEASE," Clin Microbiol Rev. 7(1):14-28).
- In addition, some biological markers like beta-2 microglobulin, neopterin, interferon, other cytokines and their receptors are being used as alternative markers (Anderson, R.E. *et al.* (1990) "LONGITUDINAL ANALYSIS OF LABORATORY MARKERS DURING HIV INFECTION," VI Intl. Conf. AIDS, San Francisco;
- Anderson, R.E. *et al.* (1990) "USE OF β_2 -MICROGLOBULIN LEVEL AND CD4 LYMPHOCYTE COUNT TO PREDICT DEVELOPMENT OF ACQUIRED IMMUNODEFICIENCY SYNDROME IN PERSONS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTIONS," Arch. Intern. Med 150:73-77).

Unfortunately, the use of known surrogate markers to evaluate HIV progression has a number of limitations. No known surrogate marker consistently reflects AIDS progression in all individuals. Additionally, there is a concern that by monitoring one surrogate marker (such as CD4⁺ titers), it is possible to overlook treatments that for some reason fail to affect the chosen marker but which would nevertheless be effective in treating the disease. For these reasons, the use of combinations of surrogate markers has been proposed. For example, a study performed to test the ability of cellular markers (i.e., the number of CD4⁺ T cells, the number of CD8⁺ T cells and the ratio of CD4⁺ T cells to CD8⁺ T cells) and serologic markers (i.e., serum levels of neopterin, beta 2-microglobulin, soluble interleukin-2 receptors, IgA, and HIV p24 antigen) to predict the progression of clinically acquired immunodeficiency syndrome (AIDS) revealed that the level of CD4⁺ T cells in combination with serum level of either neopterin or beta 2-microglobulin were most powerful predictors of progression to AIDS (Fahey, J.L. *et al.* (1990) "THE PROGNOSTIC VALUE OF CELLULAR AND SEROLOGIC MARKERS IN INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1," N Eng J Med 322(3):166-72). While the use of combinations of markers may address

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deficiencies associated with individual markers, the use of multiple assays is associated with a corresponding increase in assay complexity and potential error.

Although a marker must have a biologically relevant relationship to disease progression, the relationship cannot effectively be utilized in evaluating a treatment regimen unless the marker also responds quickly to effective treatment. For example, while the level of plasma HIV RNA and the absolute numbers of peripheral CD4⁺ T cells have become *de facto* reference markers, additional parameters are still needed in clinical practice in order to refine antiretroviral treatments and to discriminate adequately between apparently equivalent stages of disease (see, De Milito, A. *et al.* (2003) "SURROGATE MARKERS AS A GUIDE TO EVALUATE RESPONSE TO ANTIRETROVIRAL THERAPY," *Curr. Med. Chem.* 10 (5):349-365). To address these needs, prognostic markers of immune system activation that can be used to assess disease progression and which might be suitable for the monitoring and prognosis of antiretroviral therapies are desired (De Milito, A. *et al.* (2003) "SURROGATE MARKERS AS A GUIDE TO EVALUATE RESPONSE TO ANTIRETROVIRAL THERAPY," *Curr. Med. Chem.* 10 (5):349-365; Fahey, J.L. *et al.* (1990) "THE PROGNOSTIC VALUE OF CELLULAR AND SEROLOGIC MARKERS IN INFECTION WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1," *N Eng J Med* 322(3):166-72; Mellors, J.W. *et al.* (1998) "PLASMA VIRAL LOAD AND 20 CD4⁺ LYMPHOCYTES AS PROGNOSTIC MARKERS OF HIV-1 INFECTION," *Ann Intern Med.* 126(12):946-54; Cozzi Lepri, A. *et al.* (1998) "THE RELATIVE PROGNOSTIC VALUE OF PLASMA HIV RNA LEVELS AND CD4 LYMPHOCYTE COUNTS IN ADVANCED HIV INFECTION," *AIDS* 12(13):1639-43).

TNF-Related Apoptosis-Inducing Ligand (TRAIL)

25 TNF-Related Apoptosis-Inducing Ligand (TRAIL) is a member of the TNF superfamily. It induces apoptosis in human tumor cell lines and in virus-infected cells, but does not induce apoptosis in normal cells (Wiley, S. R. *et al.* (1995) "IDENTIFICATION AND CHARACTERIZATION OF A NEW MEMBER OF THE TNF FAMILY THAT INDUCES APOPTOSIS," *Immunity* 3, 673-682; Griffith, T. S., *et al.*

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(1998) "INTRACELLULAR REGULATION OF TRAIL-INDUCED APOPTOSIS IN HUMAN MELANOMA CELLS," *J Immunol* 161, 2833-28340; Herbeuval, J. P. *et al.* (2003) "MACROPHAGES FROM CANCER PATIENTS: ANALYSIS OF TRAIL, TRAIL RECEPTORS, AND COLON TUMOR CELL APOPTOSIS," *J Natl Cancer Inst* 95, 611-621; Jeremias, I. *et al.* (1998) "TRAIL/APO-2-LIGAND-INDUCED APOPTOSIS IN HUMAN T CELLS," *Eur J Immunol* 28, 143-152; Clarke, P. *et al.* (2003) "TWO DISTINCT PHASES OF VIRUS-INDUCED NF-KAPPAB-REGULATION ENHANCE TRAIL-MEDIATED APOPTOSIS IN VIRUS-INFECTED CELLS," *J Biol Chem*; Gura, T. (1997) "HOW TRAIL KILLS CANCER CELLS, BUT NOT NORMAL CELLS," *Science* 277:768). TRAIL has been implicated in apoptosis of multiple cell types in HIV-infected patients, including CD4⁺ and CD8⁺ T cells (Lum, J. J. *et al.* (2001) "INDUCTION OF CELL DEATH IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED MACROPHAGES AND RESTING MEMORY CD4 T CELLS BY TRAIL/APO21," *J Virol* 75, 11128-11136; Katsikis, P. D. *et al.* (1997) "INTERLEUKIN-1 BETA CONVERTING ENZYME-LIKE PROTEASE INVOLVEMENT IN FAS-INDUCED AND ACTIVATION-INDUCED PERIPHERAL BLOOD T CELL APOPTOSIS IN HIV INFECTION. TNF-RELATED APOPTOSIS-INDUCING LIGAND CAN MEDIATE ACTIVATION-INDUCED T CELL DEATH IN HIV INFECTION," *J Exp Med* 186, 1365-1372; Jeremias, I. *et al.* (1998) "TRAIL/APO-2-LIGAND-INDUCED APOPTOSIS IN HUMAN T CELLS," *Eur J Immunol* 28, 143-152).

The two biologically active forms of TRAIL, membrane-bound (mTRAIL) and soluble TRAIL (sTRAIL), are regulated by type I interferon (interferon-alpha and beta: IFN- α and IFN- β) (Sato, K. *et al.* (2001) "ANTIVIRAL RESPONSE BY NATURAL KILLER CELLS THROUGH TRAIL GENE INDUCTION BY IFN- α /IFN- β ," *Eur J Immunol*. 31:3138-3146). TRAIL can be secreted by leukocytes, including T lymphocytes, natural killer cells (Smyth, M.J. *et al.* (2001) "TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) CONTRIBUTES TO INTERFERON GAMMA-DEPENDENT NATURAL KILLER CELL PROTECTION FROM TUMOR METASTASIS," *J Exp Med*. 2001;193:661-670), dendritic cells (Vidalain, P.O. *et al.* (2000) "MEASLES VIRUS INDUCES FUNCTIONAL

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TRAIL PRODUCTION BY HUMAN DENDRITIC CELLS," J Virol. 74:556-559; Vidalain, P.O. *et al.* "MEASLE VIRUS-INFECTED DENDRITIC CELLS DEVELOP IMMUNOSUPPRESSIVE AND CYTOTOXIC ACTIVITIES," Immunobiology 204:629-638), monocytes and macrophages (Herbeauval, J.P. *et al.* (2003) "MACROPHAGES FROM CANCER PATIENTS: ANALYSIS OF TRAIL, TRAIL RECEPTORS, AND COLON TUMOR CELL APOPTOSIS," J Natl Cancer Inst. 95:611-621). However, the role of TRAIL in CD4+ T cell depletion in vivo during progression to AIDS remains to be established. Several reports have shown that macrophages and activated monocytes can play a role in CD4+ T cell depletion (Sperber, K. *et al.* (2003) "INDUCTION OF APOPTOSIS BY HIV-1-INFECTED MONOCYTIC CELLS," J Immunol. 70:1566-1578; Badley, A.D. *et al.* (1997) "MACROPHAGE-DEPENDENT APOPTOSIS OF CD4+ T LYMPHOCYTES FROM HIV-INFECTED INDIVIDUALS IS MEDIATED BY FASL AND TUMOR NECROSIS FACTOR. J Exp Med. 185:55-64; Miura, Y. *et al.* (2003) "TNF-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) INDUCES NEURONAL APOPTOSIS IN HIV-ENCEPHALOPATHY," J Med Dent Sci. 50:17-25). HIV-1 infection activates circulating monocytes by inducing IFN- α (Zagury, D. *et al.* (1998) "Interferon alpha and Tat involvement in the immunosuppression of uninfected T cells and C-C chemokine decline in AIDS," Proc Natl Acad Sci U S A. 95:3851-3856) and secretion of apoptotic cytokines such as tumor necrosis factor alpha (TNF- α), CD30L, and FasL (Oyaizu, N. *et al.* (1997) "MONOCYTES EXPRESS FAS LIGAND UPON CD4 CROSS-LINKING AND INDUCE CD4+ T CELLS APOPTOSIS: A POSSIBLE MECHANISM OF BYSTANDER CELL DEATH IN HIV INFECTION," J Immunol. 1997;158:2456-2463). Furthermore, antigen-presenting cells such as monocytes and dendritic cells have been implicated in AIDS pathogenesis (Badley, A.D. *et al.* (2000) "Mechanisms of HIV-associated lymphocyte apoptosis," Blood 96:2951-2964; Badley, A.D. *et al.* (1997) "MACROPHAGE-DEPENDENT APOPTOSIS OF CD4+ T LYMPHOCYTES FROM HIV-INFECTED INDIVIDUALS IS MEDIATED BY FASL AND TUMOR NECROSIS FACTOR. J Exp Med. 185:55-64).

Soluble TRAIL ("sTRAIL") is upregulated in HIV-1-infected patients (Liabakk, N.B. *et al.* (2002) "DEVELOPMENT, CHARACTERIZATION AND USE OF MONOCLONAL ANTIBODIES AGAINST STRAIL: MEASUREMENT OF STRAIL BY

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ELISA," J Immunol Methods. 259(1-2):119-28) and in vitro studies have linked TRAIL to the depletion of T cells from HIV-1-infected patients (Lum, J. J. *et al.* (2001) "INDUCTION OF CELL DEATH IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED MACROPHAGES AND RESTING MEMORY CD4 T CELLS BY 5 TRAIL/APO21," J Virol 75, 11128-36; Yang, Y. (2003) "MONOCYTES TREATED WITH HUMAN IMMUNODEFICIENCY VIRUS TAT KILL UNINFECTED CD4+ CELLS BY A TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCED LIGAND-MEDIATED MECHANISM," J. Virol 77: 6700-6708). Nonetheless, the identification of TRAIL-producing cells in vivo and the mechanism responsible for TRAIL-mediated T cell 10 death remain to be established.

Among the five TRAIL receptors described, only TRAIL-R1 death receptor 4 (DR4) and TRAIL-R2 (DR5) induce cell death upon ligation (Golstein, P. (1997) "CELL DEATH: TRAIL AND ITS RECEPTORS," Curr Biol 7, R750-3; Pan, G. *et al.* (1997) "THE RECEPTOR FOR THE CYTOTOXIC LIGAND TRAIL," Science 276, 111-15 3). DR5 gene is regulated by the transcription factor p53 which can be upregulated by type I interferons (Wu, G. S. *et al.* (1997) "KILLER/DR5 IS A DNA DAMAGE-INDUCIBLE P53-REGULATED DEATH RECEPTOR GENE," Nat Genet 17, 141-3; Takaoka, A. *et al.* (2003) "INTEGRATION OF INTERFERON-ALPHA/BETA SIGNALING 20 TO P53 RESPONSES IN TUMOUR SUPPRESSION AND ANTIVIRAL DEFENSE," Nature 424, 516-23). The other receptors, TRAIL-R3 decoy receptor 1 (DcR1), TRAIL-R4 (DcR2), and osteoprotogerin (OPG) lack a death domain and do not trigger apoptosis (Degli-Esposti, M. A. *et al.* (1997) "THE NOVEL RECEPTOR TRAIL-R4 INDUCES NF-KAPPAB AND PROTECTS AGAINST TRAIL-MEDIATED APOPTOSIS, YET 25 RETAINS AN INCOMPLETE DEATH DOMAIN," Immunity 7, 813-20). The amino acid sequence of TRAIL is known, as is the N-terminal cytoplasmic domain (residues TRAIL residues 38-281), which comprises sTRAIL. sTRAIL production is presently thought to involve a shedding mechanism that involves cleavage by proteases (see, Liabakk, N.B. *et al.* (2002) "DEVELOPMENT, CHARACTERIZATION AND USE OF MONOCLONAL ANTIBODIES AGAINST STRAIL: MEASUREMENT OF 30 STRAIL BY ELISA," J Immunol Methods. 259(1-2):119-28; Martinez-Lorenzo, M.J. *et al.* (1999) "ACTIVATED T CELLS RELEASE BIOACTIVE FAS LIGAND AND

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- APO2 LIGAND IN MICROVESICLES," J. Immunol. 163:1274-1281; Mariani, S.M. *et al.* (1998) "SURFACE EXPRESSION OF TRAIL/APO-2 LIGAND IN ACTIVATED MOUSE T AND B CELLS," Eur. J. Immunol. 28(5):1492-1498; Mariani, S.M. *et al.* (1998) "DIFFERENTIAL REGULATION OF TRAIL AND CD95 LIGAND IN TRANSFORMED
- 5 CELLS OF THE T AND B LYMPHOCYTE LINEAGE," Eur J Immunol. 28(3):973-982).

In sum, despite progress in the use of surrogate markers, such as viral load and CD4⁺ T cell count, to evaluate the progression of AIDS and other diseases whose progression involves immune system activation, a need remains for a method for more effectively monitoring the progression of such diseases. The

10 present invention is directed to such need.

Summary of the Invention:

This invention relates to methods and markers for monitoring the progression of AIDS, and other diseases whose progression involves immune system activation. In particular, the invention relates to the use of the TNF-Related

15 Apoptosis-Inducing Ligand (TRAIL) and TRAIL Compounds to monitor the progression of HIV infection, and to diagnose AIDS and other immune system diseases. Preferably, this is accomplished by assessing the presence or concentration of TRAIL and especially mTRAIL, sTRAIL, the TRAIL DR5 receptor molecule, and biological molecules that activate TRAIL or its receptor or

20 that are expressed by cells that express activated TRAIL or TRAIL DR5 receptor molecules in an individual's blood or other bodily fluids. These biological molecules include p53, α -interferon and β -interferon (IFN α/β). The invention further contemplates assessing the presence or concentration of molecules that are not TRAIL Compounds (as that term is defined herein) in an individual's blood or

25 other bodily fluids in concert with an assessment of the presence or concentration of one or more TRAIL Compound(s) in order to determine the presence or severity of a disease involving immune system activation. Such additional compounds include CD69 and HLA-DR.

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The present invention thus provides a new surrogate marker that has mechanistic implications for HIV-1 pathogenesis, and which directly correlates with viral load but not necessarily inversely with CD4⁺ T cell count. Soluble TRAIL produced by monocytes can interact with TRAIL death receptors that can 5 be induced by CD 4⁺ T cells, and contribute to apoptosis-induced loss of CD4⁺ T cells in progression to AIDS. The present invention thus permits, by measuring levels of the TNF-Related Apoptosis-Inducing Ligand, one to distinguish among HIV-1 infected patients with high viral load, HIV-1 infected patients with low viral loads and uninfected healthy individuals. The invention provides an advantage 10 over other surrogate marker (e.g., viral load measurement and CD4⁺ T cell count) in monitoring the progression of retroviral infection in that TRAIL is a death molecule involved in CD4⁺ T cell depletion in HIV/AIDS. Furthermore, TRAIL is a marker of immune function that directly correlates with HIV viral load.

In detail, the invention provides a method for determining whether a 15 mammal suffers from a disease or condition of involving immune system activation, wherein the method comprises assaying for the presence or concentration of a TRAIL Compound in a biological fluid of the mammal.

The invention particularly concerns the embodiment of such method wherein the mammal is selected from the group consisting of a human, simian, 20 feline, bovine, equine, canine, ovine or porcine mammal.

The invention particularly concerns the embodiments of such methods wherein the method comprises an immunoassay that determines the presence or concentration of the TRAIL Compound, the immunoassay comprising the steps of:
(a) contacting a sample of the biological fluid with an antibody specific 25 for the TRAIL Compound, the contacting being under conditions sufficient to permit the TRAIL Compound if present in the sample to bind to the antibody and form a TRAIL Compound – antibody complex;

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- (b) contacting the formed TRAIL Compound – antibody complex with a molecule capable of specific binding to the complex, the contacting being under conditions sufficient to permit the molecule to bind to the complex and form an extended complex; and
- 5 (c) determining the presence or concentration of the TRAIL Compound in the biological fluid by determining the presence or concentration of the formed extended complex in the sample.

The invention particularly concerns the embodiments of such methods wherein the TRAIL Compound is selected from the group consisting of sTRAIL, mTRAIL, the TRAIL DR5 receptor molecule, a biological molecule that activates TRAIL, and a biological molecule that activates the TRAIL DR5 receptor (especially, wherein the biological molecule that activates the TRAIL DR5 receptor is p53).

The invention particularly concerns the embodiments of such methods wherein the method reveals the presence of the TRAIL Compound, or wherein the method reveals the concentration of the TRAIL Compound.

The invention particularly concerns the embodiments of such methods wherein the disease or condition involving immune system activation is selected from the group consisting of HIV infection, AIDS, cancer, atherosclerosis, Alzheimer's disease, inflammation, autoimmune disorder, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant rejection, and graft vs host disease. The invention further concerns the embodiments of such methods wherein the disease or condition involving immune system activation is cancer, and particularly colon cancer, lung cancer, breast cancer, pancreatic cancer, leukemia, myeloma, skin cancer, brain cancer, cervical cancer, or testicular cancer.

The invention additionally concerns an immunoassay that determines the presence or concentration of a TRAIL Compound in a biological fluid of a mammal, wherein the immunoassay comprises the steps of:

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- 5 (a) contacting a sample of the biological fluid with an antibody specific for the TRAIL Compound, the contacting being under conditions sufficient to permit the TRAIL Compound if present in the sample to bind to the antibody and form a TRAIL Compound – antibody complex;
- 10 (b) contacting the formed TRAIL Compound – antibody complex with a molecule capable of specific binding to the complex, the contacting being under conditions sufficient to permit the molecule to bind to the complex and form an extended complex; and
- 15 (c) determining the presence or concentration of the TRAIL Compound in the biological fluid by determining the presence or concentration of the formed extended complex in the sample.

The invention additionally concerns the embodiment of such immunoassay wherein the TRAIL Compound is selected from the group consisting of sTRAIL, mTRAIL, the TRAIL DR5 receptor molecule, a biological molecule that activates TRAIL, and a biological molecule that activates the TRAIL DR5 receptor (especially, wherein the biological molecule that activates the TRAIL DR5 receptor is p53

20 The invention additionally concerns the embodiment of such immunoassay wherein the mammal is selected from the group consisting of a human, simian, feline, bovine, equine, canine, ovine or porcine mammal.

25 The invention additionally concerns the embodiments of such immunoassays wherein the immunoassay is an immunochromatographic immunoassay, wherein:

 in the step (a), the biological sample is placed in contact with a first porous carrier, the first porous carrier containing a non-immobilized, labeled antibody specific for the TRAIL Compound;

 in the step (b), the formed TRAIL Compound– antibody complex is placed in contact with a second porous carrier, the second porous carrier being in

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communication with the first porous carrier, and containing an immobilized molecule capable of specific binding to the complex; and

in the step (c), the presence or concentration of the TRAIL Compound in the biological fluid is determined by detecting the presence of the labeled antibody 5 specific for the TRAIL Compound in the second porous carrier.

The invention additionally concerns the embodiments of such immunoassays wherein the immunoassay reveals the presence of the TRAIL Compound or wherein the immunoassay reveals the concentration of the TRAIL Compound.

10 The invention additionally concerns the embodiments of such immunoassays wherein the immobilized molecule capable of specific binding to the complex is an rsTRAIL molecule.

15 The invention additionally concerns the embodiments of such immunoassays wherein the TRAIL Compound is sTRAIL, and the immobilized molecule capable of specific binding to the complex is an anti-sTRAIL antibody.

20 The invention additionally concerns the embodiments of such immunoassays wherein the immunoassay determines whether a mammal suffers from a disease or condition involving immune system activation selected from the group consisting of HIV infection, AIDS, cancer, atherosclerosis, Alzheimer's disease, inflammation, autoimmune disorder, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant rejection, and graft vs host disease. The invention further concerns the 25 embodiments of such methods wherein the disease or condition involving immune system activation is cancer, and particularly colon cancer, lung cancer, breast cancer, pancreatic cancer, leukemia, myeloma, skin cancer, brain cancer, cervical cancer, or testicular cancer.f

The invention additionally concerns a kit for measuring the presence or concentration of a TRAIL Compound in a biological fluid of a mammal, wherein

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the kit comprises a hollow casing comprising a multilayer filter system, and first and second porous carriers, wherein the second porous carrier is in communication with the first porous carrier, and the first porous carrier is in communication with the multilayer filter system, a portion of which is accessible from the casing;

5 wherein:

the first porous carrier contains a non-immobilized, labeled antibody that specifically binds the TRAIL Compound; and

the second porous carrier contains an immobilized, unlabeled molecule that binds to a TRAIL Compound – antibody complex.

10 The invention additionally concerns the embodiments of such kits wherein the kit permits the presence of the TRAIL Compound or the concentration of the TRAIL Compound to be determined.

The invention additionally concerns the embodiments of such kits wherein the immobilized molecule capable of specific binding to the complex is an
15 rsTRAIL molecule.

The invention additionally concerns the embodiments of such kits wherein the TRAIL Compound is selected from the group consisting of sTRAIL, mTRAIL, the TRAIL DR5 receptor molecule, a biological molecule that activates TRAIL, and a biological molecule that activates the TRAIL DR5 receptor (especially,
20 wherein the biological molecule that activates the TRAIL DR5 receptor is p53), and the immobilized molecule capable of specific binding to the complex is an anti-sTRAIL antibody.

The invention additionally concerns the embodiments of such kits wherein the kit is suitable for determining whether a mammal suffers from a disease or
25 condition of involving immune system activation selected from the group consisting of HIV infection, AIDS, cancer, atherosclerosis, Alzheimer's disease, inflammation, autoimmune disorder, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant rejection, and graft vs host disease. The invention further concerns the

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embodiments of such kits wherein the disease or condition involving immune system activation is cancer, and particularly colon cancer, lung cancer, breast cancer, pancreatic cancer, leukemia, myeloma, skin cancer, brain cancer, cervical cancer, or testicular cancer.

5 In light of the findings of the present invention that antibodies against IFN- α/β reduced CD4 $^{+}$ T cell death, and blocked the effect of each of the molecules required for inducing TRAIL-mediated apoptosis, one aspect of the present invention concerns the recognition that anti-type I interferon antibodies can be used as an anti-HIV-1 therapeutic agent.

10 **Brief Description of the Figures:**

Figure 1 shows increased plasma levels of TRAIL in AIDS patients.

Figure 1, Panel A shows plasma samples from 107 HIV-1-infected patients and 53 uninfected controls which are tested for their soluble TRAIL content by ELISA. Two groups of HIV-1-infected patients were defined depending on their viral load.
15 The mean values of plasma TRAIL were 852 \pm 52 pg/ml for 55 control donors, 1339 \pm 79 pg/ml for 49 HIV-1-infected patients with undetectable viral load (<50 RNA copies/ml of blood) and 2242 \pm 131 pg/ml for 58 HIV-1-infected patients with higher viral load (>50 RNA copies/ml of blood). P = p values from unpaired *t* test.
Figure 1, Panel B shows correlation between TRAIL levels observed in patients and TRAIL levels predicted by the invention. **Figure 1, Panel C** presents longitudinal data of four HIV-1-infected patients who, at time = 0, begin antiretroviral therapy. Patients were followed for 40 weeks. TRAIL level was measured by ELISA. Data are representative of 4 different group of the 8 patients tested.

25 **Figure 2** shows effects of AT-2 HIV-1 particles on TRAIL secretion.

Figure 2, Panel A shows PBMC from HIV-1 uninfected or HIV-1 infected individuals cultured for 3 days in the presence or absence of AT-2 HIV-1_{MN} or HIV-1_{Ada}. Levels of TRAIL are detected using ELISA. **Figure 2, Panel B** shows CD4 $^{+}$, CD8 $^{+}$ T cells, DC, macrophages and monocytes from infected and

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noninfected individuals cultured for 3 days in presence of AT-2 HIV-1_{MN}. Mean values (+/- standard errors) are shown for six independent experiments for each condition tested. **Figure 2, Panel C** shows monocytes from HIV-1 uninfected donors cultured for 1 day in the presence of microvesicles (controls) or AT-2 HIV-1_{MN}. Cells are analyzed for mTRAIL by FACS. Monocytes exposed to AT-2 HIV-1_{MN} exhibit increased expression of mTRAIL compared to controls ($p<0.0001$). Data are representative of 6 independent experiments performed. **Figure 2, Panel D** shows monocytes from HIV-1 uninfected donors cultured for 1 day in the presence of microvesicles or AT-2 HIV-1_{MN}. TRAIL mRNA level quantified by real time PCR. Mean values with standard errors are shown for four independent experiments. **Figure 2, Panel E** shows monocytes from HIV-1 uninfected donors cultured in the presence of different concentration of AT-2 HIV-1_{MN} and sTRAIL quantified by ELISA. Mean values with standard errors are shown for six independent experiments.

15 **Figure 3** shows effect of IFN type I antibodies on TRAIL production by monocytes from HIV-1 uninfected donors. **Figure 3, Panel A** shows monocytes cultured for 3 days in the presence of AT-2 HIV-1_{MN} with blocking antibodies against IFN- α , IFN- β or both. The level of sTRAIL is quantified by ELISA. **Figure 3, Panel B** shows monocytes cultured for 1 day in the presence of AT-2 HIV-1_{MN}, with or without blocking antibodies against IFN- α and IFN- β . The level of TRAIL mRNA is quantified by real time PCR analysis. **Figure 3, Panel C** shows monocytes cultured for 3 days in the presence of recombinant IFN- α or IFN- β . The level of sTRAIL is quantified by ELISA. Mean values with standard errors presented in **Figure 3, Panels A, B, and C** are representative of four independent experiments for each condition are tested. **Figure 3, Panel D** shows IFN- α levels by monocytes cultured for 1 (open bars) or 3 (closed bars) days in presence of microvesicles (control), AT-2 HIV-1_{MN} or AT-2 HIV-1_{Ada}. **Figure 3, Panel E** shows STAT1 and STAT2 expression. Monocytes were cultured 24h in the presence of AT-2 HIV-1_{MN}, AT-2 HIV-1_{Ada} or recombinant IFN- α/β (10 ng/mL) and production of STAT1 and STAT2 was analyzed by western-

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blot. β -actin was used as a loading control. **Figure 3, Panel F** shows the results of a blocking assay: monocytes were cultured for 3 days with AT-2 HIV-1_{MN} and in presence of: (1) isotype control antibody; (2) the CD4 binding inhibitor soluble CD4 (2 μ g/ml); (3) the CXCR4 inhibitor AMD-3100 (2 μ g/ml); (4) the fusion inhibitor T20 (2 μ g/ml). Monocytes cultured without AT-2 HIV-1_{MN} were used as control. TRAIL level was quantified by ELISA. Mean values with standard errors are shown for 3 independent experiments for each condition tested.

Figure 4 shows effect of infectious HIV-1 on TRAIL production by monocytes. **Figure 4, Panel A** shows monocytes cultured for 3 days in the presence of AT-2 HIV-1_{MN}, HIV-1_{MN} or infectious HIV-1_{LAV}. sTRAIL level is determined by ELISA. Mean values with standard errors are shown for six independent experiments for each condition tested. **Figure 4, Panel B** shows monocytes from HIV-1- donors cultured 3 days in the presence of live HIV-1_{MN} with blocking antibodies against IFN α , IFN β or both. The level of sTRAIL is quantified by ELISA. Mean values with standard errors are shown for 6 independent experiments for each condition tested. **Figure 4, Panel C** shows monocytes from HIV-1 $^+$ donors cultured for 1 day in the presence of microvesicles (controls) or live HIV-1_{MN}. Cells are analyzed for mTRAIL by FACS. Monocytes exposed to HIV-1_{MN} overexpressed mTRAIL compared to controls ($p<0.0001$).
Data are representative of 6 independent experiments performed.

Figure 5 shows effect of infectious HIV-1 on sTRAIL production in tonsil culture. Ex vivo human lymphoid tissue were culture for 12 days in presence or absence of HIV-1_{LAV}. sTRAIL was measured at day 3, 6, 9 and 12 by ELISA. Culture medium was changed at 3-day intervals.

Figure 6 shows an analysis of TRAIL and DR5 in HIV-1-infected patients and HIV-1-uninfected controls: **Panel A:** DR5 mRNA analysis of PBMC from 9 HIV-1-uninfected controls and 16 HIV-1-infected patients. Horizontal bars indicates mean values that were significantly different, $p=0.03$. **Panel B:** Plasma TRAIL levels and flow cytometric analysis of Annexin V $^+$ DR5 $^+$ CD4 $^+$ T cells

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from 16 uninfected control donors and 22 HIV-1-infected patients. p values were calculated using a two-tailed Student's *t* test. **Panel C:** Linear regression analysis of DR5 mediated cell death (DR5⁺ AnnexinV⁺) among apoptotic cells (AnnexinV⁺) was performed in the same cohort of patients (n=22) and controls (n=16).

Figure 7 shows annexin V and STAT expression in T cells: **Figure 7, Panel A:** Flow cytometric analysis of positively-selected CD4⁺ and CD8⁺ T cells from healthy, HIV-1-uninfected blood donors cultured for 1 (left) and 6 (right) days alone or in the presence of microvesicles (controls) or with: HIV-1_{MN}; HIV-1_{Ada}; influenza A virus (Flu); HIV-1_{MN} plus Flu; or HIV-1_{Ada} plus Flu. Infectious HIV-1 was used in the example illustrated. Very similar results were obtained when AT-2 HIV-1 was used. * represents p values <0.05 between treated T cells (HIV-1, and HIV-1⁺Flu) compared to control T cells, using a two-tailed Student's *t* test. **Figure 7, Panel B:** Annexin V/Propidium double-positive cells resulting from culture of CD4⁺ or CD8⁺ T cells with AT-2 HIV-1_{MN} plus Flu or AT-2 HIV-1_{Ada} plus Flu. **Figure 7, Panel C:** Effect of IFN- α/β -specific antibodies on inhibition of apoptosis in CD4⁺ T cells cultured with infectious (HIV-1) or noninfectious (AT-2 HIV-1) plus Flu. Effect of culturing CD4⁺ T cells with Flu, AT-2 HIV-1_{MN} or AT-2 HIV-1_{MN} plus Flu on IFN- α production (**Figure 7, Panel D**) and phosphorylated STAT1 activation (**Figure 7, Panel E**). **Figure 7, Panel F:** Western blot analysis of STAT1 and STAT2 protein after exposure of CD4⁺ T cells to AT-2 HIV-1_{MN}, Flu, AT-2 HIV-1_{MN} plus Flu, and effect of anti-IFN- α/β antibodies on STAT expression. Data of **Figure 7, Panel A** are mean values with standard errors of 6 independent experiments for each condition tested. Data of **Figure 7, Panels B, C, D, E and F** are representative of 4 independent experiments.

Figure 8 shows an analysis of TRAIL expression and production: **Figure 8, Panel A:** Flow cytometric analysis of CD4⁺ and CD8⁺ T cells cultured with Flu, infectious HIV-1_{MN}, or HIV-1_{MN} plus Flu. **Figure 8, Panel B:** TRAIL gene expression after culture of CD4⁺ T cells with AT-2 HIV-1_{MN} plus Flu , and effect of

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anti-IFN- α/β antibodies. **Figure 8, Panel C:** Effect of anti-IFN- α/β antibodies on mTRAIL expression induced by culture with AT-2 HIV-1_{MN} plus Flu. **Figure 8, Panel D:** Effect of culturing CD4 $^{+}$ T cells with IFN- α , IFN- β or IFN- α/β on the percentage of CD4 $^{+}$ T cells expressing mTRAIL. **Figure 8, Panel E:** Western blot analysis of TRAIL production after culture of CD4 $^{+}$ T cells with AT-2 HIV-1_{MN}, Flu, AT-2 HIV-1_{MN} plus Flu, AT-2 HIV-1_{MN} plus Flu plus anti-IFN- α/β antibodies, or rIFN- α/β . **Figure 8, Panel F:** Effect of anti-IFN- α/β antibodies, monoclonal RIK-2 anti-TRAIL antibody, anti-DR5 antibody, and soluble CD4-IgG (sCD4) on the percentage of Annexin V $^{+}$ cells resulting from culture of CD4 $^{+}$ T cells with AT-2 HIV-1_{MN} plus Flu Data of panel **Figure 8, Panels B and F** are mean values with standard errors of 6 independent experiments for each condition tested. Data of panels **Figure 8, Panels A, C, D and E** are representative of 4 independent experiments.

Figure 9 shows DR5 expression and p53 transcription and synthesis:
15 **Figure 9, Panel A:** Flow cytometric analysis of DR5 expression on CD4 $^{+}$ and CD8 $^{+}$ T cells after culture with Flu, infectious HIV-1_{MN}, or Flu plus infectious HIV-1_{MN} (Identical results were obtained using AT-2 HIV-1_{MN}). **Figure 9, Panel B:** Two-color flow cytometric analysis of Annexin V/DR5 double positive CD4 $^{+}$ T cells cultured alone (control) or with AT-2 HIV-1_{MN} plus Flu. **Figure 9, Panel C:** DR5 gene expression in CD4 $^{+}$ T cells cultured with AT-2 HIV-1_{MN} plus Flu, and inhibition by anti-IFN- α/β antibodies. **Figure 9, Panel D:** Flow cytometric analysis of: AT-2 HIV-1_{MN} plus Flu-induced DR5 expression by CD4 $^{+}$ T cells and inhibition by anti-IFN- α/β antibodies (upper) and lack of effect of recombinant IFN- α/β plus HIV-1_{MN} on DR5 expression (middle) and apoptosis (lower) of CD4 $^{+}$ T cells. **Figure 9, Panel E:** Western blot analysis of DR5 protein production by CD4 $^{+}$ T cells cultured with AT-2 HIV-1_{MN}, Flu, AT-2 HIV-1_{MN} plus Flu or rIFN- α/β , and inhibition of DR5 by anti-IFN- α/β antibodies. **Figure 9, Panel F:** Effect of culture of CD4 $^{+}$ T cells with AT-2 HIV-1_{MN} plus Flu on p53 gene expression and inhibition by anti-IFN- α/β antibodies. **Figure 9, Panel G:** Effect of culture of CD4 $^{+}$ T cells with AT-2 HIV-1_{MN}, Flu, or AT-2 HIV-1_{MN} plus Flu on p53 protein

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production. **Figure 9, Panel H:** Western blot analysis of p53 production by CD4⁺ T cells cultured with AT-2 HIV-1_{MN}, Flu, AT-2 HIV-1_{MN} plus Flu or rIFN- α/β ; and inhibition by anti-IFN- α/β antibodies. Data of **Figure 9, Panels C, F and G** are mean values with standard errors of 6 independent experiments for each condition tested. Data of **Figure 9, Panels A, B, D, E and H** are representative of 4 independent experiments.

Figure 10 illustrates a “Two-Hit” model of type I interferon-dependent TRAIL~DR5-mediated selective apoptosis of CD4⁺ T cells in AIDS progression. **Top:** Exposure of CD4⁺ T cells to either infectious or noninfectious HIV-1 or to other viruses induces expression of mTRAIL and activation of STAT1 but not apoptosis. **Bottom:** Activation of STAT2 and expression of p53 and DR5 requires two hits that involve either infectious or noninfectious HIV-1 (first hit) plus a second event or virus (second hit) (our examples: influenza or measles viruses). Exposure of CD4⁺ T cells to both hits is required to activate STAT2 (also increased STAT1) and p53 that results in DR5 expression. Each of these virus-induced events is blocked by antibodies against IFN- α/β . None of the above virus-induced changes were observed in CD8⁺ T cells. Because rIFN- α/β did not replace the second virus for inducing p53, DR5 and apoptosis, “?” indicates a signal or event of the two-hit model (that triggers p53 and DR5). pTRAIL = plasma TRAIL, mTRAIL = membrane TRAIL.

Description of the Preferred Embodiments:

As discussed above, only two surrogate markers (i.e., HIV-1 viral load and CD4⁺ T cell count) have previously been recognized as sufficiently correlative of AIDS progression to be employed clinically. The present invention relates to methods and compositions suitable for monitoring the progression of AIDS, and other diseases whose progression involves immune system activation.

One aspect of the present invention derives from the recognition that the “TNF-Related Apoptosis-Inducing Ligand” (“TRAIL”) Compounds can be employed as a surrogate marker for the progression of AIDS and other diseases

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whose progression involves immune system activation. The positive correlation between sTRAIL and HIV progression (i.e. the marker directly correlates with viral load but not with CD4 count) has mechanistic implications for HIV-1 pathogenesis. Because of the selectivity of TRAIL and its receptor for CD4⁺ T cells, the use of TRAIL Compounds as surrogate marker(s) for evaluating the progression of AIDS and other diseases of immune system activation provides an advantage over viral load and CD4 determinations.

The depletion of CD4⁺ T cells that results from HIV-1 infection remains unsolved despite more than 20 years of investigation of AIDS pathogenesis. The percentage of CD4⁺ T cells that is HIV-1 infected prior to and during the early decrease in CD4⁺ T cell numbers cannot account for the severe loss of this cell population (Grivel, J.C. *et al.* (2000) "HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INDUCES APOPTOSIS IN CD4(+) BUT NOT IN CD8(+) T CELLS IN EX VIVO-INFECTED HUMAN LYMPHOID TISSUE," *J Virol.* 74:8077-8084; Schnittman, S.M. *et al.* (1990) "INCREASING VIRAL BURDEN IN CD4⁺ T CELLS FROM PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION REFLECTS RAPIDLY PROGRESSIVE IMMUNOSUPPRESSION AND CLINICAL DISEASE," *Ann Intern Med.* 113:438-443). Other studies have implicated "bystander" apoptotic death of activated, uninfected T cells during progression to AIDS (Badley, A.D. *et al.* (2000) "Mechanisms of HIV-associated lymphocyte apoptosis," *Blood* 96:2951-2964). TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily (Wiley, S.R. *et al.* (1995) "IDENTIFICATION AND CHARACTERIZATION OF A NEW MEMBER OF THE TNF FAMILY THAT INDUCES APOPTOSIS," *Immunity* 3:673-682), may be involved in CD4⁺ T cell depletion, since TRAIL, produced by monocytes exposed to the HIV-1 Tat protein, killed uninfected CD4⁺ T cells (Yang, Y. *et al.* (2003) "MONOCYTES TREATED WITH HUMAN IMMUNODEFICIENCY VIRUS TAT KILL UNINFECTED CD4(+) CELLS BY A TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCED LIGAND-MEDIATED MECHANISM," *J Virol.* 77:6700-6708). Moreover, soluble TRAIL was found in HIV-1-infected patients (Liabakk, N.B. *et al.* (2002) "DEVELOPMENT, CHARACTERIZATION AND USE OF MONOCLONAL ANTIBODIES AGAINST STRAIL: MEASUREMENT OF STRAIL BY ELISA," *J Immunol Methods*.

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259:119-128; Stylianou, E. *et al.* "RAISED SERUM LEVELS OF INTERLEUKIN-18 IS ASSOCIATED WITH DISEASE PROGRESSION AND MAY CONTRIBUTE TO VIROLOGICAL TREATMENT FAILURE IN HIV-1-INFECTED PATIENTS," *Clin Exp Immunol.* 132:462-466), and may be responsible for the death of neurons in AIDS patients leading to dementia (Ryan, L.A. *et al.* (2004) "TNF-RELATED APOPTOSIS-INDUCING LIGAND MEDIATES HUMAN NEURONAL APOPTOSIS: LINKS TO HIV-1-ASSOCIATED DEMENTIA," *J Neuroimmunol.* 148:127-139). TRAIL induces apoptosis in human tumor cell lines (Griffith, T.S. *et al.* (1998) "INTRACELLULAR REGULATION OF TRAIL-INDUCED APOPTOSIS IN HUMAN MELANOMA CELLS," *J Immunol.* 161:2833-2840) and in infected cells (Jeremias, I. *et al.* (1998) "TRAIL/Apo-2-ligand-induced apoptosis in human T cells," *Eur J Immunol.* 28:143-152; Clarke, P. *et al.* (2003) "TWO DISTINCT PHASES OF VIRUS-INDUCED NF-KAPPAB-REGULATION ENHANCE TRAIL-MEDIATED APOPTOSIS IN VIRUS-INFECTED CELLS," *J Biol Chem.* 278(20):18092-18100 [Epub Mar 13. 2003]) but not in normal cells (Gura, T. (1997) "HOW TRAIL KILLS CANCER CELLS, BUT NOT NORMAL CELLS," *Science* 277:768).

As used herein, the term "TRAIL Compound" is intended to include not only the naturally occurring or recombinant fragments of TRAIL (such as mTRAIL and sTRAIL,), but also the TRAIL DR5 receptor molecule, and biological molecules that activate TRAIL (such as IFN α/β) or its receptor or that are expressed by cells that express activated TRAIL or TRAIL DR5 receptor molecules or fragments of such molecules.

Preferred TRAIL Compounds

TRAIL (sTRAIL / mTRAIL / rsTRAIL)

25 TRAIL, its naturally occurring fragments (such as m-TRAIL or sTRAIL) or polynucleotide molecules that encode such molecules, or fragments of any such molecules are preferred TRAIL Compounds of the present invention. TRAIL is a membrane-associated protein composed of multiple domains: an N-terminal cytoplasmic domain, an internal transmembrane domain, and a C-terminal

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extracellular domain (amino acid residues 38-281 of **SEQ ID NO:1**); the C-terminal extracellular domain being responsible for inducing apoptosis (Wiley, S. R. *et al.* (1995) "IDENTIFICATION AND CHARACTERIZATION OF A NEW MEMBER OF THE TNF FAMILY THAT INDUCES APOPTOSIS," *Immunity* 3, 673-682). Although TRAIL is thus generally membrane-bound, under certain circumstances bioactive TRAIL is released from cells (Liabakk, N.B. *et al.* (2002) "DEVELOPMENT, CHARACTERIZATION AND USE OF MONOCLONAL ANTIBODIES AGAINST STRAIL: MEASUREMENT OF STRAIL BY ELISA," *J Immunol Methods*. 259(1-2):119-28; Martinez-Lorenzo, M.J. *et al.* (1999) "ACTIVATED T CELLS RELEASE BIOACTIVE FAS LIGAND AND APO2 LIGAND IN MICROVESICLES," *J. Immunol.* 163:1274-1281.

The amino acid sequence of TRAIL (**SEQ ID NO:1**) is presented below, with the N-terminal cytoplasmic domain (residues 38-281) underlined:

SEQ ID NO:1					
15	MAMMEVQGGP 1	SLGQTCVLIV 10	I FTVLLQLSLC 20	VAVTYVYFTN 30	ELKQMQDKYS 40
	KSGIACFLKE 51	DDSYWDPNDE 60	ESMNNSPCWQV 70	KWQLRQLVRK 80	MILRTSEETI 90
	STVQEKEQQNI 101	SPLVRERGPQ 110	RVAAHITGTR 120	GRSNNTLSSPN 130	SKNEKALGRK 140
20	INSWESSSRSG 151	HSFLSNLHLR 160	NGELVIHEKG 170	FYYIYSQTYF 180	RFQEEIKENT 190
	KNDKQMVQYI 201	YKYTSYPDPI 210	LLMKSARNSC 220	WSKDAEYGLY 230	SIYQGGIFEL 240
25	KENDRIFVSV 251	TNEHLIDMDH 260	EASFFGAFLV 270	G 280	281

A nucleic acid molecule encoding TRAIL is presented below as **SEQ ID NO:2**. TRAIL is encoded by residues 88-930 of **SEQ ID NO:2** (the start and stop codons of TRAIL are underlined). The sequence encoding sTRAIL (residues 38-281 of **SEQ ID NO:1**) is encoded by residues 199-930 of **SEQ ID NO:2**.

SEQ ID NO:2

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1 cctcaactgac tataaaaagaa tagagaagga agggcttcag tgaccggctg cctggctgac
61 ttacagcagt cagactctga caggatcATG gctatgtgg aggtccagggggacccagc
121 ctggacaga ctgcgtgc gatcgatgc ttcacagtgc tcctgcagtc tctctgtgt
5 181 gctgttaactt acgtgtactt taccaacgag ctgaaggcaga tgcaggacaa gtactccaaa
241 agtggcattt cttgtttctt aaaagaagat gacagttatt gggaccccaa tgacgaagag
301 agtatgaaca gccccctgctg gcaagtcaag tggcaactcc gtcagctcgt tagaaagatg
361 atttgagaa cctctgagga aaccatttct acagttcaag aaagcaaca aaatatttct
421 cccttagtga gagaagaggg tcctcagaga gtagcagct acataactgg gaccgaggga
10 481 agaagcaaca catttgtcttc tccaactcc aagaatgaaa gggctctggg ccgcaaaaata
541 aactcctggg aatcatcaag gagtgggcat tcattcctgta gcaacttgca cttgagggaaat
601 ggtgaactgg tcatccatgta aaagggttt tactacatct atccaaac atactttcga
661 tttcaggggg aataaaaga aaacacaaag aacgacaaaac aatggtcca atatattttac
721 aaatacacaa gtttatcctgta cctattattg ttgatgaaaa gtgctagaaaa tagttgtttgg
15 781 tctaaagat gagaatatgg acttattcc atttatcaag ggggaaatatt tgagcttaag
841 gaaaatgaca gatttttttgt ttctagtaaca aatgagcact tgatagacat ggaccatgaa
901 gccagttttt tcgggccctt tttagttggc TActgacct ggaaagaaaa agcaataacc
961 tcaaagtgac tattcagttt tcaggatgat acactatgaa gatgtttcaaa aaatctgac
20 1021 caaacaaac aaacagaaaa cagaaaaacaa aaaaccctt atgcaatctg agtagagcag
1081 ccacaaccaa aaatttctac aacacacac gtttctgaaag tgactcactt atccaaagaa
1141 aatgaaat tgaaagat cttcaggact ctacctcata tcagttttgct agcagaaatc
1201 tgaagactgt tcagcttcca aacattaat gcaatgtttaa catttctgt ctttataatc
1261 tactcctgt taagactgt tgaagaaag ccaacatcca tctctcaatg agtgtattacc
1321 agtagtagcc tccaggtttc cttaaaggggac aacatcctt agtcaaaag gagaagagggc
25 1381 accactaaaaa gatcgcagtt tgccctggtc agtggctcac acctgttaatc ccaacattt
1441 gggaaccccaa ggtgggtag tcacgagatc aagagatca gaccatagt accacatatg
1501 tgaaacccca tctctact gta aagtgcaaaa attagctgg tgttggca catgcctgt a
1561 gtcccagcta cttgagag gtgggcag gatcgtttg aacccgggg gcagaggt ttg
1621 cagtgtgg tgagatcg gccacatcc gccctggca cagag ggag gcattggttca
30 1681 aaaaaaaaaaaaa aaaaaaaaaaaaa cttcagta g tacgtttat tttttcaat aaatttctat
1741 tacagtat gt caaaaaaaaaaaa aaaaaaaaaaaaa
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In one embodiment, invention contemplates methods and compositions for monitoring the presence of biologically active forms of TRAIL, and in particular, for monitoring biologically active forms of TRAIL that are membrane associated or naturally secreted from cells. Such molecules include membrane bound TRAIL (“mTRAIL”) and soluble (i.e., non-membrane bound) TRAIL (“sTRAIL”). As used herein, the term “TRAIL Compound” is intended to refer to naturally occurring or recombinant fragments of TRAIL. sTRAIL is the release form of membrane TRAIL, and corresponds to the extracellular domain of TRAIL (amino acid residues 38-281 of **SEQ ID NO:1**); it is encoded by residues 199-930 of **SEQ ID NO:2**). Without intending to be bound thereby, the production of sTRAIL may involve a shedding mechanism that involves cleavage by proteases (see, Liabakk, N.B. *et al.* (2002) “DEVELOPMENT, CHARACTERIZATION AND USE OF

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- MONOCLONAL ANTIBODIES AGAINST sTRAIL: MEASUREMENT OF sTRAIL BY ELISA," J Immunol Methods. 259(1-2):119-28; Martinez-Lorenzo, M.J. *et al.* (1999) "ACTIVATED T CELLS RELEASE BIOACTIVE FAS LIGAND AND APO2 LIGAND IN MICROVESICLES," J. Immunol. 163:1274-1281; Mariani, S.M. *et al.* (1998)
- 5 "SURFACE EXPRESSION OF TRAIL/APO-2 LIGAND IN ACTIVATED MOUSE T AND B CELLS," Eur. J. Immunol. 28(5):1492-1498; Mariani, S.M. *et al.* (1998) "DIFFERENTIAL REGULATION OF TRAIL AND CD95 LIGAND IN TRANSFORMED CELLS OF THE T AND B LYMPHOCYTE LINEAGE," Eur J Immunol. 28(3):973-982). sTRAIL has been used as a surrogate marker for assessing hepatitis B viral
- 10 infection (Han, L.H. *et al.* (2002) "DETECTION OF SOLUBLE TRAIL IN HBV INFECTED PATIENTS AND ITS CLINICAL IMPLICATIONS," World J Gastroenterol. 8(6):1077-1080), and multiple sclerosis (Wandinger, K.P. *et al.* (2003) "TNF-RELATED APOPTOSIS INDUCING LIGAND (TRAIL) AS A POTENTIAL RESPONSE MARKER FOR INTERFERON-BETA TREATMENT IN MULTIPLE SCLEROSIS," Lancet 361(9374):2036-2043). Recombinant forms of sTRAIL (denoted herein as "rsTRAIL") may be produced by expressing a TRAIL fragment comprising the C-terminal extracellular domain. For example, Wiley, S. R. *et al.* expressed a recombinant sTRAIL comprising amino acid residues 95-281 of **SEQ ID NO:1** (U.S Patent No. 5,763,223; Wiley, S. R. *et al.* (1995) "IDENTIFICATION AND
- 15 20 CHARACTERIZATION OF A NEW MEMBER OF THE TNF FAMILY THAT INDUCES APOPTOSIS," Immunity 3, 673-682). Other rsTRAIL molecules comprising the C-terminal extracellular domain can be employed (for example, rsTRAIL molecules comprising amino acid residues 101-281, or 114-281, of **SEQ ID NO:1**) to induce apoptosis. See also, Wang, L.H. *et al.* (2002) "DENSITY CULTIVATION FOR
- 25 PREPARATION OF RECOMBINANT SOLUBLE HUMAN TRAIL. Acad. J. Sec. Mil. Med. Univ. 23(2):132-135; Wang, X.J. *et al.* (2000) "THE CLONING, EXPRESSION AND REFOLDING OF TNF-RELATED APOPTOSIS INDUCING LIGAND," J. Beijing Med. Univ. 32(5):387-390. sTRAIL has been expressed in *Escherichia coli* (Xia, X.X. *et al.* (2004) "Purification and Characterization of Recombinant sTRAIL
- 30 Expressed in *Escherichia coli*," Acta Biochimica Biophysica Sinica 36(2):118-122). sTRAIL ELISA detection kits are available commercially (Diacclone).

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Antibodies to sTRAIL and to TRAIL can be readily produced, or obtained commercially (Diaclone; Alexis Corporation; Trinoca Biochem).

TRAIL DR5 Receptor

The TRAIL DR5 receptor and polynucleotides that encode the TRAIL DR5 receptor, or fragments of such molecules, are TRAIL Compounds of the present invention. The amino acid sequence of the TRAIL DR5 receptor is provided below as **SEQ ID NO:11**. A nucleic acid sequence that encodes the TRAIL DR5 receptor is provided below as **SEQ ID NO:12** (see, GenBank Accession Nos. AAB71412 and AF020501, herein incorporated by reference). The start and stop codons for the TRAIL DR5 receptor are underlined.

SEQ ID NO:11

	MEQRGQNAPA	ASGARKRHGP	GPREARGARP	GLRVPKTLVL	VVAAVLLLVS
	10	20	30	40	50
15	AESALITQQD	LAPQQRVAPQ	QKRSSPSEG	CPPGHHISED	GRDCISCKYG
	60	70	80	90	100
	QDYSTHWNDL	LFCLRCTRCD	SGEVELSPCT	TTRNTVCQCE	EGTFREEDSP
	110	120	130	140	150
	EMCRKCRTGC	PRGMVKVGDC	TPWSDIECVH	KESGIIIGVT	VAAVVLIVAV
	160	170	180	190	200
20	FVCKSLLWKK	VLPYLGICS	GGGGDPERVD	RSSQRPGGAED	NVLNEIVSIL
	210	220	230	240	250
	QPTQVPEQEM	EVQEPAEPTG	VNMLSPGESE	HLLEPAEAER	SQRRRLLVPA
	260	270	280	290	300
25	NEGDPTETLR	QCFDDFADLV	PFDSWEPLMR	KLGLMDNEIK	VAKAEAAGHR
	310	320	330	340	350
	DLYTMLIKW	VNKTRGDASV	HTLLDALETL	GERLAKQKIE	DHLLSSGKFM
	360	370	380	390	400
	YLEGNADSAM	S			
	410	411			

SEQ ID NO:12

1 **ATG**gaacaac ggggacagaa cccccggcc gcttcggggg cccggaaaag gcacggccca
 61 ggacccaggg aggcgcgggg agccaggcct gggctccggg tccccaaagac ccttgtgctc
 121 gttgtcgccg cggtcctgct gttggtctca gctgagtctg ctctgatcac ccaacaagac
 5 181 cttagctcccc agcagagagt ggcggccacaa caaaagaggt ccagcccttc agagggattg
 241 tgtccacctg gacaccataat ctcagaagac ggttagagatt gcatctcctg caaatatggg
 301 cagactata gcactcaactg gaatgacctc cttttctgct tgcgctgcac caggtgtgat
 361 tcaggtgaag tggagctaag tccctgcacc acgaccagaa acacagtgtg tcagtgcgaa
 421 gaaggcacct tccgggaaga agattctcct gagatgtgcc ggaagtgccg cacagggtgt
 481 cccagaggga tggtcaaggt cggtgattgt acaccctgga gtgacatcga atgtgtccac
 541 aaagaatcag gcatcatcat aggagtcaca gttcagccg tagtcttgat tgtggctgtg
 601 ttgtttgca agtctttact gtggaaagaaa gtccttcctt acctgaaagg catctgctca
 661 ggtgggtgtg gggaccctga gcgtgtggac agaagctcac aacgacctgg ggctgaggac
 721 aatgtcctca atgagatcgt gaggatctt cagcccaccc aggtccctga gcagggaaatg
 781 gaagtccagg agccagcaga gccaacaggt gtcaacatgt tgcccccgg ggagtccagag
 841 catctgctgg aaccggcaga agctgaaagg tctcagagga ggaggctgct ggttccagca
 901 aatgaaggta atcccactga gactctgaga cagtgttgc atgactttgc agacttgggt
 961 cccttgact cctgggagcc gctcatgagg aagttgggccc tcatggacaa tgagataaaag
 1021 gtggctaaag ctgaggcagc gggccacagg gacaccttg acacgatgct gataaagtgg
 1081 gtcaacaaaaa ccggggcgaga tgcctctgtc cacaccctgc tggatgcctt ggagacgctg
 1141 ggagagagac ttgccaagca gaagattgag gaccacttgt tgagctctgg aaagttcatg
 1201 tatctagaag gtaatgcaga ctctgccccat tcc**TAA**

The TRAIL DR5 receptor is known to be involved in apoptosis, and to be affected by p53 (Golstein, P. (1997) "CELL DEATH: TRAIL AND ITS RECEPTORS," Curr Biol 7, R750-3; Pan, G. *et al.* (1997) "THE RECEPTOR FOR THE CYTOTOXIC LIGAND TRAIL," Science 276, 111-113; Wu, G. S. *et al.* (1997) "KILLER/DR5 IS A DNA DAMAGE-INDUCIBLE P53-REGULATED DEATH RECEPTOR GENE," Nat Genet 17, 141-3; Takaoka, A. *et al.* (2003) "INTEGRATION OF INTERFERON-ALPHA/BETA SIGNALING TO P53 RESPONSES IN TUMOUR SUPPRESSION AND ANTIVIRAL DEFENSE," Nature 424, 516-23); Miranda-Carus, M.E. *et al.* (2004) "RHEUMATOID ARTHRITIS SYNOVIAL FLUID FIBROBLASTS EXPRESS TRAIL-R2 (DR5) THAT IS FUNCTIONALLY ACTIVE," Arthritis Rheum. 50(9):2786-2793; Ren, Y.G. *et al.* (2004) "DIFFERENTIAL REGULATION OF THE TRAIL DEATH RECEPTORS DR4 AND DR5 BY THE SIGNAL RECOGNITION PARTICLE," Mol Biol Cell. 35 15(11):5064-74. Epub 2004 Sep 08; Dicker, F. *et al.* (2004) "FAS-LIGAND (CD178) AND (TRAIL) SYNERGISTICALLY INDUCE APOPTOSIS OF CD40-ACTIVATED CHRONIC LYMPHOCYTIC LEUKEMIA B CELLS," Blood. 2004 Aug 31 [Epub ahead of print]; Liu, X. *et al.* (2004) "p53 UPREGULATES DEATH RECEPTOR 4 EXPRESSION THROUGH AN INTRONIC P53 BINDING SITE," Cancer Res. 64(15):5078-5083;

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Sheikh, M.S. (2004) "MYC TAGGING ALONG THE TRAIL TO DEATH RECEPTOR 5," Cell Cycle. 2004 Jul 8;3(7) [Epub ahead of print]; Aggarwal, B.B. *et al.* (2004) "REGULATION OF TRAIL-INDUCED APOPTOSIS BY ECTOPIC EXPRESSION OF ANTI-APOPTOTIC FACTORS," Vitam Horm. 67:453-483.

5 It has been proposed that TRAIL DR5 may be a potential anti-cancer therapy (Yagita, H. *et al.* (2004) "TRAIL AND ITS RECEPTORS AS TARGETS FOR CANCER THERAPY," Cancer Sci. 95(10):777-783; Delmas, D. *et al.* (2004) "REDISTRIBUTION OF CD95, DR4 AND DR5 IN RAFTS ACCOUNTS FOR THE SYNERGISTIC TOXICITY OF RESVERATROL AND DEATH RECEPTOR LIGANDS IN
10 COLON CARCINOMA CELLS," Oncogene. 2004 Oct 11 [Epub ahead of print]; Crowder, C. *et al.* (2004) "PML MEDIATES IFN{ALPHA} INDUCED APOPTOSIS IN MYELOMA BY REGULATING TRAIL INDUCTION," Blood 2004 Sep 30 [Epub ahead of print].

P53

15 The p53 protein and polynucleotides that encode the p53 protein, or fragments of such molecules, are TRAIL Compounds of the present invention. The p53 protein is encoded by the p53 gene, which is a very well described cancer gene (see, e.g., Vogelstein, B. *et al.* (2004) "CANCER GENES AND THE PATHWAYS THEY CONTROL," Nat Med. 10(8):789-799; Okada, H. *et al.* (2004) "PATHWAYS OF
20 APOPTOTIC AND NON-APOPTOTIC DEATH IN TUMOUR CELLS," Nat Rev Cancer. 4(8):592-603). The amino acid sequence of the human p53 protein is provided below as **SEQ ID NO:13**. A nucleotide sequence that encodes this protein is provided below as **SEQ ID NO:14** (see, GenBank Accession Nos. K03199 and AAA59989, herein incorporated by reference). The start and stop codons for p53
25 are underlined.

SEQ ID NO:13:

MEEPQSDPSV	EPPLSQETFS	DLWKLLPENN	VLSPLPSQAM	DDLMLSPDDI
10	20	30	40	50
EQWFTEDPGP	DEAPRMPEAA	PPVAPAPAAP	TPAAPAPAPS	WPLSSSVPSQ
30	60	70	80	90

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	KTYQGSYGF	LGFLHSGTAK	SVTCTYSPAL	NKMFQLAKT	CPVQLWVDST	
	110	120	130	140	150	
	PPPGTRVRAM	AIYKQSQHMT	EVVRRCPHHE	RCSDSDGLAP	PQHLIRVEGN	
	160	170	180	190	200	
5	LRVEYLDDRN	TFRHSVVVPY	EPPEVGSDCT	TIHYNYMCNS	SCMGGMNRRP	
	210	220	230	240	250	
	ILTIITLEDS	SGNLLGRNSF	EVHVCACPGR	DRRTEEENLR	KKGEPHHELP	
	260	270	280	290	300	
10	PGSTKRALPN	NTSSSPQPKK	KPLDGEYFTL	QIRGRERFEM	FRELNEALEL	
	310	320	330	340	350	
	KDAQAGKEPG	GSRAHSSHLSK	SKKGQSTSRRH	KKLMFKTEGP	DSD	
	360	370	380	390	393	

SEQ ID NO:14:

15 1 gtcgaccctt tccacccctg gaagatggaa ataaacctgc gtgtgggtgg agtgttagga
 61 caaaaaaaaaaaa aaaaaaaaaaag tctagagcca ccgtccaggg agcaggtagc tgctgggctc
 121 cggggacact ttgcgttcgg gctgggagcg tgctttccac gacggtgaca cgcttccctg
 181 gattggcagc cagactgcct tccgggtcac tgcc**ATG**gag gagccgcagt cagatcctag
 241 cgtcgagccc cctctgagtc aggaaacatt ttcatggaccta tggaaactac ttccctgaaaa
 301 caacgttctg tcccccttgc cgtcccaagc aatggatgtat ttgtatgtgt ccccgacga
 361 tattgaacaa tggttcactg aagacccagg tccagatgaa gctccagaa tgccagaggc
 421 tgctcccccc gttggccctg caccacgcgttccataccg gctggccctg caccagcccc
 481 ctctggccc ctgtcatctt ctgtcccttc ccagaaaacc taccaggggc gctacggttt
 541 ccgtctgggc ttcttgcatt ctggacagc caagtctgtg acttgcacgt actccctgc
 601 cctcaacaag atgtttgcc aactggccaa gacctgcctt gtgcagctgt gggttgattc
 661 cacacccccc cccggcaccg gcgtccgcgc catggccatc tacaagcagt cacagcacat
 721 gacggaggtt gtgaggcgct gccccacca tgagcgtgc tcagatagcg atggctggc
 781 ccctccctcag catcttatcc gagtttgcgt gtggagttt tgatgacag
 841 aaacactttt cgacatagtg ttgttgcctt ctatgagcc cctgaggtt gctctgactg
 901 taccaccatc cactacaact acatgttaa cagttcctgc atggccggca tgaaccggag
 961 gcccatcctc accatcatca cacttggaaa ctccagggtt aatctactgg gacggacacag
 1021 ctttgaggtt catgtttgtt cctgtccctgg gagagaccgg cgcacagagg aagagaatct
 1081 ccgcaagaaa ggggagccct accacgagct gccccccagg agcactaagc gacactgccc
 1141 caacaacacc agctcctctc cccagccaaa gaagaaacca ctggatggag aatatttcac
 1201 ccttcagatc cgtgggcgtt agcgttccga gatgttccga gagctgtatg aggccttgg
 1261 actcaaggat gcccaggctg ggaaggagcc agggggggagc agggctcaact ccagccacct
 1321 gaagtccaaa aagggtcagt ctacccctgg ccataaaaaaa ctcatgttca agacagaagg
 1381 gcctgactca gac**TGA**catt ctccacttct tgttccccac tgacagcctc ccaccccccatt
 1441 ctctccctcc cctgtccattt tgggttttgg gtctttgaac cttgtttgc aataggtgt
 1501 cgtcagaagc acccaggact tccatggct ttgttccggg gctccactga acaagttggc
 1561 ctgcactggt gttttgtt ggggaggagg atggggagta ggacatcca gcttagattt
 1621 taaggtttt actgtgaggg atgtttggg gatgttaagaa atgttcttgc agttaagggt
 1681 tagtttacaa tcagccacat tctaggttagg gacccacttc accgtactaa ccagggaagc
 1741 tgtccctcac tggtaattc

The expression of the p53 protein is affected by HIV-1 (Bakhanashvili, M.
 45 et al. (2004) "P53 IN CYTOPLASM MAY ENHANCE THE ACCURACY OF DNA

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- SYNTHESIS BY HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 REVERSE TRANSCRIPTASE," Oncogene. 23(41):6890-6899; Makino, E. *et al.* (2004) "INTRODUCTION OF AN N-TERMINAL PEPTIDE OF S100C/A11 INTO HUMAN CELLS INDUCES APOPTOTIC CELL DEATH," J Mol Med. 82(9):612-620. Epub 2004 Jul 06;
- 5 Garden, G.A. *et al.* (2004) "HIV ASSOCIATED NEURODEGENERATION REQUIRES P53 IN NEURONS AND MICROGLIA," FASEB J. 18(10):1141-1143. Epub 2004 May 20; Oberst, A. *et al.* (2003) "A PLACE TO DIE FOR: APOPTOSIS IN CANCER AND INFECTION, CAPRI 2002," Cell Death Differ. 10(3):393-395; Little, R.F. *et al.* (2003) "HIGHLY EFFECTIVE TREATMENT OF ACQUIRED IMMUNODEFICIENCY
- 10 SYNDROME-RELATED LYMPHOMA WITH DOSE-ADJUSTED EPOCH: IMPACT OF ANTIRETROVIRAL THERAPY SUSPENSION AND TUMOR BIOLOGY," Blood. 101(12):4653-4659. Epub 2003 Feb 27; Castedo, M. *et al.* (2002) "Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe," Cell Death Differ. 2002 Dec;9(12):1287-1293; Mahomed, A. *et al.* (2002) "HUMAN
- 15 IMMUNODEFICIENCY VIRUS INFECTION, BCL-2, P53 PROTEIN, AND KI-67 ANALYSIS IN OCULAR SURFACE SQUAMOUS NEOPLASIA," Arch Ophthalmol. 2002 May;120(5):554-558; Selliah, N. *et al.* (2001) "BIOCHEMICAL MECHANISMS OF HIV INDUCED T CELL APOPTOSIS," Cell Death Differ. 8(2):127-136; Wattre, P. *et al.* (1996) "APOPTOSIS AND HUMAN VIRAL INFECTIONS," Ann Biol Clin (Paris)
- 20 54(5):189-197; Gualberto, A. *et al.* (1995) "P53 AND SP1 INTERACT AND COOPERATE IN THE TUMOR NECROSIS FACTOR-INDUCED TRANSCRIPTIONAL ACTIVATION OF THE HIV-1 LONG TERMINAL REPEAT," J Biol Chem. 270(34):19680-19683; Duan, L. *et al.* (1994) "THE TUMOR SUPPRESSOR PROTEIN P53 STRONGLY ALTERS HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 REPLICATION,"
- 25 J Virol. 68(7):4302-4313). Indeed, HIV-1 infected patients with ocular surface squamous neoplasia (OSSN) were commonly found to express p53 (Mahomed, A. *et al.* (2002) ("HUMAN IMMUNODEFICIENCY VIRUS INFECTION, BCL-2, P53 PROTEIN, AND KI-67 ANALYSIS IN OCULAR SURFACE SQUAMOUS NEOPLASIA," Arch Ophthalmol. 2002 May;120(5):554-558). Gualberto, A. *et al.* (1995) ("P53
- 30 AND SP1 INTERACT AND COOPERATE IN THE TUMOR NECROSIS FACTOR-INDUCED TRANSCRIPTIONAL ACTIVATION OF THE HIV-1 LONG TERMINAL REPEAT," J Biol

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Chem. 270(34):19680-19683) discloses a synergistic role for p53 and Sp1 in the mechanism of TNF induction of HIV-1 LTR-mediated transcription and suggest that Sp1 may play an important role in modulating certain functions of p53.

5 **Additional Compounds Whose Assay May Be Conducted In Concert With The Assessment Of The Concentration Or Presence Of TRAIL Compounds**

CD69

CD69 is an early activation marker of peripheral lymphocyte cells (Fogli, M. et al. (2004) "SIGNIFICANT NK CELL ACTIVATION ASSOCIATED WITH 10 DECREASED CYTOLYTIC FUNCTION IN PERIPHERAL BLOOD OF HIV-1-INFECTED PATIENTS," Eur J Immunol. 34(8):2313-2321). CD69 is discussed in U.S. Patent 6,593,124.

Assays for CD69, and polynucleotides that encode CD69, or fragments of such molecules, may be used in concert with assays of TRAIL Compounds in 15 order to monitor the progression of AIDS, and other diseases whose progression involves immune system activation. The amino acid sequence of CD69 is provided by **SEQ ID NO:15**. The sequence of a polynucleotide that encodes CD69 is provided by **SEQ ID NO:16** (see, GenBank Accession Nos. NM 001781 and NP 001772, herein incorporated by reference). The start and stop codons for 20 CD69 are underlined.

SEQ ID NO:15:

MSSENCFVAE	NSSLHPESGQ	ENDATSPHFS	TRHEGSFQVP	VLCAVMNVVF
10	20	30	40	50
ITILIIIALIA	LSVGQYNCPG	QYTFSMPSDS	HVSSCSEDWV	GYQRKCYFIS
25	60	70	80	90
TVKRWSWTSAQ	NACSEHGATL	AVIDSEKDMN	FLKRYAGREE	HWVGLKKKEPG
110	120	130	140	150
HPWKWSNGKE	FNNWFNVTGS	DKCVFLKNTE	VSSMECEKNL	YWICNKPYK
160	170	180	190	199

SEQ ID NO:16:

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1 agactcaaca agagctccag caaagacttt cactgttagct tgacttgacc tgagattaac
61 tagggaatct tgagaataaaa gATGagctct gaaaatttgt tcgttagcaga gaacagctct
121 ttgcacatccgg agagtggaca agaaaaatgtat gccaccagtc cccatttctc aacacgtcat
5 181 gaagggtcct tccaagttcc tgtcctgtgt gctgtaatgta atgtggtctt catcaccatt
241 ttaatcatag ctctcattgc cttatcagtgc gccaataaca attgtccagg ccaatacaca
301 ttctcaatgc catcagacag ccatgtttct tcattgtctg aggactgggt tggctaccag
361 agggaaatgtct acttttatttc tactgtgaag aggagctggaa cttcagccccaa aaatgcttgc
421 tctgaacatg gtgctactct tgctgtcattt gattctgaaa aggacatgaa ctttctaaaaa
10 481 cgatacgcag gtagagagga acactgggtt ggactgaaaaa aggaacctgg tcacccatgg
541 aagtggtaaa atggcaaaaga atttaacaac tggtaacaac ttacagggtc tgacaagtgt
601 gttttctga aaaacacacaga ggtcagcagc atggaaatgtt agaagaattt atactggata
661 tgtaacaaac cttacaaaTA Ataaggaaac atggttcaattt attgacttattt atagaatggaa
721 actcaaggaa atctgtgtca gtggatgctg ctctgtggtc cgaagtcttc catagagact
781 ttgtgaaaaaa aaattttataa gtgtcttggg aattttcttc caaaacagaac tatggaaaaaa
841 aaggaagaaaa ttccaggaaaa atctgcactg tggctttta ttgccatgag cttagaagcat
901 cacaggttga ccaataacca tgcccaagaa tgagaagaat gactatgcaa cctttggatg
961 cactttatat tattttgaat ccagaaataaa tgaaataactt aggcgtggac ttactattt
1021 ttgtgaaatg actaccaaca gtgagagccc ttcatgcattt tgcaactactg gaaggagttt
1081 gatgtggta ctagatactg aatgtaaacaa aaggaattt ggctggtaac atagggttttt
1141 agtctaattt aatcccttaa actcagggag catttataaa tggacaaaatg cttatgaaac
1201 taagattttgt aatattttctc tctttttaga gaaatttgcc aattttactt gtatttttc
1261 cccaaaaaga atgggatgtat cgtgtattta ttttttactt tcctcagctg tagacagggtc
1321 ctttcgatg gtacatattt cttgccttt ataatctttt atacagtgtc ttacagagaa
1381 aagacataag caaagactat gaggaatatt tgcaagacat agaatagtgt tgaaaaatgt
1441 gcaatatgtat atgtggcaaa tctctattag gaaatattctt gtaatcttca gaccttagat
1501 aataactatgtc ttataatagg tttgtgactt tccttaatca attctattac gtgcaataact
1561 tcaataacttc attttaaaata tttttatgtt caataaaaatg tattttttt tattttgtgt
1621 tcagtacaat tataagctgt ttttatatat gtgaaataaa agtagaataaa acacaaaaaaa
30 1681 aaaaaaaaaa aaaaaaaaaa aa

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Stevceva, L. *et al.* (2004) (“FUNCTIONAL SIMIAN IMMUNODEFICIENCY VIRUS GAG-SPECIFIC CD8+ INTRAEPIHELIAL LYMPHOCYTES IN THE MUCOSAE OF SIVMAC251- OR SIMIAN-HUMAN IMMUNODEFICIENCY VIRUS KU2-INFECTED MACAQUES,” Virology 319(2):190-200) disclose that SIV-infected intraepithelial lymphocytes (IELs) expressed CD69 and produced IFN- γ . A relationship between immune activation antigens and HIV pathogenesis has recently been described (Dunn, S.J. *et al.* (2004) “IDENTIFICATION OF CELL SURFACE TARGETS FOR HIV-1 THERAPEUTICS USING GENETIC SCREENS,” Virology 321(2):260-273); Sousa, A.E. *et al.* (2002) “CD4 T CELL DEPLETION IS LINKED DIRECTLY TO IMMUNE ACTIVATION IN THE PATHOGENESIS OF HIV-1 AND HIV-2 BUT ONLY INDIRECTLY TO THE VIRAL LOAD,” J Immunol. 169(6):3400-3406; Palmer, B.E. *et al.* (2002) “DISCORDANCE BETWEEN FREQUENCY OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)-SPECIFIC GAMMA INTERFERON-PRODUCING CD4(+) T CELLS AND

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- HIV-1-SPECIFIC LYMPHOPROLIFERATION IN HIV-1-INFECTED SUBJECTS WITH ACTIVE VIRAL REPLICATION," J Virol. 76(12):5925-36; Trimble, L.A. *et al.* (2000) "HUMAN IMMUNODEFICIENCY VIRUS-SPECIFIC CIRCULATING CD8 T LYMPHOCYTES HAVE DOWN-MODULATED CD3ZETA AND CD28, KEY SIGNALING MOLECULES FOR 5 T-CELL ACTIVATION," J Virol. 74(16):7320-7330; Rosok, B.I. *et al.* (1998) "CORRELATES OF APOPTOSIS OF CD4+ AND CD8+ T CELLS IN TONSILLAR TISSUE IN HIV TYPE 1 INFECTION," Aids Res Hum Retroviruses 14(18):1635-1643; Voiculescu, C. *et al.* (1998) "EXPRESSION OF ACTIVATION SURFACE MARKERS, INTERLEUKIN-2 SYNTHESIS AND APOPTOSIS RATE IN FRESH OR CULTURED 10 LYMPHOCYTES FROM HIV-INFECTED CHILDREN," Roum Arch Microbiol Immunol. 57(1):33-44).

CD69 expression may be used in the diagnosis of HIV infection (Nielsen, S.D. *et al.* (1998) "EXPRESSION OF THE ACTIVATION ANTIGEN CD69 PREDICTS FUNCTIONALITY OF IN VITRO EXPANDED PERIPHERAL BLOOD MONONUCLEAR 15 CELLS (PBMC) FROM HEALTHY DONORS AND HIV-INFECTED PATIENTS," Clin Exp Immunol. 114(1):66-72; Prince, H.E. *et al.* (1997) "CD69 EXPRESSION RELIABLY PREDICTS THE ANTI-CD3-INDUCED PROLIFERATIVE RESPONSE OF LYMPHOCYTES FROM HUMAN IMMUNODEFICIENCY VIRUS TYPE 1-INFECTED PATIENTS," Clin Diagn Lab Immunol. 4(2):217-222; Perfetto, S.P. *et al.* (1997) "MEASUREMENT OF 20 CD69 INDUCTION IN THE ASSESSMENT OF IMMUNE FUNCTION IN ASYMPTOMATIC HIV-INFECTED INDIVIDUALS," Cytometry. 30(1):1-9; Krowka, J.F. *et al.* (1996) "EXPRESSION OF CD69 AFTER IN VITRO STIMULATION: A RAPID METHOD FOR QUANTITATING IMPAIRED LYMPHOCYTE RESPONSES IN HIV-INFECTED 25 INDIVIDUALS," J Acquir Immune Defic Syndr Hum Retrovirol. 11(1):95-104). In accord with the principles of the present invention, assays of CD69 may be used in concert with the TRAIL Compounds of the present invention to monitor the progression of AIDS, and other diseases whose progression involves immune system activation.

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HLA-DR

- The HLA-DR antigen is part of the major histocompatibility complex (MHC) that identifies self from non-self and is pivotal in immune recognition of foreign tissue and infective agents and presents a variety of cellular proteins
- 5 necessary for immunity (Andersson, G. (1998) "EVOLUTION OF THE HUMAN HLA-DR REGION," Front Biosci. 27;3:d739-745; Auger, I. *et al.* (1997) "HLA-DR AND THE DEVELOPMENT OF RHEUMATOID ARTHRITIS," Autoimmunity 26(2):123-128; Cabrera, T. *et al.* (1995) "BIOLOGICAL IMPLICATIONS OF HLA-DR EXPRESSION IN TUMOURS," Scand J Immunol. 41(4):398-406; Klajman, A. *et al.* (1990) "THE
- 10 IMPORTANCE OF HLA-DR IN AUTOIMMUNE DISEASES," Isr J Med Sci. 26(12):691-692; Redman, C.W. (1983) "HLA-DR ANTIGEN ON HUMAN TROPHOBlast: A REVIEW," Am J Reprod Immunol. 3(4):175-177; Charron, D.J. (1982) "HLA-DR ANTIGENS: CELLULAR EXPRESSION AND MOLECULAR STRUCTURE," Ann Immunol (Paris). 133D(2):155-169; Tiwari, J.L. *et al.* (1981) "HLA-DR AND DISEASE
- 15 ASSOCIATIONS," Prog Clin Biol Res. 58:151-163).

Assays for HLA-DR (especially HLA-DR alpha), and polynucleotides that encode HLA-DR or fragments of such molecules, may be used in concert with assays of TRAIL Compounds in order to monitor the progression of AIDS, and other diseases whose progression involves immune system activation. The amino acid sequence of HLA-DR alpha is provided by **SEQ ID NO:17**. The sequence of a polynucleotide that encodes HLA-DR alpha is provided by **SEQ ID NO:18** (see, GenBank Accession Nos. NM_019111 and NP_061984, herein incorporated by reference). The start and stop codons for HLA-DR are underlined.

SEQ ID NO:17:

25	MAISGVPVLG	FFIIAVLMSA	QESWAIKEEH	VIIQAEFYLN	PDQSGEFMFD
	10	20	30	40	50
	FDGDEIFHVD	MAKKETVWRLL	EEFGRFASFE	AQGALANIAAV	DKANLEIMTK
	60	70	80	90	100
30	RSNYTPITNV	PPEVTVLTNS	PVELREPNVL	ICFIDKFTPP	VVNVTWLRNG
	110	120	130	140	150

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KPVTTGVSET	VFLPREDHLF	RKFHYLPFLP	STEDVYDCRV	EHWGLDEPLL
160	170	180	190	200

KHWEFDAPSP	LPETTENVVC	ALGLTVGLVG	IIIGTIFIIK	GLRKSNAER
210	220	230	240	250

5 GPL
 254

SEQ ID NO:18:

1	acattcttctt ttcttttatt cttgtctgtt ctgcctcaact cccgagctct actgactccc
61	aacagagcgc ccaagaagaa a <u>ATG</u> ccata agtgaggatcc ctgtgttagg attttcatc
10	121 atagctgtgc ttagtggcg tcaggaaatca tgggctatca aagaagaaca tgtgatcatc
	181 caggccgagt tctatctgaa tcctgaccaa tcaggcgagt ttatgttga ctgtatgg
	241 gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggct tgaagaattt
	301 ggacgatttg ccagcttga ggctcaaggt gcattggcca acatagctgt ggacaaagcc
15	361 aacctggaaa tcatgacaaaa ggcgtccaaac tatactccga tcaccaatgt acctccagag
	421 gtaactgtgc tcacgaacag ccctgtggaa ctgagagagc ccaacgtcct catctgttcc
	481 atagacaagt tcaccccaacc agtggtaat gtcacgtggc ttcgaaatgg aaaacctgtc
	541 accacaggag tgcagagac agtcttcctg cccagggaaag accaccttt ccgcaagttc
	601 cactatctcc ctttcctgccc ctcaactgag gacgtttacg actgcagggt ggagcactgg
20	661 ggcttggatg agccttcttca agcactgg gagttgtatg ctccaagccc tctcccagag
	721 actacagaga acgtgggtgtg tgccctggc ctgactgtgg gtctgggtgg catcattatt
	781 gggaccatct tcatcatcaa gggattgcgc aaaagcaatg cagcagaacg cagggggccct
	841 ctg <u>TAA</u> ggca catggagggtg atgggttttc ttagagagaa gatcaactgaa gaaacttctg
	901 cttaatggc ttacaaagc tggcaatatt acaatccttgc acctcaatgt aagcagtcat
25	961 cttcagcatt ttccagccct atagccaccc caagtgtgg tatgcctt cgattgctcc
	1021 gtactctaacc atctagctgg cttccctgtc tattgcctt tcccttatct attttcctct
	1081 atttcctatc attttattat caccatgcaat tgcctctggataaaaacata caggagtctg
	1141 tctctgtat ggaatgcccc atggggcatc tcttgtgtac ttattgttta agtttcctc
	1201 aaactgtgtat ttttctgaac acaataaaact attttcatgt aacatgggtgg aaaaaaaaaaa
	1261 aaaaaaaaaaa

30	Higher levels of HLA-DR have been found in HIV-1 infected individuals, relative to uninfected individuals (Khudiakova, N.E. (2004) ("SOLUBLE HLA CLASS I AND HLA CLASS II ANTIGENS IN THE BLOOD OF HIV INFECTED PATIENTS," Zh Mikrobiol Epidemiol Immunobiol. (1):42-45). Additionally, Resino, S. <i>et al.</i> (2003) reported that children having low viral loads exhibited enhanced HLA-DR expression relative to children having higher viral loads (Resino, S. <i>et al.</i> (2003) "CHARACTERIZING THE IMMUNE SYSTEM AFTER LONG-TERM UNDETECTABLE VIRAL LOAD IN HIV-1-INFECTED CHILDREN," J Clin Immunol. 23(4):279-289). This finding of an inverse correlation between viral load and HLA-DR expression appears to be found in multiple reports (see, for example, Sindhu, S.T. <i>et al.</i> (2003) "VIRUS LOAD CORRELATES INVERSELY WITH
35	
40	

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THE EXPRESSION OF CYTOTOXIC T LYMPHOCYTE ACTIVATION MARKERS IN HIV-1-INFECTED/AIDS PATIENTS SHOWING MHC-UNRESTRICTED CTL-MEDIATED LYSIS," Clin Exp Immunol. 132(1):120-127). However, it has also been reported that untreated AIDS patients had higher levels of HLA-DR than HAART-treated patients (see, Lange, C.G. *et al.* (2002) "IMPACT OF SUPPRESSION OF VIRAL REPLICATION BY HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON IMMUNE FUNCTION AND PHENOTYPE IN CHRONIC HIV-1 INFECTION," J Acquir Immune Defic Syndr. 30(1):33-40; Resino, S. (2001) "RELATIONSHIP BETWEEN T-CELLS SUBSETS AND PROGNOSTIC MARKERS IN HIV-1-INFECTED CHILDREN," Med Clin Barc). 2001 Jul 14;117(6):201-206).

It has been reported that HLA-DR may be used to predict HIV-progression (Gascon, R.L. *et al.* (2002) "INCREASED HLA-DR EXPRESSION ON PERIPHERAL BLOOD MONOCYTES IN SUBSETS OF SUBJECTS WITH PRIMARY HIV INFECTION IS ASSOCIATED WITH ELEVATED CD4 T-CELL APOPTOSIS AND CD4 T-CELL DEPLETION," J Acquir Immune Defic Syndr. 30(2):146-153; Choi, B.S. *et al.* (2002) "THE CD28/HLA-DR EXPRESSIONS ON CD4+T BUT NOT CD8+T CELLS ARE SIGNIFICANT PREDICTORS FOR PROGRESSION TO AIDS," Clin Exp Immunol. 2002 Jan;127(1):137-144; Leng, Q. *et al.* (2001) "IMMUNE ACTIVATION CORRELATES BETTER THAN HIV PLASMA VIRAL LOAD WITH CD4 T-CELL DECLINE DURING HIV INFECTION," J Acquir Immune Defic Syndr. 27(4):389-397; [Authors Not Listed] (1999) "T-CELL ACTIVATION PREDICTIVE OF POOR SURVIVAL IN ADVANCED DISEASE," AIDS Treat News. 317:3; Aldhous, M.C. *et al.* (1996) "CD4 AND CD8 LYMPHOCYTES IN DIAGNOSIS AND DISEASE PROGRESSION OF PEDIATRIC HIV INFECTION," Pediatr AIDS HIV Infect. 7(1):20-30).

25 **α -Interferon / β -interferon (IFN α/β)**

Type I interferon (interferon-alpha and beta: IFN- α and IFN- β) regulate membrane-bound (mTRAIL) and soluble TRAIL (sTRAIL) (Sato, K. *et al.* (2001) "ANTIVIRAL RESPONSE BY NATURAL KILLER CELLS THROUGH TRAIL GENE INDUCTION BY IFN-ALPHA/BETA," Eur J Immunol. 31:3138-3146), and are TRAIL

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Compounds of the present invention. The amino acid sequence of interferon alpha is provided by **SEQ ID NO:19**. The sequence of a polynucleotide that encodes interferon alpha is provided by **SEQ ID NO:20** (see, GenBank Accession No. AL390882, herein incorporated by reference). The gene encoding IFN α is located at positions 1806-2829 of **SEQ ID NO:20**. Position numbering is from AL390882. The start and stop codons for interferon alpha are underlined.

SEQ ID NO:19:

MALSFSLMA	VLVLSYKSIC	SLGCDLPQTH	SLGNRRALIL	LAQMGRISPF
10	20	30	40	50
10 SCLKDRHDFG	FPQEEFDGNQ	FQKAQAIISVL	HEMIQQTFNL	FSTKDSSATW
60	70	80	90	100
EQLSLEKFST	ELNQQLNDLE	ACVIQEVGVE	ETPLMNVDI	LAVKKYFQRI
110	120	130	140	150
TLYLTEKKYS	PCAWEVVRAE	IMRSFSLSKI	FQERLRRKE	
15 160	170	180	189	

SEQ ID NO:20:

1801 caaggttcaa gtttacccat ctcaagttagc ctagcaatat tggcaacatc cca <u>ATG</u> gcc
1861 tgtcttttc ttactgatg gccgtgctgg tgctcagcta caaatccatc tgttctctgg
1921 gctgtgatct gcctcagacc cacaggctgg gtaataggag ggccttgata ctccctggcac
20 1981 aaatgggaag aatctctcct ttctcctgcc tgaaggacag acatgacttt ggattcccc
2041 aggaggagtt tggatggcaac cagttccaga aggctcaagc catctctgtc ctccatgaga
2101 tgatccagca gaccttcaat ctcttcagca caaaggactc atctgctact tggaaacaga
2161 gcctcctaga aaaattttcc actgaactta accagcagct gaatgacactg gaaggctgcg
2221 tgatacagga gtttgggggtg gaagagactc ccctgtatgaa tgtggactcc atccctggctg
2281 tgaagaaaata cttccaaaga atcactctt atctgacaga gaagaaaatac agcccttgtg
2341 cctggaggt tgcagagca gaaatcatga gatccttctc tttatcaaaa attttcaag
2401 aaagattaag gaggaaggaa <u>TGA</u> aacctgt ttcaacatgg aaatgatctg tattgactaa
2461 tacaccagtc cacacttcttca tgacttctgc catttcaaag actcatttct cctataacca
2521 ccgcatgagt tgaatcaaaa tttttagatc ttttcaggag tggtaaggaaa catcatgttt
2581 acctgtgcag gcaactgtcc tttacagatg accatgtga tagatcta tatcttatcta
2641 ttgaaatatt tatttattttt ttagatttaa attattttttgc tccatgtaat attatgtgt
2701 cttttacatt gtgttatatc aaaatatgtt atttataattt agtcaatata ttattttctt
2761 ttttatttttatttttactttaaaacttctta tatttattttgt ttatttttta ataaagaaat
2821 accaagccca

35 The amino acid sequence of interferon beta is provided by **SEQ ID NO:21**. The sequence of a polynucleotide that encodes interferon beta is provided by **SEQ ID NO:22** (see, GenBank Accession No. AL390882, herein incorporated by reference). The gene encoding IFN β is located at positions 90503-91361 of **SEQ**

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ID NO:22. Position numbering is from AL390882. The start and stop codons for interferon beta are underlined.

SEQ ID NO:21:

	MTNKCLLQIA	LLLCFSTTAL	SMSYNLLGFL	QRSSNFQCQK	LLWQLNGRLE
5	10	20	30	40	50
	YCLKDRMNFD	IPEEKQLQQ	FQKEDAALT	YEMLQNIFAI	FRQDSSSTGW
	60	70	80	90	100
	NETIVENLLA	NVYHQINHLK	TVLEEKLEKE	DFTRGKLMSS	LHLKRYYGRI
	110	120	130	140	150
10	LHYLKAKEYS	HCAWTIVRVE	ILRNFYFINR	LTGYLRN	
	160	170	180	187	

SEQ ID NO:22:

15	90481	aggaccatct	catataaaata	ggccatataccc	atggagaaaag	gacattctaa	ctgcacacccc
	90541	tgcgttgttt	tgcgttgttt	caacaggtag	taggcacac	tgttcgtgtt	gtcaac <u>ATG</u> a
	90601	ccaacaagtg	tctcctccaa	attgtctcc	tgttgtgtt	ctccactaca	gcttttcca
	90661	ttagtaccaa	cttgcttggaa	ttcctacaaa	gaagcagca	ttttcagtgt	cagaagctcc
	90721	tgtggcaatt	gaatggggagg	cttgaatact	gcctcaagga	caggatgaac	tttgacatcc
	90781	ctgaggagat	taagcagctg	cagcagttcc	agaaggagga	cgccgcattt	accatctatg
20	90841	agatgtccca	gaacatcttt	gctattttca	gacaagattt	atctagca	ggcttggaaatg
	90901	agactattgt	tgagaacctc	ctggctaatg	tctatcatca	gataaaaccat	ctgaagacag
	90961	tccttggaaaga	aaaactggag	aaagaagatt	tccaccagggg	aaaactcatg	agcagtctgc
	91021	acctggaaag	atattatggg	aggattctgc	attacctgaa	ggccaaggag	tacagtca
	91081	gtgcctggac	catagtca	gtggaaatcc	taaggaactt	ttacttcatt	aacagactta
	91141	caggttacct	ccgaaac <u>TGA</u>	agatctcc	gcctgtgcct	ctgggactgg	acaattgttt
25	91201	caagcattct	tcaaccaggca	gatgtgtttt	aagtgtactga	tggcta	atgtcatatg
	91261	aaaggacact	agaagatttt	gaaattttta	ttaaaattatg	agtttatttt	atttattttaa
	91321	attttatttt	ggaaaataaa	ttatttttgg	tgcaaaagtc	aacatggcag	tttttaatttc

Preferred Methods for the Use of TRAIL Compounds

The present invention provides a method for determining whether a
30 mammal (especially a human, simian, feline, bovine, equine, canine, ovine or
porcine mammal) suffers from a disease or condition of involving immune system
activation, wherein the method comprises assaying for the presence or
concentration of one or more TRAIL Compound(s) in a biological fluid of said
mammal. As used herein, examples of such diseases and conditions whose
35 progression involves “immune system activation” include HIV (FIV, SIV, etc.)
infection, AIDS, certain cancers (e.g., colon cancer, lung cancer, breast cancer,

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pancreatic cancer, leukemia, myeloma, skin cancer, brain cancer, cervical cancer, testicular cancer, etc.), atherosclerosis, Alzheimer's disease, inflammatory diseases and autoimmune disorders (especially, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant

- 5 rejection, graft vs. host disease, etc.

In one embodiment, the methods and compositions of the present invention may be used to diagnose whether such a mammal is healthy, or suffers from such diseases and conditions. In an alternative embodiment, the methods and compositions of the present invention may be used to monitor the progression, 10 stage, or severity of a disease or condition in humans and other mammals known to suffer from such a disease or condition.

The present invention particularly provides a method for diagnosing AIDS and other diseases and conditions involving immune system activation. In a preferred embodiment, the present invention permits one to distinguish among 15 HIV-1-infected patients with high viral loads, patients with low viral loads and uninfected healthy control blood donors versus by measuring plasma levels of one or more TRAIL Compounds, and to relate the quantity of viral particles in an individual to the level(s) of TRAIL Compounds expressed or secreted by cells (e.g., monocytes) and to recognize that the quantity of HIV-1-mediated TRAIL 20 Compound production by such cells is regulated by type I interferon.

In accord with a second aspect of the present invention, since TRAIL Compound expression/production is a marker of immune system activation (e.g., TRAIL is produced after HIV-1 infection), the TRAIL Compounds can be used as markers for evaluating the progression of all diseases and conditions involving 25 immune system activation.

In one embodiment, the invention measures the presence or concentration of a TRAIL Compound in a fluid of a patient. In another preferred embodiment the invention measures the presence or concentration of sTRAIL or mTRAIL in a fluid of a patient. Suitable fluid can comprise blood, serum, plasma, urine, saliva,

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semen, cerebrospinal fluid, lymph, etc. Preferably, such fluid will be blood, serum, or plasma. The determination of the presence or concentration of sTRAIL may be accomplished using any molecule capable of specific binding with sTRAIL (e.g., a receptor or ligand of sTRAIL). In preferred embodiments, the invention will
5 employ an immunoassay to determine the concentration or presence of sTRAIL in such a fluid. Such assays use the immunospecific binding of an antibody and an antigen to facilitate the detection of the sTRAIL. As used herein the term “antibody” is intended to encompass both naturally produced immunoglobulins (e.g., IgG, etc.), as well as fragments of such molecules (e.g., Fab, F(ab)₂, etc.) that
10 are capable of specifically binding an antigen, as well as recombinant antigen-binding molecules (e.g., humanized antibodies, single chain antibodies, etc.). In a preferred embodiment, the invention will employ a PE-conjugated mouse IgG1 anti-human TRAIL monoclonal antibody.

Any of a wide variety of assay formats may be used in accordance with the
15 methods of the present invention. Such formats may be heterogeneous or homogeneous, sequential or simultaneous, competitive or noncompetitive. U.S. Patent Nos. 5,563,036; 5,627,080; 5,633,141; 5,679,525; 5,691,147; 5,698,411; 5,747,352; 5,811,526; 5,851,778; and 5,976,822 illustrate several different assay formats and applications. Such assays can be formatted to be quantitative, so as to
20 measure the concentration or amount of the selected TRAIL Compound, or they may be formatted to be qualitative, so as to measure the presence or absence of the selected TRAIL Compound. Enzymatic-binding assay formats (e.g., Enzyme-Linked Immunosorbent Assay (ELISA)), are particularly preferred.

Heterogeneous immunoassay techniques typically involve the use of a solid
25 phase material to which the reaction product becomes bound, but may be adapted to involve the binding of non-immobilized antigens and antibodies (i.e., a solution-phase immunoassay). The reaction product is separated from excess sample, assay reagents, and other substances by removing the solid phase from the reaction mixture (e.g., by washing). One type of solid phase immunoassay that may be
30 used in accordance with the present invention is a sandwich immunoassay. In a

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typical sandwich assay, the more TRAIL Compound analyte (e.g., sTRAIL, mTRAIL, etc.) present in the sample, the greater the amount of label present on the solid phase. This type of assay format is generally preferred, especially for the visualization of low analyte concentrations, because the appearance of label on the
5 solid phase is more readily detected.

In accordance with a preferred embodiment of such a heterogeneous assay, a “first” antibody bound to a solid support (i.e., immobilized) is incubated in the presence of a biological sample being tested for the presence of sTRAIL or mTRAIL. A “second” antibody, which has been detectably labeled and which is
10 capable of specifically binding with the TRAIL Compound is provided. In one sub-embodiment, the “first” antibody will be an anti-TRAIL antibody; alternatively, it may be an anti-“first” antibody. In either case, the presence of a TRAIL Compound in the sample being tested results in the immobilization of the detectably labeled “second” antibody. The extent of such immobilization is
15 proportional to the amount of TRAIL Compound present in the sample.

As will be appreciated, the “first” antibody may be incubated with the biological sample in an unbound state and then subsequently bound to the solid support (i.e., immobilizable). The supports are then preferably extensively treated (e.g., by washing, etc.) to substantially remove unbound detectably labeled
20 antibody that may be present. Sandwich assay formats are described by Schuurs *et al.* U.S. Patent Nos. 3,791,932 and 4,016,043, and by Pankratz, *et al.*, U.S. Patent No. 5,876,935. The “second” antibody may be a natural immunoglobulin isolated from nonhuman primates (e.g., anti-human IgG murine antibody, anti-human IgG goat antibody, etc.), or can be produced recombinantly or synthetically. It may be
25 an intact immunoglobulin, or an immunoglobulin fragment (e.g., FAb, F(Ab)₂, etc.). As desired, other binding molecules (capable of binding to the TRAIL Compound employed) may be employed in concert with or in lieu of such second antibodies.

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To eliminate the bound-free separation step and reduce the time and equipment needed for a chemical binding assay, a homogeneous assay format may alternatively be employed. In such assays, one component of the binding pair may still be immobilized; however, the presence of the second component of the
5 binding pair is detected without a bound-free separation. Examples of homogeneous optical methods are the EMIT method of Syva, Inc. (Sunnyvale, CA), which operates through detection of fluorescence quenching; the laser nephelometry latex particle agglutination method of Behringwerke (Marburg, Germany), which operates by detecting changes in light scatter; the LPIA latex
10 particle agglutination method of Mitsubishi Chemical Industries (Tokyo, Japan); the TDX fluorescence depolarization method of Abbott Laboratories (Abbott Park, IL); and the fluorescence energy transfer method of Cis Bio International (Paris, France). Any of such assays may be adapted for use in accordance with the objectives of the present invention.

15 The binding assay of the present invention may be configured as a competitive assay. In a competitive assay, the more sTRAIL or mTRAIL present in the test sample, the lower the amount of label present on the solid phase.

In a manner similar to the sandwich assay, a competitive assay can be conducted by providing a defined amount of a labeled selected TRAIL Compound
20 (such as sTRAIL, mTRAIL or rsTRAIL) and determining whether the fluid being tested contains a TRAIL Compound that would compete with the labeled antibody for binding to the support. In such a competitive assay, the amount of captured label is inversely proportional to the amount of analyte present in the test sample. Smith (U.S. Patent No. 4,401,764) describes a competitive assay format using a mixed binding complex that can bind analyte or labeled analyte but in which the analyte and labeled analyte cannot simultaneously bind the complex. Clagett (U.S.
25 Patent No. 4,746,631) describes an immunoassay method using a reaction chamber in which an analyte/ligand/marker conjugate is displaced from the reaction surface in the presence of test sample analyte and in which the displaced analyte/ligand/marker conjugate is immobilized at a second reaction site. The
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conjugate includes biotin, bovine serum albumin, and synthetic peptides as the ligand component of the conjugate, and enzymes, chemiluminescent materials, enzyme inhibitors, and radionucleotides as the marker component of the conjugate.

Li (U.S. Patent No. 4,661,444) describes a competitive immunoassay using a conjugate of an anti-idiotype antibody and a second antibody, specific for a detectable label, in which the detectable response is inversely related to the presence of analyte in the sample. Allen (European Patent Appln. No. 177,191) describes a binding assay involving a conjugate of a ligand analog and a second reagent, such as fluorescein, in which the conjugate competes with the analyte (ligand) in binding to a labeled binding partner specific for the ligand, and in which the resultant labeled conjugate is then separated from the reaction mixture by means of solid phase carrying a binding partner for the second reagent. This binding assay format combines the use of a competitive binding technique and a reverse sandwich assay configuration; i.e., the binding of conjugate to the labeled binding member prior to separating conjugate from the mixture by the binding of the conjugate to the solid phase. The assay result, however, is determined as in a conventional competitive assay in which the amount of label bound to the solid phase is inversely proportional to the amount of analyte in the test sample.

Chieregatt *et al.* (GB Patent No. 2,084,317) describe a similar assay format using an indirectly labeled binding partner specific for the analyte. Mochida *et al.* (U.S. Patent No. 4,185,084) also describe the use of a double-antigen conjugate that competes with an antigen analyte for binding to an immobilized antibody and that is then labeled. This method also results in the detection of label on a solid phase in which the amount of label is inversely proportional to the amount of analyte in the test sample. Sadeh *et al.* (U.S. Patent No. 4,243,749) describe a similar enzyme immunoassay in which a hapten conjugate competes with analyte for binding to an antibody immobilized on a solid phase. Any of such variant assays may be used in accordance with the present invention.

In all such assay formats, at least one component of the assay reagents (e.g., a TRAIL Compound, an antibody, etc.) will preferably be labeled or otherwise rendered detectable by the evolution or quenching of light. Any of a variety of

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detectable moieties (e.g., a fluorescent moiety, a radioactive moiety, an enzyme, a magnetic or paramagnetic particle or bead, a chemiluminescent moiety, etc.) may be employed. Radioisotopic-binding assay formats (e.g., a radioimmunoassay, etc.) employ a radioisotope as such label; the signal is detectable by the evolution 5 of light in the presence of a fluorescent or fluorogenic moiety (see Lucas *et al.* (U.S. Patent No. 5,698,411) and Landrum *et al.* (U.S. Patent No. 5,976,822)). Enzymatic-binding assay formats (e.g., an ELISA, etc.) employ an enzyme as a label; the signal is detectable by the evolution of color or light in the presence of a chromogenic or fluorogenic moiety. Other labels, such as paramagnetic labels, 10 materials used as colored particles, latex particles, colloidal metals such as selenium and gold, and dye particles (see U.S. Patent Nos. 4,313,734; 4,373,932, and 5,501,985) may also be employed. The use of enzymes (especially alkaline phosphatase, β -galactosidase, horse radish peroxidase, or urease) as the detectable label is preferred.

15 The presence of enzymatic labels may be detected through the use of chromogenic substrates (including those that evolve or adsorb fluorescent, UV, visible light, etc.) in response to catalysis by the enzyme label. Detection of label can be accomplished using multiple detectors, multipass filters, gratings, or spectrally distinct fluors (see e.g., U.S. Patent No. 5,759,781), etc. It is particularly 20 preferred to employ peroxidase as an enzyme label, especially in concert with the chromogenic substrate 3, 3', 5, 5'-tetramethylbenzidine (TMB). In the case of labeling of the antibodies with peroxidase as enzyme, it is possible to use the periodate technique (Nakane, P.K. *et al.* (1974) "PEROXIDASE-LABELED ANTIBODY. A NEW METHOD OF CONJUGATION," J Histochem Cytochem. 22:1084- 25 90) or a method reported in which the partners are linked with a heterobifunctional reagent (Ishikawa, E. *et al.* (1983) "ENZYME-LABELING OF ANTIBODIES AND THEIR FRAGMENTS FOR ENZYME IMMUNOASSAY AND IMMUNOHISTOCHEMICAL STAINING," J Immunoassay. 4(3):209-327).

30 Any of a wide variety of solid supports may be employed in the immunoassays of the present invention. Suitable materials for the solid support are

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synthetics such as polystyrene, polyvinyl chloride, polyamide, or other synthetic polymers, natural polymers such as cellulose, as well as derivatized natural polymers such as cellulose acetate or nitrocellulose, and glass, especially glass fibers. The support can take the form of spheres, rods, tubes, and microassay or 5 microtiter plates. Sheet-like structures such as paper strips, small plates, and membranes are likewise suitable. The surface of the carriers can be permeable and impermeable for aqueous solutions. For the method of the present invention, the substrate is coated on to a solid matrix. Any standard immunoassay solid matrix such as a microtitre plate, beads and the like can be used.

10 Although the foregoing description pertains to assaying for the presence of a TRAIL Compound (e.g., sTRAIL) in a biological sample that is a fluid, it will be appreciated that any biological sample that may be rendered fluidic (e.g., tissue or biopsy extracts, cellular extracts, extracts of feces, sputum, etc.) may likewise be employed in the assays of the present invention.

15 Materials for use in the assays of the invention are ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement; one or more containers means vials, tubes and the like; each of the containers means comprising one of the separate elements to be used in the method. For example, in one embodiment, one 20 of the containers means may comprise as a first element a TRAIL Compound (especially, sTRAIL, mTRAIL or rsTRAIL) bound to a solid support. A second container may comprise as a second element a detectably labeled antibody, preferably in lyophilized form, or in solution, that specifically binds to the employed TRAIL Compound. In using the kit, a user adds to a container a premeasured amount of a sample suspected of containing a measurable yet 25 unknown amount of a TRAIL Compound, and a premeasured amount of support-bound TRAIL Compound and the detectably labeled antibody. To the extent that a TRAIL Compound (e.g., sTRAIL, etc.) is present in the sample being tested, it will compete for antibody binding to the immobilized TRAIL Compound. After an appropriate time for incubation, an immune complex is formed and is separated 30

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from the supernatant fluid, and the immune complex or the supernatant fluid are detected, as by radioactive counting, addition of an enzyme substrate, and color development, or by inclusion of a chemical label (e.g., colloidal gold, latex beads, etc.). Thus, the amount of labeled antibody bound to the support will be inversely proportional to the amount of the selected TRAIL Compound in the sample.

In an alternative embodiment, one of the containers means may comprise as a first element an anti-TRAIL Compound antibody bound to a solid support. A second container may comprise as a second element a detectably labeled antibody, preferably in lyophilized form, or in solution, that specifically binds to a TRAIL Compound. In using the kit, a user adds to a container a premeasured amount of a sample suspected of containing a measurable yet unknown amount of the TRAIL Compound (e.g., sTRAIL), and a premeasured amount of support-bound antibody and the detectably labeled antibody. To the extent that the TRAIL Compound is present in the sample being tested, it will bind to the immobilized antibody, and thus become itself immobilized. After an appropriate time for incubation, an immune complex is formed and is separated from the supernatant fluid, and the immune complex or the supernatant fluid are detected, as by radioactive counting, addition of an enzyme substrate, and color development, or by inclusion of a chemical label (e.g., colloidal gold, latex beads, etc.). Thus, the amount of labeled antibody bound to the support will be directly proportional to the amount of the TRAIL Compound in the sample.

In addition, the kit may also contain one or more containers, each of which comprises a (different) predetermined amount of a TRAIL Compound (e.g., sTRAIL) or anti-TRAIL Compound antibody. These latter containers can be used to prepare a standard curve into which can be interpolated the results obtained from the sample containing the unknown amount of the selected TRAIL Compound.

The present invention particularly relates to the use of immuno-chromatographic assay formats to detect a TRAIL Compound (especially sTRAIL), and particularly in the diagnosis or monitoring of HIV infection and

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AIDS progression. In a preferred immunochromatographic assay format, two contacting, but spatially distinct, porous carriers are employed. By way of illustration with respect to sTRAIL, the first such carrier may preferably contain a non-immobilized, labeled anti-sTRAIL antibody and the second such carrier will 5 preferably contain an immobilized, but unlabeled antibody. The unlabeled antibody may be an anti-sTRAIL antibody (especially one which binds to a different epitope from that recognized by the labeled antibody) or an antibody that specifically binds to the species of unlabeled antibody employed (e.g., where labeled murine anti-sTRAIL IgG antibodies are employed, the unlabeled antibody 10 may be an anti-murine IgG antibody, etc.). Other TRAIL Compounds may be employed in lieu of sTRAIL.

Preferably, the device will comprise a hollow casing constructed of, for example, a plastic material, etc., in which the first carrier will communicate indirectly with the interior of the casing via a multilayer filter system that is 15 accessible from the device (e.g., by protruding therefrom or by being incompletely covered by the device), such that a serum, plasma, or whole blood test sample can be applied directly to the filter system and will permeate therefrom into the first porous carrier. In such a device, the permeation of fluid containing the TRAIL Compound will cause the non-immobilized labeled antibody of the first carrier to 20 become bound to the migrating TRAIL Compound, and will then permeate into the second carrier. Because the second carrier contains immobilized antibody that binds the migrating complex, immune complexes entering the second carrier will be trapped therein.

Detection of labeled antibody in the carrier containing the immobilized 25 unlabeled antibody thus indicates that a TRAIL Compound is present in the sample being evaluated. The assay can be made quantitative by measuring the quantity of labeled molecules that become bound within the second carrier.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are

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provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1

TNF-related Apoptosis-inducing Ligand (TRAIL) in HIV-1-infected Patients and its in vitro Production by Antigen-presenting Cells

Since sTRAIL has been detected in HIV-1-infected patients, the levels of plasma sTRAIL from HIV-1-infected patients to those of uninfected control donors were compared in order to determine whether sTRAIL levels in AIDS patients were associated with viral load.

10 Exposure of peripheral blood T cells from HIV-1 uninfected donors to infectious HIV-1 or to HIV-1 that had been rendered noninfectious by chemical treatment (Adrithiol-2) (AT-2 HIV-1 particles) results in the expression of membrane TRAIL on CD4⁺ but not CD8⁺ T cells. Therefore, the question of whether mTRAIL and/or sTRAIL would be produced by monocytes, macrophages,
15 and/or dendritic cells from HIV-1-infected and uninfected blood donors upon exposure to AT-2 HIV-1, as well as to infectious HIV-1 was investigated. Both HIV-1_{MN} (CXCR4-tropic) and HIV-1_{Ada} (CCR5-tropic) were used to determine if TRAIL production was HIV-1 coreceptor dependent. Since type I interferons (IFN- α and IFN- β) are required for TRAIL expression and production, the
20 possibilities that inhibition of this pathway would interfere with TRAIL; and that agents that inhibit HIV-1 infection would block HIV-1-induced production of sTRAIL were also investigated. An ex vivo tonsil lymphoid tissue histoculture model was used to determine whether HIV-1 would induce TRAIL in primary human lymphoid tissue.

Methods

Patients. Peripheral blood was collected from 107 HIV-1-infected patients. Longitudinal study of 8 HIV-1-infected patients who, at time = 0, begin antiretroviral therapy. Patients were followed for 40 weeks. One patient (I) was not on antiretroviral therapy (ART) prior to the commencement of the study, and was then started on Tenofivir, D4T, and Kaletra. The patient went off ART and did

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not start another regimen prior to the end of the study. A second patient (II) was naïve prior to starting the study and was enrolled into a study that included Trizivir, Combivir, and Sustiva. Therapy continued through week 40. A third patient (III) was naïve prior to starting the study and was enrolled in a study that
5 included Trizivir, Combivir, and Sustiva. The patient was switched to DDI, Combivir, and Sustiva, which continued through week 40. A fourth patient (IV) was on ART prior to the commencement of the study and was switched to Kaletra and Sustiva at the start of TRAIL study.

Preparation of AT-2-inactivated virions. HIV-1_{MN} (X4-tropic) and HIV-1_{Ada} (R5-tropic) are propagated as described by Rossio, J. L. *et al.* (1998) (“INACTIVATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTIVITY WITH PRESERVATION OF CONFORMATIONAL AND FUNCTIONAL INTEGRITY OF VIRION SURFACE PROTEINS,” *J Virol* 72, 7992-8001). Viral supernatants are inactivated with 1 mM AT-2 for 18 h at 4°C before purification. Virus preparations are
15 produced by sucrose gradient banding in a continuous-flow centrifuge (Rossio, J. L. *et al.* (1998) “INACTIVATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTIVITY WITH PRESERVATION OF CONFORMATIONAL AND FUNCTIONAL INTEGRITY OF VIRION SURFACE PROTEINS,” *J Virol* 72, 7992-8001; Arthur, L. O. *et al.* (1998) “CHEMICAL INACTIVATION OF RETROVIRAL INFECTIVITY BY TARGETING
20 NUCLEOCAPSID PROTEIN ZINC FINGERS: A CANDIDATE SIV VACCINE,” *AIDS Res Hum Retroviruses* 14 Suppl 3, S311-9). Microvesicles, used as a control reagent, are isolated from supernatants of uninfected cell cultures in a manner identical to that used for virus preparation from infected cells (Bess, J.W. *et al.* (1997) “MICROVESICLES ARE A SOURCE OF CONTAMINATING CELLULAR PROTEINS FOUND
25 IN PURIFIED HIV-1 PREPARATIONS,” *Virology* 230:134-144).

Isolation and culture of PBMC and T cells. PBMC are isolated by density centrifugation (Ficoll-Hypaque: Uppsala, Sweden) from citrate-anti-coagulated peripheral blood obtained from healthy, HIV-1-seronegative donors by the Department of Blood Transfusion, NIH, Bethesda. Cells are cultured in RPMI
30 medium (Invitrogen, Gaithersburg, MD) with 10% fetal bovine serum (Sigma, St.

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Louis, MO). CD4⁺ and CD8⁺ cells were purified from PBMC by positive selection with anti-CD4⁺ or anti-CD8⁺ beads (Miltenyi Biotech, Auburn, CA).

Heparinized venous blood was collected from HIV-1-infected patients. CD4⁺ and CD8⁺ cells are purified from PBMC by positive selection with anti-CD4 or anti-CD8 beads (Miltenyi Biotech, Auburn, CA). Macrophages and dendritic cells are generated using monocytes as described by Chomarat, P. (2000) ("IL-6 SWITCHES THE DIFFERENTIATION OF MONOCYTES FROM DENDRITIC CELLS TO MACROPHAGES," Nat Immunol. 1:510-514). Cells were incubated with AT-2 HIV-1 at a final concentration of 50 ng/mL p24 equivalent. Infectious HIV-1MN and HIV-1LAV were used at the same concentration. For all the experiments, T cells isolated by both negative and positive selection are used interchangeably, without noticeable differences in the experimental outcome.

Ex vivo human lymphoid tissue preparation and infection. Human tonsils were obtained from patients undergoing routine tonsillectomy within 6 h from surgery. Tissues were kept in PBS and washed in complete culture medium composed of RPMI 1640 (Invitrogen) containing 15% heat-inactivated FCS (Sigma), non-essential amino acids (1mM), sodium pyruvate 1mM, L-glutamine (292 µg/ml), amphotericin B (2,5 µg/ml) (Gibco BRL, Grand Island, NJ), timentin (310 µg/ml; SmithKline Bechman, Philadelphia, PA), and gentamicin (50 µg/ml; Quality Control, Inc., MD). Tissues were then sectioned into 2 mm³ blocks and placed on top of medium-hydrated collagen sponge gel (Gelfoam, Pharmacia & Upjohn) in complete medium at the air-liquid interface in 6 well plate (Costar, Cambridge, MA) with 9 blocks per sponge in each well in 4 ml of meidum. 24 h later, the culture supernatant was replaced with fresh medium containing 1% Pen/Strep (Gibco BRL), and each tissue block was infected with 1 ng p24_{gag} of an HIV-1 LAV.04 viral stock (3µl, 120 TCID₅₀). Culture medium was sampled and changed every 3 days.

Incubations of PBMC and isolated T cell subsets with viruses. Cells are cultured at 1 x 10⁶ cells/ml in RPMI 10% FCS in 12-well plates. For incubation

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with AT-2 HIV-1, the inactivated virus preparations are added at the initiation of culture to a final concentration of 50 ng/mL p24 equivalent. Infectious HIV-1_{MN} and HIV-1_{LAV} are used at the same concentration.

Plasma quantification of HIV-1 RNA. Plasma HIV-1 RNA is measured by

- 5 quantitative RT-PCR and all data are expressed in copies/ml (Amplicor Monitor; Roche Diagnostic Systems, Brancburg, NY, USA; detection limit 50 copies/ml).

Example 2
Flow Cytometry Investigation

Membrane TRAIL detection. Monocytes are cultured for 24 h in the absence or
10 presence of AT-2 HIV-1_{MN}, AT-2 HIV-1_{Ada} and infectious HIV-1_{MN} viruses. The cells are washed twice in PBS. Membrane TRAIL expression is determined by incubating cells for 20 minutes at room temperature with PE-conjugated mouse IgG1 anti-human TRAIL monoclonal antibody, (clone B-S23 Diaclone, Besançon France) or with relevant control isotype-matched antibodies (at 5 µg/mL each); in
15 PBS containing 2% mouse serum (SIGMA, St. Louis, MO).

Data acquisition and analysis. Cells are acquired on FACSCalibur flow
cytometer (Becton Dickinson), using CellQuest software (Becton Dickinson
Immunocytometry System). Samples are gated on viable cells by forward and
side light scatters, and at least 50,000 live cell events are acquired for each sample.
20 Acquired data is analyzed using Cellquest software (Becton Dickinson).

Soluble TRAIL measurement. Levels of soluble TRAIL are measured in serum of HIV⁺ or HIV⁻ individuals, in PBMC and monocytes supernatants with the commercial TRAIL ELISA kit (Diaclone, Besancon, France), according to the manufacturer's instructions.

25 **Blocking assay.** To block TRAIL production, monocytes are cultured 3 days with AT-2 HIV-1_{MN/Ada} in the presence of of CD4 binding inhibitor soluble CD4 (2 µg/ml), or the fusion inhibitor T20 (2 µg/ml), or the CXCR4 inhibitor AMD-310 (2 µg/ml) (AIDS Research and Reagents Program, NIAID, Bethesda, MD), or in

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presence of both sheep polyclonal anti-human IFN- α (2000 IFN- α neutralizing U/ml) and sheep polyclonal anti-human IFN- β antibodies (500 IFN- β neutralizing U/ml) (BioSource International, Camarillo, CA). Recombinant IFN- α and IFN- β (Peprotech Inc, Rocky Hill, NJ) are used at 200 U/ml. TRAIL production is
5 quantified by ELISA.

Example 3
Real Time Quantitative RT-PCR

RNA extraction and reverse transcription. Total RNA is extracted from monocytes with the acid guanidium thiocyanate-phenol-chloroform method
10 (Chomczynski, P. et al. (1987) "SINGLE-STEP METHOD OF RNA ISOLATION BY ACID GUANIDINIUM THIOCYANATE-PHENOL-CHLOROFORM EXTRACTION," Anal Biochem 162:156-159), modified for TRIzol (Invitrogen, Carlsbad, CA, USA). The amount and the purity of the extracted RNAs are determined by spectrophotometric analysis at 260nm and 280nm. One μ g of total RNA is reverse
15 transcribed into first-strand cDNA in a 20 μ l final volume containing 1 μ M of random hexanucleotide primers, 1 μ M of oligo dT and 200 U of Molony murine leukemia virus reverse transcriptase. (Promega, Madison, WI, USA).

Real Time PCR. cDNA quantification for TRAIL and GAPDH is obtained with a real time PCR technique. Real time PCR is conducted with the ABI Prism
20 7900HT (Applied Biosystems, Foster City, CA, USA). All reactions are performed using a SYBR green PCR mix (QuantiTect SYBR Green PCR, Qiagen, Valencia, CA, USA), according to the following thermal profile: denaturation at 95°C for 15 sec, annealing at 60°C (61°C for TRAIL) for 15 sec, extension at 72°C for 15 sec (data collection is performed during the extension step). The primer
25 sequences are designed using the Primer Express Software v2.0 provided with the ABI Prism 7900HT

TRAIL	Forward	(SEQ ID NO:3)	GCTCTGGGCC	GCAAAAT
	Reverse	(SEQ ID NO:4)	TGCAAGTTGC	TCAGGAATGA A
GAPDH	Forward	(SEQ ID NO:9)	CCACCCATGG	CAAATTCC
30	Reverse	(SEQ ID NO:10)	TGGGATTTCC	ATTGATGACA AG

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All reactions are performed in triplicate (denaturation at 95°C for 15 sec, annealing at 60°C (61°C for TRAIL) for 15 sec, extension at 72°C for 15 sec).. Data analysis is performed with the SDS2.1 software, provided with the ABI Prism 7900HT. The threshold level is determined by the software according to the
5 optimization of the standard curve. Standards are obtained by amplification of a control sample in a PCR reaction, using the same primers, reagents and conditions optimized for the real time analysis. Arbitrary quantity values are assigned to the resulting standard and 4-fold serial dilutions are made to obtain an 8-point standard curve. Results are presented as ratios between the target gene and the GAPDH
10 mRNA.

Western blot. Monocytes were cultured with AT-2 HIV-1_{MN}, AT-2 HIV-1_{ADA}, recombinant IFN- α (10 ng/ml) or IFN- β (10 ng/ml), anti-IFN- α and anti-IFN- β (R & D Systems, Minneapolis MN). After 24 h, the cells were pelleted, washed in PBS and lysed (1% NP-40, 200mM NaCl, 50mM Tris pH 7.5, supplemented with
15 protease inhibitor cocktail [Roche, Indianapolis, IN]). Total protein was quantified by BCA (Pierce, Rockford, IL) and 10 μ g of protein was mixed 1:1 with SDS Protein Gel Buffer (Quality Biologics, Gaithersburg, MD). Samples were run on a 10% or 15% SDS-PAGE gel (Bio-Rad, Hercules, CA). After transfer, the blot was blocked in 3% milk/TBST then incubated with anti-STAT1 or anti-STAT2
20 (Upstate Biotechnology, Inc., Lake Placid, NY) antibodies followed by anti-rabbit HRP secondary antibody (Jackson, West Grove, PA). ECL was performed and bands were visualized on Hyperfilm (Amersham, Piscataway, NJ). B-actin was visualized as a loading control using a mouse monoclonal antibody (Sigma).

Statistical Analysis. All experiments were repeated a minimum of three times.
25 All comparisons of data were made with a two-sided Student's t test (data meet the requirements for a Student's t test). Differences in plasma TRAIL between HIV-1-infected patients and healthy donors were also analyzed using F-test to compare variances. All the statistic data were analyzed using Prism software (GraphPad, San Diego, CA).

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Example 4
TRAIL Levels In Plasma Of AIDS Patients And Uninfected Controls

To determine whether TRAIL is produced in AIDS patients as a result of infection with HIV-1, plasma from patients with AIDS, containing higher levels of soluble TRAIL, is tested and compared to healthy donors. As shown in **Figure 1, Panel A** the mean values of plasma sTRAIL are 852 ± 79 pg/ml for 53 control donors, 1339 ± 79 pg/ml for 49 HIV-1-infected patients with undetectable viral load (<50 mRNA copies/ml of blood) and 2240 ± 131 pg/ml for 58 HIV-1-infected patients with detectable viral load (>50 mRNA copies/ml of blood). These results suggest that in vivo TRAIL expression (and particularly sTRAIL expression) is increased in HIV-infected patients and is linked to the viral load. However, there appeared to be a saturation of the sTRAIL level in patients (12/58) with viral loads exceeding 40,000 RNA copies/mL, since higher viral loads were not associated with further increases in sTRAIL.

The correlation between viral loads and the CD4 counts of the 59 patients (with high viral load) and the TRAIL levels in plasma is determined. As shown in **Figure 1, Panel B** a positive correlation between viral load and TRAIL in plasma was found. The sTRAIL values obtained from the 58 HIV-1-infected patients with detectable viral load are plotted and compared with the predicted TRAIL values (solid diagonal line), assuming a perfect correlation between TRAIL and viral load.

The data of **Figure 1, Panel C** provide a longitudinal study of four HIV-1-infected patients who began HAART therapy and were subsequently followed for 40 weeks with measurement of plasma viral load and sTRAIL. Patient I exhibited an initial parallel drop in viral load and sTRAIL, followed by a concomitant rebound in both of these parameters. This rebound in viral load may reflect development of HIV-1 drug resistance. Patient II showed a continuous parallel drop in viral load and sTRAIL. Patient III showed three-of-four points in which changes in viral load and sTRAIL levels were similar. Patient IV, who had received ART prior to enrollment into this study, exhibited a parallel flat and low profile for both viral load and sTRAIL throughout the 40 weeks. This patient's

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- initial low viral may have been due to successful ART prior to enrollment, which continued during this ART protocol.

Thus, both cross-sectional and longitudinal association between higher plasma viral loads and elevated sTRAIL levels were observed in two different cohorts of HIV-1-infected patients, and a parallel between ART-induced reduction in viral load and sTRAIL levels was observed.

Example 5
Noninfectious HIV-1 Induces TRAIL Production By Monocytes

To study the effects of noninfectious viruses, PBMC from healthy HIV-10 uninfected donors and AIDS patients are cultured in the presence of microvesicles (negative control), AT-2 HIV-1_{MN} (CXCR4-tropic) or AT-2 HIV-1_{Ada} (CCR5-tropic) for 3-to-6 days. sTRAIL levels in the culture supernatants are determined by ELISA. TRAIL production in untreated cultures or in cultures of PBMC from control donors treated with microvesicles (n=10) is not detected. In contrast 15 unstimulated PBMC from AIDS patients produced 190 ± 10 pg/ml of sTRAIL (n=16). Exposure to AT-2 HIV-1_{MN} for 3 days induced sTRAIL production by PBMC from control donors (800 ± 160), as well as from AIDS patients (760 ± 140). Similarly, exposure to AT-2 HIV-1_{Ada} induced TRAIL production by both PBMC from controls (900 ± 300) and HIV-1-infected patients (640 ± 300) (**Figure 2, Panel** 20 **A**).

To determine which cell types present in PBMC are producing sTRAIL in response to AT-2 HIV-1, isolated CD4⁺ and CD8⁺ T cells and monocyte-derived macrophages and dendritic cells are tested for TRAIL-production after culture with AT-2 HIV-1. Monocytes from HIV-1-infected patients, but not from healthy 25 donors, produced sTRAIL (130 ± 40 pg/ml) when cultured without AT-2 HIV-1. Monocytes produced high levels of sTRAIL after exposure to AT-2 HIV-1 (MN or Ada) (**Figure 2 Panel B**). No significant differences were observed between monocytes from uninfected donors and AIDS patients. Exposure of monocyte-derived dendritic cells from HIV-1-infected but not from uninfected donors

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produced significant amounts (210 ± 20 pg/mL), although lower levels of sTRAIL than monocytes (1100 ± 210) (**Figure 2, Panel B**). Because membrane and soluble TRAIL are two forms of active TRAIL, the expression of mTRAIL was also studied. Increased expression of mTRAIL in AT-2 HIV-1-exposed monocytes 5 (p<0.0001), compared to microvesicle-treated or untreated monocytes (controls) is found. After AT-2 HIV-1_{MN} exposure for 3 days, 80±8 % of monocytes expressed mTRAIL with a MFI = 19±3 compared to 7% (MFI= 6±1) of control monocytes (**Figure 2, Panel C**). Similar results are obtained using AT-2 HIV-1_{Ada}.

Monocytes from uninfected donors treated with AT-2 HIV-1_{MN} are also 10 tested for TRAIL mRNA expression using a real time quantitative PCR TRAIL mRNA is barely detectable in untreated or microvesicles-treated monocytes, whereas exposure to AT-2 HIV-1_{MN} induced 20-fold increase in TRAIL mRNA expression (p=0.0045) (**Figure 2, Panel D**). These results indicated that 15 monocytes expressed TRAIL mRNA and produced sTRAIL and mTRAIL upon exposure to AT-2 HIV-1. In contrast, sTRAIL was not produced by CD4+ or CD8+ T cells nor by macrophages. However, CD4+ T cells express mTRAIL but not sTRAIL after exposure to AT-2 HIV-1.

To investigate whether low amount of virus induce sTRAIL production, a dose response experiment is performed in which monocytes were cultured for 3 20 days with dilutions of AT-1 HIV-1, spanning 4 logs of concentration. Very low concentrations of AT-2 HIV-1_{MN} induced measurable production of sTRAIL (**Figure 2, Panel E**). Optimal concentrations of AT-2 HIV-1_{MN} for sTRAIL production were in the 5-to-50 ng/ml (p24^{CA} equivalents) range, with no further increase in TRAIL at higher virus concentrations. Therefore, AT-2 HIV-1 was 25 used at 50 ng/ml for subsequent experiments.

Example 6

Noninfectious HIV-1 Induced TRAIL Production By The Type I IFN Pathway

To test whether type I IFN are required for TRAIL production by monocytes isolated from PBMC of control donors, an attempt was made to block

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TRAIL production using anti-IFN- α and/or anti-IFN- β antibodies. Anti-IFN- α Ab or anti-IFN- β Ab inhibited the sTRAIL production of AT-2 HIV-1-exposed monocytes by 25 \pm 11 % and 40 \pm 3 % inhibition of TRAIL production, respectively. When used together, these antibodies reduced TRAIL production by 76 \pm 4 % (n = 6 experiments) in monocytes exposed to AT-2 HIV-1_{MN} (p<0.00001) (**Figure 3, Panel A**). Similar results were obtained using HIV-1_{Ada}. The effect of Ab against type I interferons on transcription of the TRAIL gene is also tested. Addition of anti-IFN- α and - β blocking mAb decreased TRAIL mRNA expression by 70 \pm 30 % (p=0.046) (**Figure 3, Panel B**). To determine whether IFN- α and IFN- β were both involved in TRAIL production, monocytes were cultured in the presence of recombinant IFN- α or IFN- β . IFN- α induced 730 \pm 200 pg/ml and IFN- β 1650 \pm 300 pg/ml of sTRAIL (**Figure 3, Panel C**). Because AT-2 HIV-1 induction of TRAIL is IFN- α / β -dependent, it was verified that monocytes from uninfected donors cultured with AT-2 HIV-1_{MN} or AT-2 HIV-1_{Ada} produced IFN- α (**Figure 3, Panel D**). Moreover, it is demonstrated that AT-2 HIV-1_{MN} and AT-2 HIV-1_{Ada}, as well as recombinant IFN- α / β , increased monocyte expression of STAT1 and STAT2 (**Figure 3, Panel E**), the principal signaling molecules for type I IFN (Qureshi, S.A. *et al.* (1995) "TYROSINE-PHOSPHORYLATED STAT1 AND STAT2 PLUS A 48-KDA PROTEIN ALL CONTACT DNA IN FORMING INTERFERON-STIMULATED-GENE FACTOR 3," Proc Natl Acad Sci U S A. 92:3829-3833; Leung, S. *et al.* (1995) "ROLE OF STAT2 IN THE ALPHA INTERFERON SIGNALING PATHWAY," Mol Cell Biol. 15:1312-1317). Taken together, these findings indicate that AT-2 HIV-1-induced expression and production of TRAIL by monocytes is dependent on type I interferons.

Tests were conducted as to whether noninfectious HIV-1-induced production of sTRAIL would be inhibited by: 1) soluble CD4, that blocks the interaction between viral gp120 and CD4 expressed on CD4 $^{+}$ cells; 2) AMD-310, that blocks CXCR4 coreceptor binding; 3) T20, that prevents fusion of the virus with the CD4 cell membrane. The results (**Figure 3, Panel F**) demonstrate that only soluble CD4 inhibited HIV-1_{MN} from inducing sTRAIL production. These

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results are consistent with a triggering mechanism that is dependent on very early CD4/gp120 binding events, but which does not require early post-binding events such as co-receptor engagement or membrane fusion.

All of the results presented above were reproduced using the infectious
5 counterparts of HIV-1MN and HIV-1Ada. Infectious HIV-1LAV, which is commonly used in tonsil model (Grivel, J.C. *et al.* (1999) "CCR5- AND CXCR4-TROPIC HIV-1 ARE EQUALLY CYTOPATHIC FOR THEIR T-CELL TARGETS IN HUMAN LYMPHOID TISSUE," Nat Med. 1999;5:344-346), also induced TRAIL production in monocytes.

10 **Example 7**
Infectious HIV-1 Induces IFN Type I-Dependent TRAIL Production By Monocytes

The effect of infectious HIV-1 on induction of TRAIL production by monocytes, similar to that obtained using noninfectious HIV-1, is tested. PBMC
15 are cultured for 3 days in the presence of infectious HIV-1_{MN} at the same concentration used for noninfectious HIV-1. Infectious HIV-1_{LAV} is also tested for TRAIL production. As shown on **Figure 4, Panel A** HIV-1_{MN}-stimulated monocytes produced 1400±190 pg/ml of sTRAIL. This level of sTRAIL is not significantly different from the level detected after AT-2 HIV-1_{MN} exposure
20 (p<0.5). HIV-1_{LAV} produces only a slightly lower level of sTRAIL. The combination of anti-IFN α and anti-IFN β mAb reduces production of sTRAIL by monocytes cultured with HIV-1_{MN} by 90±3 % (p<0.0001) (**Figure 4, Panel B**). Infectious HIV-1_{MN} also induces mTRAIL expression on monocytes at similar levels to those induced by noninfectious virus. After exposure to infectious HIV-
25 1_{MN}, 75±8 % of monocytes expressed mTRAIL, with a MFI=15±3 (**Figure 4, Panel C**).

Example 8
Infection With HIV-1 Induces TRAIL Production In Tonsil Tissue Culture

To investigate whether TRAIL could be produced in primary lymphoid
30 organs, human tonsils were infected with infectious HIV-1_{Lav}, the HIV-1 strain

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used previously in this model (Grivel, J.C. *et al.* (1999) "CCR5- AND CXCR4-TROPIC HIV-1 ARE EQUALLY CYTOPATHIC FOR THEIR T-CELL TARGETS IN HUMAN LYMPHOID TISSUE," Nat Med. 1999;5:344-346), and the TRAIL production was measured at different times ranging from 1-to-12 days. As shown in **Figure 5**,

5 **Panels A-C**, for each of three independent experiments using tissues from three different individuals, increased TRAIL secretion after infection with HIV-1_{Lav} compared to uninfected tonsils ($p<0.001$) is observed. These results suggest that sTRAIL can be found in the primary lymphoid organs of HIV-1-infected patients, as well as in their blood (see **Figure 1**).

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Example 9

TNF-Related Apoptosis-Inducing Ligand (TRAIL) In HIV-1-Infected Patients And Its In Vitro Production By Antigen-Presenting Cells

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As indicated above, elevated sTRAIL levels are found in the plasma of HIV-1-infected patients compared to healthy control donors. After in vitro exposure to either infectious or noninfectious HIV-1, it is found that both sTRAIL and mTRAIL are produced by monocytes. Monocyte-derived dendritic cells from HIV-1-infected patients also produce low levels of sTRAIL. In contrast, sTRAIL is not produced by macrophages or by CD4⁺ or CD8⁺ T cells upon exposure to HIV-1. TRAIL production is associated with increased transcription of the TRAIL gene in monocytes exposed to HIV-1 particles, and is mediated by the type I IFN pathway. Induction of sTRAIL by HIV-1 is likely to be dependent on CD4-gp120 interaction since it is blocked by soluble CD4. Finally, the ex vivo tonsil model demonstrates that TRAIL can be produced in primary lymphoid tissue after HIV-1 infection.

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Soluble TRAIL was reported in HIV-1-infected patients (Liabakk, N.B. *et al.* (2002) "Development, Characterization And Use Of Monoclonal Antibodies Against Strail: Measurement Of Strail By ELISA," J Immunol Methods. 259:119-128) and in vitro studies linked TRAIL to the depletion of T cells from HIV-1-infected patients (Yang, Y. *et al.* (2003) "Monocytes Treated with Human Immunodeficiency Virus Tat Kill Uninfected CD4(+) Cells by a Tumor Necrosis

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Factor-Related Apoptosis-Induced Ligand-Mediated Mechanism,” J Virol. 77:6700-6708; Miura, Y. *et al.* (2001) “Critical Contribution of Tumor Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL) to Apoptosis of Human CD4(+) T Cells in HIV-1-infected hu-PBL-NOD-SCID Mice. J Exp Med. 5 2001;193:651-660)^{5,29}. However, the identification of TRAIL-producing cells in vivo, the association of viral load with the level of sTRAIL produced, and the mechanism responsible for TRAIL-mediated T cell death remain to be established. The present study demonstrates that sTRAIL can be detected in the plasma of HIV-1-infected patients. Furthermore, the amount of sTRAIL is higher in patients with 10 elevated viral load, than in patients with low or undetectable viral loads. However, plasma from all HIV-1 patients tested showed higher TRAIL content than plasma from healthy donors. Our 40-week longitudinal study of patients on ART showed striking parallel changes between viral load and TRAIL levels. Thus when ART decreased viral load, we observed a concomitant decrease in plasma TRAIL, which 15 may be one of the reasons for the efficacy of antiviral therapy.

More than 95% of HIV-1 virions detected in HIV-1-infected patients’ plasma do not possess culturable infectivity (Piatak, M, Jr. *et al.* (1993) “HIGH LEVELS OF HIV-1 IN PLASMA DURING ALL STAGES OF INFECTION DETERMINED BY COMPETITIVE PCR,” Science. 259:1749-1754), which raises the possibility that 20 noninfectious virus particles contribute to HIV-1 pathogenesis. The above Examples demonstrate that the major cellular source of TRAIL is monocytes, which produce TRAIL in response to both infectious and chemically-inactivated X4 (MN) and R5 (Ada) HIV-1. Interestingly, PBMC and DC from HIV-1-infected donors produced sTRAIL without further stimulation, suggesting that previous 25 contact with HIV-1 was sufficient to induce sTRAIL secretion. However, after 5 days of culture, sTRAIL production disappeared if the patients’ PBMC were not stimulated by HIV-1 particles, suggesting that continuous cell-virus interaction is required to maintain sTRAIL production.

Type I interferons are produced as a result of viral infection and can exert 30 anti-viral effects (Sato, K. *et al.* (2001) “ANTIVIRAL RESPONSE BY NATURAL

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- KILLER CELLS THROUGH TRAIL GENE INDUCTION BY IFN-ALPHA/BETA," Eur J Immunol. 31:3138-3146). Type I interferons also induce TRAIL expression through the interferon-stimulated response element (ISRE), which binds IFN-inducible genes (Kayagaki, N. *et al.* (1999) "TYPE I INTERFERONS (IFNS)
- 5 REGULATE TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) EXPRESSION ON HUMAN T CELLS: A NOVEL MECHANISM FOR THE ANTITUMOR EFFECTS OF TYPE I IFNS," J Exp Med. 1999;189:1451-1460. The above Examples demonstrate that in monocytes, type I interferons are induced by exposure to HIV-1 virions, thereby providing a mechanism for induction of TRAIL
- 10 production in response to HIV-1. Recombinant IFN- α or IFN- β induced sTRAIL production, while antibodies against these interferons greatly reduced both TRAIL gene transcription and production in monocytes exposed to AT-2 HIV-1. The blockade of TRAIL production by soluble CD4, strongly suggests that the interaction between viral gp120 and the CD4 molecule on monocytes is required
- 15 for HIV-1-induction of sTRAIL production. Conversely, the above-described findings that AMD-310 and T20 did not significantly inhibit sTRAIL production suggest that co-receptor binding and viral fusion with the target cell membrane are not required for HIV-1 activation of sTRAIL production by monocytes. Thus, only the earliest interaction between HIV-1 and CD4 on monocytes is important
- 20 for initiation of TRAIL production.

HIV-1 induced TRAIL production was also tested in an ex vivo human tonsil model that resembles human lymphoid organ where critical events of HIV disease occur. Increased TRAIL secretion was seen in tonsil organ cultures from three different donors after HIV-1 infection. TRAIL production was maintained over an extended time period; higher TRAIL production was observed in infected rather than in uninfected tonsil cultures after 10 days. Detection of TRAIL in these HIV-1-infected cultures is consistent with increased apoptosis of CD4 $^{+}$ T cells after HIV-1 infection of tonsil cultures (Grivel, J.C. *et al.* (2000) "HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INDUCES APOPTOSIS IN CD4(+) BUT NOT IN

25 CD8(+) T CELLS IN EX VIVO-INFECTED HUMAN LYMPHOID TISSUE," J Virol.

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74:8077-8084). Because CD4⁺ T cells express mTRAIL but do not produce sTRAIL upon exposure to HIV-1, the sTRAIL detected in plasma of HIV-1-infected patients is unlikely to have been secreted by CD4⁺ T cells, and may have been produced by monocytes and/or dendritic cells after exposure to HIV-1.

- 5 Furthermore, since B cells, present in large number in tonsil, did not produce TRAIL, it is possible that dendritic cells in the tonsil were the source of sTRAIL in the employed HIV-1-infected tonsil cultures.

The findings of the present study, in combination with the present observation that the membrane DR5 death receptors, required for induction of cell 10 death via TRAIL, are expressed on CD4⁺ but not CD8⁺ T cells from HIV-1-infected patients, suggest a plausible mechanism for preferential loss of CD4+ T cells, in HIV-1 infection. In this model sTRAIL and/or mTRAIL produced by monocytes contribute to TRAIL-mediated death of CD4⁺ T cells in HIV-1-infected patients by directly interacting with the DR5 death receptor expressed on CD4⁺ T 15 cells.

Establishment of the in vivo relevance of our in vitro findings to the loss of CD4⁺ T cells in HIV-1-infected patients may point toward novel therapeutic approaches for maintaining immune cell numbers and function. In particular, as indicated above, sCD4 was the most efficient inhibitor of TRAIL production, 20 suggesting that this reagent might provide effective therapy that would inhibit the interaction of either infectious or noninfectious HIV-1 with the CD4 molecule that results in TRAIL production by CD4-expressing cells.

In sum, there is now considerable in vitro evidence that TNF-related apoptosis-inducing ligand (TRAIL) is involved in HIV-1 pathogenesis by inducing 25 CD4+ T cell death characteristic of AIDS. Therefore, the levels of TRAIL in plasma samples from 107 HIV-1-infected and 53 uninfected controls, as well as in longitudinal plasma samples from patients who started antiretroviral therapy (ART) were tested. TRAIL was elevated in plasma of HIV-1-infected patients compared to uninfected individuals, and patients receiving ART showed decreased

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- plasma TRAIL levels that correlated with reduction in viral load. In vitro exposure to infectious and noninfectious HIV-1 induced TRAIL in monocytes and marginally in DC, but not in macrophages, or T cells. Interestingly, the HIV-1 entry inhibitor, soluble CD4, blocked HIV-1-induced production of TRAIL.
- 5 Furthermore, production and gene expression of TRAIL by monocytes were regulated by type I interferon via STAT1/STAT2 signaling molecule. Ex vivo HIV-1 infection of human tonsil lymphoid tissue also resulted in increased TRAIL production. The above examples demonstrate that plasma TRAIL is elevated in HIV-1-infected patients, and is decreased by ART therapy. The high production of
- 10 TRAIL by antigen-presenting cells may contribute to the death of CD4+ T cells during progression to AIDS.
- Example 10**
A Model for Type I Interferon-dependent CD4+ T Cell Apoptosis in AIDS: Two Hits to Death
- 15 The pathogenic mechanisms responsible for the extensive depletion of CD4⁺ T cells characteristic of AIDS have not previously been well understood. As indicated above, although HIV-1-infected T cells die, the frequency of infected cells appears to be too low to account for the depletion of CD4⁺ T cells (Harper, M.E. *et al.* (1986) "DETECTION OF LYMPHOCYTES EXPRESSING HUMAN T-
- 20 LYMPHOTROPIC VIRUS TYPE III IN LYMPH NODES AND PERIPHERAL BLOOD FROM INFECTED INDIVIDUALS BY IN SITU HYBRIDIZATION," Proc Natl Acad Sci U S A 83:772-776; Grivel, J.C. *et al.* (2000) "HUMAN IMMUNODEFICIENCY VIRUS TYPE I INDUCES APOPTOSIS IN CD4⁽⁺⁾ BUT NOT IN CD8⁽⁺⁾ T CELLS IN EX VIVO-INFECTED HUMAN LYMPHOID TISSUE," J Virol 74:8077-8084; Schnittman, S.M. *et al.* (1990) "INCREASING VIRAL BURDEN IN CD4⁺ T CELLS FROM PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION REFLECTS RAPIDLY PROGRESSIVE IMMUNOSUPPRESSION AND CLINICAL DISEASE," Ann Intern Med 113:438-443; Schnittman, S.M. *et al.* (1989) "THE RESERVOIR FOR HIV-1 IN HUMAN PERIPHERAL BLOOD IS A T CELL THAT MAINTAINS EXPRESSION OF CD4," Science 245:305-308). Therefore, indirect mechanisms involving the death of uninfected CD4⁺ T cells have been proposed (Badley, A.D. *et al.* (2000) "MECHANISMS OF
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HIV-ASSOCIATED LYMPHOCYTE APOPTOSIS," Blood 96:2951-2964; Finkel, T.H. *et al.* (1995) "APOPTOSIS OCCURS PREDOMINANTLY IN BYSTANDER CELLS AND NOT IN PRODUCTIVELY INFECTED CELLS OF HIV- AND SIV-INFECTED LYMPH NODES," Nat Med 1:129-134). Several reports suggested that activation-induced cell death
5 (AICD) of T lymphocytes might be a major contributor to CD4⁺ T cell depletion (Groux, H. *et al.* (1992) "ACTIVATION-INDUCED DEATH BY APOPTOSIS IN CD4⁺ T CELLS FROM HUMAN IMMUNODEFICIENCY VIRUS-INFECTED ASYMPTOMATIC INDIVIDUALS," J Exp Med 175:331-340; Ameisen, J.C. *et al.* (1991) "CELL DYSFUNCTION AND DEPLETION IN AIDS: THE PROGRAMMED CELL DEATH
10 HYPOTHESIS," Immunol Today 12:102-105). However, both CD4⁺ and CD8⁺ T cells are activated by HIV-1 infection (Groux, H. *et al.* (1992) "ACTIVATION-INDUCED DEATH BY APOPTOSIS IN CD4⁺ T CELLS FROM HUMAN IMMUNODEFICIENCY VIRUS-INFECTED ASYMPTOMATIC INDIVIDUALS," J Exp Med 175:331-340; Grossman, Z. *et al.* (2002). "CD4⁺ T-CELL DEPLETION IN HIV
15 INFECTION: ARE WE CLOSER TO UNDERSTANDING THE CAUSE"? Nat Med 8:319-323), and both subsets of T cells are susceptible to apoptosis (Meyaard, L. *et al.* (1992) "PROGRAMMED DEATH OF T CELLS IN HIV-1 INFECTION," Science 257:217-219). Therefore, current models of AIDS pathogenesis do not adequately account for the preferential loss of CD4⁺ T cells during progression to AIDS.

20 Type I interferons (IFN α/β) have anti-viral activity, including against HIV-1 (Yamamoto, J.K. *et al.* (1986) "HUMAN ALPHA- AND BETA-INTERFERON BUT NOT GAMMA- SUPPRESS THE IN VITRO REPLICATION OF LAV, HTLV-III, AND ARV-2," J Interferon Res 6:143-152). A recent study indicated that murine viral infections that induce IFN- α/β production, activate STAT1 and STAT2, resulting
25 in expression and synthesis of p53 and apoptosis of virus-infected cells by an yet undefined (Takaoka, A. *et al.* (2003) "INTEGRATION OF INTERFERON-ALPHA/BETA SIGNALLING TO P53 RESPONSES IN TUMOUR SUPPRESSION AND ANTIVIRAL DEFENSE," Nature 424:516-523) but important mechanism that can control viral infection (Vilcek, J. (2003) "BOOSTING P53 WITH INTERFERON AND VIRUSES," Nat
30 Immunol 4:825-826). However, if this cascade of type I interferon-dependent

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events leading to apoptosis of virally-infected cells also occurs when a virus interacts with target cells without productively infecting them, then these virus-exposed uninfected target cells might be signaled to die along with infected cells. In HIV-1 infection, where a large but variable percentage of viral particles are defective (Dimitrov, D.S. (1993) "QUANTITATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION KINETICS," *J Virol* 67:2182-2190; Piatak, M., Jr. *et al.* (1993) "HIGH LEVELS OF HIV-1 IN PLASMA DURING ALL STAGES OF INFECTION DETERMINED BY COMPETITIVE PCR," *Science* 259:1749-1754), interaction of CD4⁺ T cells with viral particles might induce apoptosis of CD4⁺ T cells without evidence of extensive HIV infection.

As indicated above, TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily (Wiley, S.R. *et al.* (1995) "IDENTIFICATION AND CHARACTERIZATION OF A NEW MEMBER OF THE TNF FAMILY THAT INDUCES APOPTOSIS," *Immunity* 3:673-682), induces apoptosis in human tumor cell lines (Griffith, T.S. *et al.* (1998) "INTRACELLULAR REGULATION OF TRAIL-INDUCED APOPTOSIS IN HUMAN MELANOMA CELLS," *J Immunol* 161:2833-2840; Herbeauval, J.P. *et al.* (2003) "MACROPHAGES FROM CANCER PATIENTS: ANALYSIS OF TRAIL, TRAIL RECEPTORS, AND COLON TUMOR CELL APOPTOSIS," *J Natl Cancer Inst* 95:611-621), and in virus-infected cells (Jeremias, I. *et al.* (1998) "TRAIL/APO-2-LIGAND-INDUCED APOPTOSIS IN HUMAN T CELLS," *Eur J Immunol* 28:143-152; Clarke, P. *et al.* (2003) "TWO DISTINCT PHASES OF VIRUS-INDUCED NF-KAPPAB-REGULATION ENHANCE TRAIL-MEDIATED APOPTOSIS IN VIRUS-INFECTED CELLS," *J Biol Chem.* 278(20):18092-100. Epub 2003 Mar 13), but not in normal cells (Gura, T. (1997) "How TRAIL KILLS CANCER CELLS, BUT NOT NORMAL CELLS," *Science* 277:768). TRAIL has five receptors, two (DR4 and DR5) that induce cell death, and three others that lack the death domain (Pan, G. *et al.* (1997) "THE RECEPTOR FOR THE CYTOTOXIC LIGAND TRAIL," *Science* 276:111-113). The TRAIL gene is regulated by type I interferons (Gong, B. *et al.* (2000) "GENOMIC ORGANIZATION AND TRANSCRIPTIONAL REGULATION OF HUMAN APO2/TRAIL GENE," *Biochem Biophys Res Commun* 278:747-752), and the DR5 gene is transcriptionally regulated by p53 (Wu, G.S. *et al.* (1997) "KILLER/DR5 IS A

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- DNA DAMAGE-INDUCIBLE P53-REGULATED DEATH RECEPTOR GENE," Nat Genet 17:141-143). TRAIL may be involved in CD4⁺ T cell depletion, since TRAIL, produced by monocytes exposed to the HIV-1 Tat protein, killed uninfected CD4⁺ T cells (Yang, Y. *et al.* (2003) "MONOCYTES TREATED WITH HUMAN
- 5 IMMUNODEFICIENCY VIRUS TAT KILL UNINFECTED CD4(⁺) CELLS BY A TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCED LIGAND-MEDIATED MECHANISM," J Virol 77:6700-6708). Moreover, soluble TRAIL was found in HIV-1-infected patients (Liabakk, N.B. *et al.* (2002) "DEVELOPMENT, CHARACTERIZATION AND USE OF MONOCLONAL ANTIBODIES AGAINST STRAIL:
- 10 MEASUREMENT OF STRAIL BY ELISA," J Immunol Methods 259:119-128; Stylianou, E. *et al.* (2003) "RAISED SERUM LEVELS OF INTERLEUKIN-18 IS ASSOCIATED WITH DISEASE PROGRESSION AND MAY CONTRIBUTE TO VIROLOGICAL TREATMENT FAILURE IN HIV-1-INFECTED PATIENTS," Clin Exp Immunol 132:462-466), and may be responsible for the death of neurons in AIDS
- 15 patients leading to dementia (Ryan, L.A. *et al.* (2004) "TNF-RELATED APOPTOSIS-INDUCING LIGAND MEDIATES HUMAN NEURONAL APOPTOSIS: LINKS TO HIV-1 ASSOCIATED DEMENTIA," J Neuroimmunol 148:127-139). TRAIL has been implicated, *in vitro*, in the apoptosis of HIV-1-infected cells (Lum, J.J. *et al.* (2001) "INDUCTION OF CELL DEATH IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED
- 20 MACROPHAGES AND RESTING MEMORY CD4 T CELLS BY TRAIL/APO2L," J Virol 75:11128-11136), including CD4⁺ and CD8⁺ T cells (Jeremias, I. *et al.* (1998) "TRAIL/APO-2-LIGAND-INDUCED APOPTOSIS IN HUMAN T CELLS," Eur J Immunol 28:143-152; Katsikis, P.D. *et al.* (1997) "INTERLEUKIN-1 BETA CONVERTING ENZYME-LIKE PROTEASE INVOLVEMENT IN FAS-INDUCED AND ACTIVATION-
- 25 INDUCED PERIPHERAL BLOOD T CELL APOPTOSIS IN HIV INFECTION. TNF- RELATED APOPTOSIS-INDUCING LIGAND CAN MEDIATE ACTIVATION-INDUCED T CELL DEATH IN HIV INFECTION," J Exp Med 186:1365-1372). However, TRAIL and DR5 expression on CD4⁺ T cells in HIV-1-infected patients remain to be determined.
- 30 The results provided in this Example demonstrate that HIV-1-infected patients exhibited: 1) elevated levels of TRAIL in their plasma, 2) increased

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percentage of CD4⁺ T cells that expressed DR5 and the apoptotic marker Annexin V. Because a higher percentage of apoptotic CD4⁺ T cells was found than is known to be infected (see above), the possibility that uninfected CD4⁺ T cells can undergo TRAIL-mediated apoptotic death as the result of a noninfectious interaction with 5 HIV-1 was investigated. To accomplish this, purified CD4⁺ and CD8⁺ T cells from HIV-1-seronegative blood donors were exposed to HIV-1 that had been rendered noninfectious with aldrithiol-2 (AT-2), an HIV-1 inactivation strategy that maintains the integrity of the viral envelope (Rossio, J.L. *et al.* (1998) "INACTIVATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTIVITY WITH 10 PRESERVATION OF CONFORMATIONAL AND FUNCTIONAL INTEGRITY OF VIRION SURFACE PROTEINS," *J Virol* 72:7992-8001; Arthur, L.O. *et al.* (1998) "CHEMICAL INACTIVATION OF RETROVIRAL INFECTIVITY BY TARGETING NUCLEOCAPSID PROTEIN ZINC FINGERS: A CANDIDATE SIV VACCINE," *AIDS Res Hum Retroviruses* 14 Suppl 3:S311-319). Therefore, the expression of TRAIL, STAT1, 15 STAT2, p53 and DR5, as well as apoptosis in CD4⁺ and CD8⁺ T cells cultured with infectious or noninfectious HIV-1 was studied. The dependence of this T cell death cascade on type I interferons was investigated, and the dependence of apoptosis on HIV-1 binding to the CD4 molecule expressed by T cells was tested. A two-hit model is presented in which infectious or noninfectious HIV-1 (first hit) 20 can activate type I interferons and TRAIL, and in synergy with a second infection or event (second hit), result in p53 and DR5-mediated apoptosis of CD4⁺ T cells.

Materials and Methods

HIV-1 positive patients. Venous blood was collected from 48 HIV-1-infected patients.

25 *Preparation of AT-2-inactivated virions.* HIV-1_{MN} (X4-tropic) and HIV-1_{Ada} (R5-tropic) were propagated as described above.

Isolation and culture of PBMC and T cells. PMBC and T cells were isolated as described above. CD4⁺ and CD8⁺ cells were purified from PBMC by either positive selection with anti-CD4 or anti-CD8 beads or by negative selection

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using cocktail of antibodies that bind all but CD4⁺ or CD8⁺ T cells (Miltenyi Biotech, Auburn, CA). For all the experiments, T cells isolated by both negative and positive selection were used interchangeably, without noticeable differences in the experimental outcome. The purity of CD4⁺ and CD8⁺ cells after purification
5 was >98% and >99%, respectively.

Incubation of PBMC and isolated T cell subsets with viruses. Cells were cultured at 1 X 10⁶ cells/ml in RPMI 10% FCS in 12-well plates. For incubation with AT-2 HIV-1, the inactivated virus preparations were added at the initiation of culture at a final concentration of 500 ng/mL p24^{CA} equivalent. Infectious HIV-1_{MN}
10 was also used at 500ng/mL p24^{CA} equivalent. The ability of the infectious HIV-1 to actually infect cultures was confirmed by measuring p24 levels in the 6-day culture supernatants by ELISA (Beckman-Coulter, Miami, FL). Infectious influenza A (PR8) virus with a hemagglutinin A titer of 1:8192, was used at a 1/400 dilution in culture wells. Edmonston "wild-type" (EDW) measles virus
15 produced in Vero cell with a titer of 4.6 x 10⁶ pfu/ml was used at a 1/500 dilution.

IFN- α detection. CD4⁺ and CD8⁺ T cells from HIV-1-seronegative donors were cultured for 24 h with or without influenza A and/or AT-2 HIV-1 viruses. Supernatants were collected and tested for IFN- α by ELISA (R & D Systems, Minneapolis, MN).

20 *Apoptosis study.* Cells were centrifuged, washed in Annexin buffer (1.4 M NaCl, 25 mM CaCl₂, and 100 mM MOPS, pH 7.14), and incubated for 20 minutes with medium only (negative control) or with FITC-conjugated Annexin V (Annexin V-FITC) (CALTAG, Burlingame, CA) in the dark at room temperature. After two washes, propidium iodide (100 μ g/mL) (CALTAG) was added and
25 analysis of apoptotic cells was confined to propidium iodide-negative cells to eliminate necrotic cells and membrane fragments that might bind Annexin V-FITC.

Detection of Membrane TRAIL. Isolated CD4⁺ and CD8⁺ T cells were cultured 24 h in absence or presence of influenza A virus and AT-2 HIV-1_{MN} or

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AT-2 HIV-1_{Ada}. The cells were washed twice in PBS. Membrane TRAIL expression was determined by incubating cells for 20 minutes at room temperature with PE-conjugated mouse IgG1 anti-human TRAIL monoclonal antibody (RIK-2), (Ebioscience, San Diego, CA) or with control isotype-matched antibodies (at 5 µg/mL each) in PBS containing 2% mouse serum (SIGMA, St. Louis, MO). Cells were washed twice in ice-cold PBS and analyzed by flow cytometry. The same protocol was used for the staining of DR5 (Ebioscience), CD4, and CD8 (BD Bioscience, San Jose, CA). For STAT1 detection, an intracellular staining was performed using Fix and Perm kit (Caltag) and STAT1 phosphorylated monoclonal antibody (BD Bioscience), according to the manufacturer's instructions.

Data acquisition and analysis. Cells were acquired on FACSCalibur flow cytometer (Becton Dickinson), using CellQuest software (Becton Dickinson Immunocytometry System). Samples were gated on viable cells by forward and side light scatters, and at least 50,000 live cell events were acquired for each sample. Acquired data were analyzed using Cellquest software (Becton Dickinson).

RNA extraction and reverse transcription. Total RNA was extracted from CD4⁺ T cells with after 12 h of culture with HIV-1MN and influenza viruses, using the acid guanidium thiocyanate-phenol-chloroform method (Chomczynski, P. et al (1987) "SINGLE-STEP METHOD OF RNA ISOLATION BY ACID GUANIDINIUM 20 THIOCYANATE-PHENOL-CHLOROFORM EXTRACTION," Anal Biochem 162:156-159), modified for TRIzol (Invitrogen, Carlsbad, CA, USA).

Real Time PCR. cDNA quantification for TRAIL, DR-5, p53 and GAPDH was obtained with a real time PCR technique. Real time PCR was conducted as described above. Primers for TRAIL and GAPDH are as described above. Primers for DR-5 are: Forward (**SEQ ID NO:5**) 5'-GGGCCACAGGGACACCTT-3'; Reverse (**SEQ ID NO:6**) 5'-GCATCTGCCCGGTTT-3'. Primers for p53 are: Forward (**SEQ ID NO:7**) 5'-CATGAGCGCTGCTCAGATAG-3'; Reverse (**SEQ ID NO:8**) 5'-ACACGCAAATTCCCTCAC-3'.

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All reactions were performed in triplicate. Data analysis was performed with the SDS2.1 software. The threshold level was determined by the software according to the optimization of the standard curve. Standards were obtained by amplification of a control sample in a PCR reaction, using the same primers, 5 reagents and conditions optimized for the real time analysis. Arbitrary quantity values were assigned to the resulting standard and 4-fold serial dilution were made to obtain a 8-point standard curve. Results are presented as ratios between the target gene and the GAPDH mRNA.

Blocking assays. Cells were cultured 24 h with AT-2 HIV-1 and influenza 10 A virus in the presence of mouse blocking monoclonal anti-TRAIL (RIK-2) (Ebioscience) (Kayagaki, N. et al. (1999) "INVOLVEMENT OF TNF-RELATED APOPTOSIS-INDUCING LIGAND IN HUMAN CD4⁺ T CELL-MEDIATED CYTOTOXICITY," J Immunol 162:2639-2647), anti-DR5 (Imgenex, San Diego, CA) at 1, 5 and 10 µg/mL, soluble CD4 (AIDS Reagent Program, Bethesda, MD)at 2 15 µg/mL, or the combination of sheep polyclonal anti-human IFN-α (2000 IFN-α neutralizing U/mL) and sheep polyclonal anti-human IFN-β (500 IFN-β neutralizing U/mL) antibodies (BioSource International, Camarillo, CA). Mouse IgG isotype control antibody (BD Bioscience) was used at 5 µg/ml. Apoptosis was assessed by the Annexin V method described above. Results shown are for 5 20 µg/mL (anti-TRAIL) and 1 µg/mL (anti-DR5).

Nuclear p53 measurements. CD4⁺ T cells were culture for 24 h with or without influenza A and AT-2 HIV-1 viruses. Cells were lysed and nuclear p 53 was quantified by ELISA (Active Motif, Carlsbad, CA, USA) according to the according to the manufacturer's instructions.

25 *Western Blot.* PBMC's from uninfected donors were separated from whole blood by Ficoll centrifugation and CD4⁺ T cells were isolated by magnetic beads (Miltenyi, Auburn , CA). Cells were then resuspended in DMEM supplemented with 10% FBS (Hyclone, Logan, UT) and 1% Pen-Strep-Glut (Invitrogen, Carlsbad, CA) and stimulated with influenza virus, X4 AT-2 HIV-1, recombinant

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IFN- α (100 ng/ml) and IFN- β (100 ng/ml), anti-IFN- α and anti-IFN- β (R & D Systems) either singly or in combination. After 24 h, the cells were pelleted, washed in PBS and lysed (1% NP-40, 200mM NaCl, 50mM Tris pH 7.5, supplemented with protease inhibitor cocktail [Roche, Indianapolis, IN]). Total protein in the lysate was quantified using BCA (Pierce, Rockford, IL) and 10 μ g of protein was mixed 1:1 with SDS Protein Gel Buffer (Quality Biologics, Gaithersburg, MD). The samples were run on a 10% or 15% SDS-PAGE gel (Bio-Rad, Hercules, CA). After transfer to a nitrocellulose membrane, the blot was blocked in 5% milk/TBST then incubated with either anti-STAT1, anti-STAT2 (Upstate Biotechnology, Inc., Lake Placid, NY), anti-TRAIL or anti-DR5 (Imgenix, San Diego, CA), anti-p53 (Calbiochem, La Jolla, CA), monoclonal antibodies followed by either anti-mouse or anti-rabbit HRP conjugated secondary antibody (Jackson, West Grove, PA). ECL was performed and bands were visualized on Hyperfilm (Amersham, Piscataway, NJ). β -actin was visualized as a loading control using a monoclonal antibody (Sigma, St. Louis, MO).

Statistical analysis. All the experiments were repeated at least four times and the p values (p) were calculated using a two-tailed Student's *t* test. The linear regression analysis of DR5 mediated cell death (DR5 $^+$ AnnexinV $^+$) among apoptotic cells (AnnexinV $^+$) was performed with Microsoft Excel.

20 Results

Detection of TRAIL and DR5 in HIV-1-infected patients and uninfected controls. The levels of DR5 mRNA level in PBMC were compared from 16 HIV-1-infected patients and 9 HIV-1-uninfected controls. PBMC from HIV-1-infected patients expressed 2-fold more DR5 mRNA than PBMC from HIV-1-uninfected controls ($p=0.03$) (**Figure 6, Panel A**). An additional 22 patients and 16 controls were tested for plasma TRAIL levels, and for the percentage of CD4 $^+$ T cells that were positive for Annexin V and DR5. Elevated levels of plasma TRAIL were detected in the HIV-1-infected patients (2617 ± 729 pg/ml) compared to controls, (730 ± 320 pg/ml) ($p=0.0001$). An increase in the percentage of CD4 $^+$ T cells that

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expressed the Annexin V ($p=3\times 10^{-5}$) and DR5 ($p=2\times 10^{-6}$) was observed (**Figure 6, Panel B**). Furthermore, a strong correlation is found between DR5 expression and apoptosis in CD4⁺ T cells from HIV-1-infected patients ($R^2=0.67$, $p=3.10^{-6}$). In contrast, there was no correlation between these parameters in HIV- healthy
5 individuals ($R^2=0.17$, $p=0.11$) (**Figure 6, Panel C**). This result indicates that 67% of the death of CD4⁺ T cells in these patients can be explained by DR5 expression, while no death of CD4⁺ T cells can be attributed to DR5 expression in healthy individuals. These findings of increased plasma TRAIL levels combined with increased DR5 gene and protein expression in CD4⁺ T cells from HIV-1-infected
10 patients suggest the participation of TRAIL-mediated apoptosis in CD4⁺ T cell depletion in AIDS.

Annexin V and STAT expression in virus-exposed T cells. To determine the role of HIV-1 in the expression of TRAIL and DR5 observed in HIV-1-infected patients, peripheral blood T cells were cultured from HIV-1-seronegative donors
15 with infectious or noninfectious HIV-1. The effects of infectious HIV-1_{MN} (X4) and HIV-1_{Ada} (R5), as well as their noninfectious viral counterparts that had been inactivated by aldrithiol 2 (AT-2 HIV-1) (Arthur, L.O. *et al.* (1998) "CHEMICAL INACTIVATION OF RETROVIRAL INFECTIVITY BY TARGETING NUCLEOCAPSID PROTEIN ZINC FINGERS: A CANDIDATE SIV VACCINE," AIDS Res Hum
20 Retroviruses 14 Suppl 3:S311-319) were tested, on T cell apoptosis. CD4⁺ and CD8⁺ T cells were positively-selected from PBMC and cultured for one-to-six days with these HIV-1 preparations, or with microvesicles (control), and the percentage of Annexin V-positive T cells was determined, using multicolor flow cytometry. Infectious HIV-1_{MN} or HIV-1_{Ada} did not induce significant apoptosis of either CD4⁺
25 or CD8⁺ T cells after one day. However, it was found that 32% and 31% of CD4⁺ T cells were Annexin V positive when cultured 6 days with HIV-1_{MN} or HIV-1_{Ada}, respectively (**Figure 7, Panel A**). This apoptosis was approximately two fold above T cells cultured with microvesicles (16%). This difference was significant at $p=0.04$ for HIV-1_{MN} and $p= 0.03$ HIV-1_{Ada}.

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T cells from HIV-1-seronegative donors were then co-cultured with HIV-1 plus a second virus to determine whether this viral contribution would induce apoptosis. Influenza A virus was chosen as the second virus because it was shown to co-localize with HIV-1 in mucosa-associated lymphoid tissue in vivo (Doherty, 5 P.C. (1995) "ANATOMICAL ENVIRONMENT AS A DETERMINANT IN VIRAL IMMUNITY," J Immunol 155:1023-1027), and induced death of CD4⁺ T cells in PBMC from asymptomatic HIV-1-infected individuals (Clerici, M. *et al.* (1996) "ANTIGEN-STIMULATED APOPTOTIC T-CELL DEATH IN HIV INFECTION IS SELECTIVE FOR CD4⁺ T CELLS, MODULATED BY CYTOKINES AND EFFECTED BY 10 LYMPHOTOXIN," Aids 10:603-611). T cells cultured with infectious Flu alone did not undergo apoptosis. However, co-culture of infectious HIV-1 plus Flu virus induced the death of 45-50% (day 1) and 90-95% (day 6) of CD4⁺ T cells, which are significantly less than CD4⁺ T cells cultured with microvesicles (controls) (p<0.001). In contrast less than 15% and 30% of CD8⁺ T cells on the same days 15 (**Figure 7, Panel A**). Identical results were obtained using noninfectious AT-2 HIV-1_{MN} and AT-2 HIV-1_{Ada}. Similar effects are observed between HIV-1 and measles virus, indicating that induction of CD4⁺ T cell death was not unique to Flu virus. It was verified that 28-30% of Annexin V⁺ CD4⁺, but only 3-6% of CD8⁺ T cells progressed to late stage apoptosis using propidium iodide (**Figure 7, Panel 20 B**). Because virus infections induce type I interferons, which can be involved in apoptosis (Takaoka, A. *et al.* (2003) "INTEGRATION OF INTERFERON-ALPHA/BETA SIGNALLING TO P53 RESPONSES IN TUMOUR SUPPRESSION AND ANTIVIRAL DEFENSE," Nature 424:516-523), the role of type I interferons in apoptosis of HIV-1-exposed T cells was investigated. Antibodies against IFN- α/β dramatically 25 reduced death of CD4⁺ T cells cultured with infectious HIV-1 and Flu virus, or with AT-2 HIV-1_{MN} and Flu virus (**Figure 7, Panel C**). **Figure 7, Panel C** also illustrates how similar the flow cytometric Annexin V curves are between infectious (upper) and noninfectious HIV-1 (lower) plus Flu.

The data of **Figure 7, Panel C** strongly suggest that type I interferons are 30 important in the CD4⁺ T cell death that is induced by either infectious or noninfectious HIV-1 plus Flu. In fact, the comparison of the flow cytometry data

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of Annexin V⁺ CD4⁺ T cells was identical irrespective of whether infectious HIV-1 plus Flu or noninfectious AT-2 HIV-1 plus Flu were used to induce apoptosis. Furthermore, an equal inhibition of death was observed with anti-IFN α/β mAbs, indicating that type I interferons were required for inducing apoptosis by either 5 infectious or noninfectious HIV-1 plus Flu in the above-described system. If apoptosis of CD4⁺ T cells is inhibited by type I interferon-specific antibodies, then IFN- α or IFN- β might be detectable in our culture supernatants. Therefore, tests were conducted to determine whether enriched CD4⁺ or CD8⁺ T cells would produce IFN- α when culture with AT-2 HIV-1, Flu virus or the combination of 10 both. It was found that CD4⁺ but not CD8⁺ cells produced IFN- α after exposure to AT-2 HIV-1, Flu and the combination of these viruses (**Figure 7, Panel D**).

Because STAT1 and STAT2 are signaling molecules for type I interferons (Qureshi, S.A. *et al.* (1995) "TYROSINE-PHOSPHORYLATED STAT1 AND STAT2 PLUS A 48-KDA PROTEIN ALL CONTACT DNA IN FORMING INTERFERON-STIMULATED- 15 GENE FACTOR 3," Proc Natl Acad Sci U S A 92:3829-3833; Leung, S. *et al.* (1995) "ROLE OF STAT2 IN THE ALPHA INTERFERON SIGNALING PATHWAY," Mol Cell Biol 15:1312-1317), tests were conducted to determine whether STAT1 and STAT2 would be affected by co-culture of CD4⁺ T cells with HIV-1 and Flu. Flow cytometric and western blot analyses showed that STAT1 and STAT2 were 20 modulated by exposure of T cells to HIV-1 and Flu virus. AT-2 HIV-1_{MN}, Flu or AT-2 HIV-1_{MN} plus Flu induced similar levels of phosphorylated STAT1 expression on CD4⁺ T cells (**Figure 7, Panel E**), with a slightly higher percentage of STAT1⁺ CD4⁺ T cells resulting from culture with AT-2 HIV-1_{MN} plus Flu. Western blot analysis confirmed that Flu plus AT-2 HIV-1_{MN} was required to 25 increase STAT1, and to induce high levels of STAT2, and that both STAT1 and STAT 2 production were inhibited by anti IFN- α/β antibodies (**Figure 7, Panel F**). Thus, type I interferons are involved in the death of CD4⁺ T cells exposed to HIV-1 and Flu virus.

Analysis of TRAIL expression and production in virus-exposed T cells.

30 Since interferons regulate TRAIL expression (Gong, B. *et al.* (2000) "GENOMIC

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- ORGANIZATION AND TRANSCRIPTIONAL REGULATION OF HUMAN APO2/TRAIL GENE," Biochem Biophys Res Commun 278:747-752), and because TRAIL was detected in the plasma of HIV-1-infected patients, TRAIL expression was measured on T cells cultured with HIV-1 and Flu virus alone or in combination.
- 5 Membrane TRAIL (mTRAIL) was detected on CD4⁺ (**Figure 8, Panel A**) but not CD8⁺ T cells cultured with Flu or HIV-1_{MN} alone, or with HIV-1_{MN} plus Flu. Noninfectious AT-2 HIV-1 also induced TRAIL expression on CD4⁺ T cells (data not shown). Exposure of CD4⁺ T cells to HIV-1_{MN} plus Flu induced TRAIL mRNA expression that was blocked by IFN- α/β antibodies (**Figure 8, Panel B**).
- 10 Anti-IFN- α/β antibodies also inhibited virus-induced expression of mTRAIL, suggesting that type I interferons are paramount for the induction of TRAIL in virus exposed-T cells (**Figure 8, Panel C**). IFN- α and/or IFN- β both induced mTRAIL expression on CD4⁺ T cells when added exogenously (**D**). Western blot analysis verified that TRAIL was produced upon culture of CD4⁺ T cells with AT-
- 15 2 HIV-1_{MN}, Flu, AT-2 HIV-1_{MN} plus Flu or IFN- α/β (**Figure 8, Panel E**). Antibodies against IFN- α/β greatly inhibited TRAIL production by AT-2 HIV-1_{MN} plus Flu (**Figure 8, Panel E**). The involvement of TRAIL in apoptosis of CD4⁺ T cells exposed to HIV-1_{MN} plus Flu was confirmed by experiments in which anti-IFN- α/β antibodies, the TRAIL-specific RIK-2 monoclonal antibody, and anti-DR5
- 20 antibody each inhibited CD4⁺ T cell apoptosis by 75% (p= 0.0001), 40% (p= 0.02) and 60 % (p= 0.03), respectively (**Figure 8, Panel F**). Soluble CD4 inhibited CD4⁺ T cell apoptosis by 90% (p= 0.0001) (**Figure 8, Panel F**), which demonstrates the involvement of the interaction between HIV-1 and CD4 for inducing apoptosis. Considered together, these results indicate that: (1) Flu- and/or HIV-1-induced
- 25 TRAIL expression and (2) the interaction between HIV-1 and CD4 is essential for inducing CD4⁺ T cell death via a type I interferon dependent mechanism.

DR5 expression and p53 transcription and synthesis in virus-exposed T cells. TRAIL-mediated apoptosis requires the expression of cellular TRAIL death receptors. Therefore, DR5 expression was measured on CD4⁺ and CD8⁺ T cells after culture with HIV-1_{MN}, Flu or HIV-1_{MN} plus Flu. In contrast to TRAIL, which

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was induced by either HIV-1_{MN} or Flu alone, DR5 was selectively expressed only on CD4⁺ T cells cultured with both HIV-1_{MN} and Flu (**Figure 9, Panel A**). DR4 was not detected on CD4⁺ T cells, and CD8⁺ T cells did not express DR molecules. Involvement of DR5 was confirmed by its expression on the majority of cells
5 undergoing apoptosis after culture with AT-2 HIV-1_{MN} plus Flu (**Figure 9, Panel B**). In addition, culture of CD4⁺ T cells with AT-2 HIV-1_{MN} plus Flu induced DR5 mRNA and protein expression, both of which were blocked by anti-IFN-α/β antibodies (**Figure 9, Panels C and D** (upper panel)). Western blot and real time PCR analysis showed that both Flu and HIV-1_{MN} were required for DR5 expression
10 (**Figure 9, Panels C and E**), and that DR5 gene and protein expression were inhibited by anti-IFN-α/β antibodies (**Figure 9, Panels C and E**). However, addition of exogenous IFN-α/β did not induce DR5 (**Figure 9, Panel E**). Similarly, recombinant IFN-α/β in combination with HIV-1 did not induce DR5 or apoptosis (**Figure 9, Panel D** (middle and lower panels)) indicating that expression
15 of TRAIL (see **Figure 8, Panel D**) and DR5 have different requirements. Therefore, DR5 expression is the limiting component of TRAIL-mediated death in HIV-1-exposed cells.

DR5 expression is regulated by the tumor suppressor p53 (24, 40). Therefore, we tested whether p53 gene expression and synthesis would be induced
20 by Flu, AT-2 HIV-1_{MN} or AT-2 HIV-1_{MN} plus Flu, and whether p53 induction would be inhibited by IFN-α/β antibodies. Both p53 gene expression (Fig. 4F) and p53 production (Fig. 4G,H) required CD4⁺ T cell stimulation with AT-2 HIV-1_{MN} plus Flu, and were blocked by anti-IFN-α/β. Thus, similar to the other components of this cascade to death, p53 activation and synthesis, which are necessary for DR5 expression (Wu, G.S. *et al.* (1997) "KILLER/DR5 IS A DNA DAMAGE-INDUCIBLE P53-REGULATED DEATH RECEPTOR GENE," Nat Genet 17:141-143; Sheikh, M.S. *et al.* (1998) "p53-DEPENDENT AND -INDEPENDENT REGULATION OF THE DEATH
25 RECEPTOR KILLER/DR5 GENE EXPRESSION IN RESPONSE TO GENOTOXIC STRESS AND TUMOR NECROSIS FACTOR ALPHA," Cancer Res 58:1593-1598), are type I
30 interferon-dependent.

In sum, as reported above, HIV-1-infected patients have more TRAIL in their plasma and more CD4⁺ T cells expressing DR5 and the apoptotic marker Annexin V than healthy donors. Furthermore, a strong correlation has been uncovered between DR5 expression and apoptosis in CD4 T cells from HIV-1-
5 infected patients ($r^2=0.67$, $p=3.10^{-6}$). In contrast, there was no correlation between these parameters in HIV⁻ healthy individuals ($r^2=0.17$, $p=0.11$). These results strongly suggest that TRAIL/DR5-mediated apoptosis is the major death mechanism that contribute to CD4⁺ T cell depletion in HIV-1-infected patients.
The above-presented in vitro results demonstrate that CD4⁺ T cell death occurs
10 upon in vitro exposure of T cells to either infectious or noninfectious HIV-1 plus a second virus. While not intending to be limited thereby, the results suggest a model in which death is mediated by a cascade of molecular events, each of which is dependent on type I interferons. Thus, STAT1, STAT2, TRAIL, DR5, p53 and apoptosis were all inhibited by antibodies against IFN- α/β , and indicate a central
15 role for type I interferons in this HIV-1-associated death process. A recent study reported that the interaction of cell lines transfected with the gp120/41 HIV-1 envelope complex and CD4 results in expression of p53, leading to the expression of the proapoptotic protein Puma and apoptosis (Perfettini, J.L. *et al.* (2004) "NF-
{KAPPA}B AND P53 ARE THE DOMINANT APOPTOSIS-INDUCING TRANSCRIPTION
20 FACTORS ELICITED BY THE HIV-1 ENVELOPE," *J Exp Med* 199:629-640). The Puma model may be relevant here in that it revealed HIV-1-induced apoptosis of CD4 expressing cells in the absence of infection via a p53-dependent mechanism.

The present demonstration that apoptosis was partially inhibited by anti-TRAIL and anti-DR5 antibodies and almost completely inhibited by soluble CD4 indicate that: (1) TRAIL and its receptors are involved in this model of CD4⁺ T cell death; and (2) the interaction between HIV-1 and the CD4 molecule expressed on CD4⁺ T cells is essential for induction of apoptosis. The present findings that X4 and R5 co-receptor-using HIV-1 isolates were equally effective for inducing TRAIL and synergizing with Flu to induce DR5 and apoptosis indicates that discriminatory co-receptor recognition is not important to the present invention.
30 The finding that anti-TRAIL and anti-DR5 antibody did not completely block

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apoptosis raises the possibility that other death mechanisms may have contributed to the high levels of CD4⁺T cell death that we observed. However, antibodies against TNF, FASL and TWEAK had no effect in the proposed model.

Furthermore, an earlier study reported that AT-2 HIV-1-induced T cell death that
5 was mediated by an unidentified non-Fas-FasL mechanism (Esser, M.T. *et al* (2001) "PARTIAL ACTIVATION AND INDUCTION OF APOPTOSIS IN CD4(+) AND CD8(+) T LYMPHOCYTES BY CONFORMATIONALLY AUTHENTIC NONINFECTIOUS HUMAN IMMUNODEFICIENCY VIRUS TYPE 1," *J Virol* 75:1152-1164).

Opportunistic infectious agents have been suggested to exacerbate HIV-1
10 disease by activating CD4⁺ T cells, rendering T helper cells susceptible to HIV-1 infection (Donovan, R.M. *et al.* (1996). "CHANGES IN VIRUS LOAD MARKERS DURING AIDS-ASSOCIATED OPPORTUNISTIC DISEASES IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PERSONS," *J Infect Dis* 174:401-403), resulting in HIV-1 cytopathic death. Without intending to be bound thereby, the
15 present findings provide a new mechanism by which preferential CD4⁺ T cell apoptosis can occur. This mechanism involves the synergistic effects of either infectious or noninfectious HIV-1 and a second event (viral infection), both of which are essential for activating rate-limiting expression of DR5 death receptor molecule by a type I interferon-dependent mechanism. Although influenza A virus
20 was employed as the second virus (because it co-localizes with HIV-1 in primary lymphoid tissue (Doherty, P.C. (1995) "ANATOMICAL ENVIRONMENT AS A DETERMINANT IN VIRAL IMMUNITY," *J Immunol* 155:1023-1027)), the second event was not limited to influenza; measles virus, for example, also synergized with AT-2 HIV-1 to induce p53, DR5 and apoptosis of CD4⁺ T cells. Although
25 influenza and measles virus infections have not been associated with AIDS progression, both of these viruses fulfill the requirement of activating p53 and DR5 in presence of HIV-1. Thus, they provide an example of one way to selectively induce rapid and extensive CD4⁺ T cell death upon exposure to HIV-1. Because our findings show that p53 expression required two viruses to induce DR5, other
30 infection could provide the second event to induce DR5 and accelerate T cell depletion. Alternatively, it is possible that, in synergy with HIV-1, other non-viral

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events would induce p53 and DR5 resulting in apoptosis. These other events might include agents that would induce DNA damage, since p53 is induced by DNA damage (Wu, G.S. *et al.* (1997) "KILLER/DR5 IS A DNA DAMAGE-INDUCIBLE P53-REGULATED DEATH RECEPTOR GENE," Nat Genet 17:141-143).

- 5 The present finding that CD4⁺ T cells cultured with AT-2 HIV-1 plus recombinant IFN- α/β did not induce apoptosis suggests that type I interferons (induced by HIV-1) are required but not sufficient for inducing CD4⁺ T cell death. The central event for CD4⁺ T cell death in our findings was activation of p53 by the combined effect of HIV-1 and a second virus leading to the selective expression of DR5 on CD4⁺ T
10 cells.

The present demonstration that CD4⁺ T cells from HIV-1 patients were double-positive for Annexin V and DR5 and that TRAIL was detected in patients' plasma is consistent with the present in vitro data and suggests that the above-suggested in vitro model has clinical relevance. In contrast to the slow decline in
15 CD4 count during progression to AIDS, the accelerated in vitro model of TRAIL/DR5-mediated death suggested by such model occurred within six days. Part of this rapid CD4⁺ depletion could be due to the fact that T cell repopulation did not occur in the above-reported in vitro studies, in contrast to the in vivo setting in which CD4⁺ T cell repopulation was reported (Wei, X. *et al.* (1995)
20 "VIRAL DYNAMICS IN HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION," Nature 373:117-122; Ho, D.D. *et al.* (1995) "RAPID TURNOVER OF PLASMA VIRIONS AND CD4 LYMPHOCYTES IN HIV-1 INFECTION," Nature 373:123-126). The fact that preferential apoptosis of CD4⁺ T cells was induced using non-infectious as well as infectious HIV-1 indicates that this synergistic effect is not
25 dependent on productive HIV-1-infection, and demonstrates that uninfected CD4⁺ T cells die in the suggested model (see report that uninfected CD4⁺ T cells can undergo bystander death during HIV-1 infection (Finkel, T.H. *et al.* (1995)
"APOPTOSIS OCCURS PREDOMINANTLY IN BYSTANDER CELLS AND NOT IN PRODUCTIVELY INFECTED CELLS OF HIV- AND SIV-INFECTED LYMPH NODES," Nat
30 Med 1:129-134). The present findings also raise the possibility that non-infectious HIV-1 particles, which constitute a large portion of the plasma virus (Dimitrov,

D.S. (1993) "QUANTITATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 INFECTION KINETICS," J Virol 67:2182-2190; Piatak, M., Jr. *et al.* (1993) "HIGH LEVELS OF HIV-1 IN PLASMA DURING ALL STAGES OF INFECTION DETERMINED BY COMPETITIVE PCR," Science 259:1749-1754), contribute to the decline in CD4 count by a similar mechanism. Nevertheless, it should be emphasized that the development of AIDS requires infectious HIV-1, which is the source of infectious and non-infectious virus particles that can induce apoptotic signals in CD4⁺ T cells.

- The present invention demonstrates that cultures of T cells enriched for CD4⁺ by 98-99% produced IFN- α when cultured with HIV-1 and/or Flu.
- 10 However, type I IFN production could be due to CD4⁺ monocytes. IFN- α was reported to inhibit HIV-1 replication in vitro (Yamamoto, J.K. *et al.* (1986) "HUMAN ALPHA- AND BETA-INTERFERON BUT NOT GAMMA- SUPPRESS THE IN VITRO REPLICATION OF LAV, HTLV-III, AND ARV-2," J Interferon Res 6:143-152), and has been used for therapy in AIDS patients with Kaposi's sarcoma
- 15 (Frissen, P.H. *et al.* (1997) "HIGH-DOSE INTERFERON-ALPHA2A EXERTS POTENT ACTIVITY AGAINST HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 NOT ASSOCIATED WITH ANTITUMOR ACTIVITY IN SUBJECTS WITH KAPOSI'S SARCOMA," J Infect Dis 176:811-814). Type I interferons produced by plasmacytoid dendritic cells have been suggested to protect against AIDS progression (Soumelis, V. *et al.* (2001)
- 20 "DEPLETION OF CIRCULATING NATURAL TYPE 1 INTERFERON-PRODUCING CELLS IN HIV-INFECTED AIDS PATIENTS," Blood 98:906-912; Siegal, F.P. *et al.* (1999) "THE NATURE OF THE PRINCIPAL TYPE 1 INTERFERON-PRODUCING CELLS IN HUMAN BLOOD," Science 284:1835-1837). However, type I interferons induced in lymph nodes of macaques infected with SIV did not control viral replication (Abel, K. *et al.* (2002) "THE RELATIONSHIP BETWEEN SIMIAN IMMUNODEFICIENCY VIRUS RNA LEVELS AND THE mRNA LEVELS OF ALPHA/BETA INTERFERONS (IFN-ALPHA/BETA) AND IFN-ALPHA/BETA-INDUCIBLE MX IN LYMPHOID TISSUES OF RHESUS MACAQUES DURING ACUTE AND CHRONIC INFECTION," J Virol 76:8433-8445). The present findings raise the possibility that type I interferons can be
- 25 harmful by setting the stage for the death of uninfected CD4⁺ T cells in patients whose CD4⁺ T cells have been activated to express TRAIL and DR5. In light of

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the present findings that antibodies against IFN- α/β reduced CD4 $^{+}$ T cell death, and blocked the effect of each of the molecules required for inducing TRAIL-mediated apoptosis, one aspect of the present invention concerns the recognition that anti-type I interferon antibodies can be used as an anti-HIV-1 therapeutic agent. See also reports that immune suppression is induced by IFN- α and can be blocked by anti-IFN- α antibodies (Zagury, D. *et al.* (1998) "INTERFERON ALPHA AND TAT INVOLVEMENT IN THE IMMUNOSUPPRESSION OF UNINFECTED T CELLS AND C-C CHEMOKINE DECLINE IN AIDS," Proc Natl Acad Sci U S A 95:3851-3856), and that immunization of AIDS patients against IFN- α reduced the rate of HIV-1 disease progression (Gringeri, A. *et al.* (1999) "ACTIVE ANTI-INTERFERON-ALPHA IMMUNIZATION: A EUROPEAN-ISRAELI, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL IN 242 HIV-1--INFECTED PATIENTS (THE EURIS STUDY)," J Acquir Immune Defic Syndr Hum Retrovirol 20:358-370.

The above-described two-hit model, based on the above-described data is illustrated in **Figure 10**. The model illustrates the requirement for STAT1 and STAT2 activation, as well as the induction of type I interferons, TRAIL, p53 and DR5 in CD4 $^{+}$ T cells. The top panel of **Figure 10** shows that only one hit is needed to induce TRAIL, but not p53 or DR5. The bottom panel of **Figure 10** indicates that two hits are required to induce the entire cascade of molecular events that are necessary for TRAIL-mediated death of CD4 $^{+}$ T cells. Both membrane TRAIL (mTRAIL), which is detected on CD4 $^{+}$ T cells after exposure to HIV-1 or influenza virus, and plasma TRAIL (pTRAIL) that is detected in patients can interact with DR5 that is expressed on CD4 $^{+}$ T cells, resulting TRAIL-mediated apoptosis. It is also noteworthy that exposure of monocytes from healthy blood donors to AT-2 HIV-1 results in soluble TRAIL production. Based on the above-presented findings, it is concluded that exposure to either infectious or noninfectious HIV-1 (first hit) in conjunction with a second virus (second hit) can induce the selective expression of TRAIL, p53 and DR5 on CD4 $^{+}$ T cells, resulting in apoptosis of uninfected CD4 $^{+}$ but not CD8 $^{+}$ T lymphocytes. The above-presented results indicate that HIV-1 and a second p53-activating event synergize to initiate a type I interferon-dependent cascade of molecular events that leads to

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TRAIL, p53 and DR5 expression, resulting in preferential apoptosis of uninfected CD4⁺ T cells. Thus, the present invention provide a mechanistic explanation for CD4⁺ T cell death that is independent of productive HIV-1 infection, but which may contribute to CD4⁺ T cell depletion in HIV-1-infected patients.

What Is Claimed Is:

- Claim 1. A method for determining whether a mammal suffers from a disease or condition involving immune system activation, wherein said method comprises assaying for the presence or concentration of a TRAIL Compound in a biological fluid of said mammal.
- 5
- Claim 2. The method of claim 1, wherein said mammal is selected from the group consisting of a human, simian, feline, bovine, equine, canine, ovine or porcine mammal.
- Claim 3. The method of claim 1, wherein said TRAIL compound is sTRAIL.
- 10 Claim 4. The method of claim 1, wherein said method comprises an immunoassay that determines the presence or concentration of said TRAIL Compound, said immunoassay comprising the steps of:
- (a) contacting a sample of said biological fluid with an antibody specific for said TRAIL Compound, said contacting being under conditions sufficient to permit said TRAIL Compound if present in said sample to bind to said antibody and form a TRAIL Compound – antibody complex;
- 15 (b) contacting said formed TRAIL Compound – antibody complex with a molecule capable of specific binding to said complex, said contacting being under conditions sufficient to permit said molecule to bind to said complex and form an extended complex; and
- (c) determining the presence or concentration of said TRAIL Compound in said biological fluid by determining the presence or concentration of said formed extended complex in said sample.
- 20
- 25
- Claim 5. The method of claim 4, wherein said TRAIL Compound is selected from the group consisting of sTRAIL, mTRAIL, the TRAIL DRS

receptor molecule, a biological molecule that activates TRAIL, and a biological molecule that activates the TRAIL DR5 receptor.

- Claim 6. The method of claim 5, wherein said biological molecule that activates the TRAIL DR5 receptor is p53.
- 5 Claim 7. The method of claim 4, wherein said method reveals the presence of said TRAIL Compound.
- Claim 8. The method of claim 4, wherein said method reveals the concentration of said TRAIL Compound.
- 10 Claim 9. The method of claim 1, wherein said disease or condition involving immune system activation is selected from the group consisting of HIV infection, AIDS, cancer, atherosclerosis, Alzheimer's disease, inflammation, autoimmune disorder, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant rejection, and graft vs host disease.
- 15 Claim 10. The method of claim 9, wherein said disease or condition involving immune system activation is HIV infection or AIDS.
- Claim 11. An immunoassay that determines the presence or concentration of a TRAIL Compound in a biological fluid of a mammal, wherein said immunoassay comprises the steps of:
- 20 (a) contacting a sample of said biological fluid with an antibody specific for said TRAIL Compound, said contacting being under conditions sufficient to permit said TRAIL Compound if present in said sample to bind to said antibody and form a TRAIL Compound – antibody complex;
- 25 (b) contacting said formed TRAIL Compound – antibody complex with a molecule capable of specific binding to said complex, said contacting being under conditions sufficient

to permit said molecule to bind to said complex and form an extended complex; and

- 5 (c) determining the presence or concentration of said TRAIL Compound in said biological fluid by determining the presence or concentration of said formed extended complex in said sample.

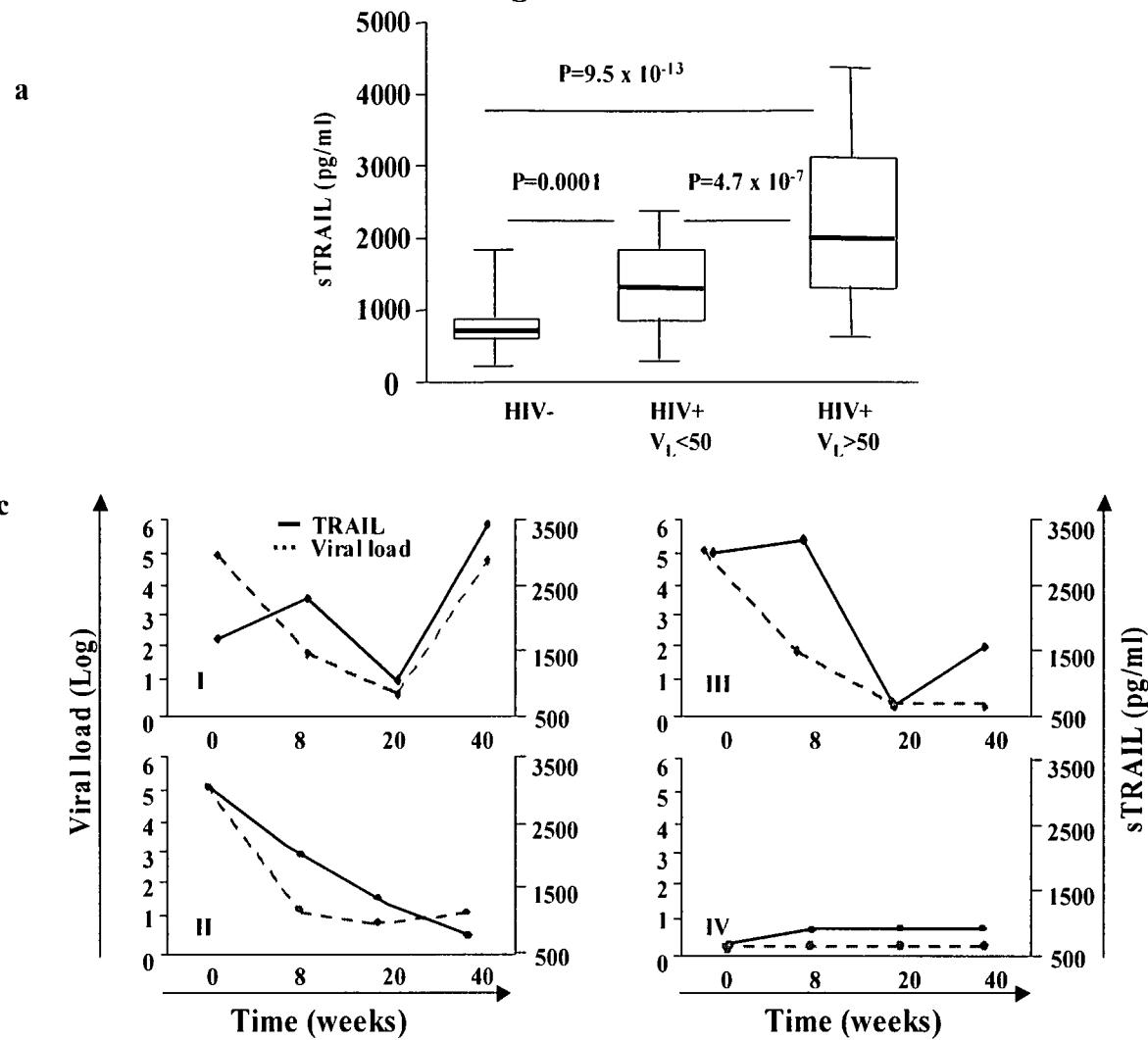
- Claim 12. The immunoassay of claim 11, wherein said mammal is selected from the group consisting of a human, simian, feline, bovine, equine, canine, ovine or porcine mammal.
- 10 Claim 13. The immunoassay of claim 11, wherein said TRAIL Compound is selected from the group consisting of sTRAIL, mTRAIL, the TRAIL DR5 receptor molecule, a biological molecule that activates TRAIL, and a biological molecule that activates the TRAIL DR5 receptor.
- 15 Claim 14. The immunoassay of claim 13, wherein said biological molecule that activates the TRAIL DR5 receptor is p53.
- Claim 15. The immunoassay of claim 11, wherein said immunoassay reveals the presence of said TRAIL Compound.
- 20 Claim 16. The immunoassay of claim 11, wherein said immunoassay reveals the concentration of said TRAIL Compound.
- Claim 17. The immunoassay of claim 11, wherein said molecule capable of specific binding to said complex is an rsTRAIL molecule.
- 25 Claim 18. The immunoassay of claim 11, wherein said TRAIL Compound is sTRAIL, and said molecule capable of specific binding to said complex is an anti-sTRAIL antibody.

- Claim 19. The immunoassay of claim 11, wherein said immunoassay determines whether a mammal suffers from a disease or condition involving immune system activation selected from the group consisting of HIV infection, AIDS, cancer, atherosclerosis, Alzheimer's disease, inflammation, autoimmune disorder, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant rejection, and graft vs host disease.
- 5
- Claim 20. The immunoassay of claim 19, wherein said disease or condition involving immune system activation is HIV infection or AIDS.
- 10
- Claim 21. The immunoassay of claim 11, wherein said immunoassay is an immunochromatographic immunoassay, wherein:
- 15 in said step (a), said biological sample is placed in contact with a first porous carrier, said first porous carrier containing a non-immobilized, labeled antibody specific for said TRAIL Compound;
- in said step (b), said formed TRAIL Compound– antibody complex is placed in contact with a second porous carrier, said second porous carrier being in communication with said first porous carrier, and containing an immobilized molecule capable of specific binding to said complex; and
- 20 in said step (c), the presence or concentration of said TRAIL Compound in said biological fluid is determined by detecting the presence of said labeled antibody specific for said TRAIL Compound in said second porous carrier.
- 25
- Claim 22. The immunoassay of claim 21, wherein said immunoassay reveals the presence of said TRAIL Compound.
- Claim 23. The immunoassay of claim 21, wherein said immunoassay reveals the concentration of said TRAIL Compound.

- Claim 24. The immunoassay of claim 21, wherein said immobilized molecule capable of specific binding to said complex is an rsTRAIL molecule.
- Claim 25. The immunoassay of claim 21, wherein said TRAIL Compound is sTRAIL, and said immobilized molecule capable of specific binding to said complex is an anti-sTRAIL antibody.
- Claim 26. The immunoassay of claim 21, wherein said immunoassay determines whether a mammal suffers from a disease or condition involving immune system activation selected from the group consisting of HIV infection, AIDS, cancer, atherosclerosis, Alzheimer's disease, inflammation, autoimmune disorder, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant rejection, and graft vs host disease.
- Claim 27. The immunoassay of claim 26, wherein said disease or condition involving immune system activation is HIV infection or AIDS.
- Claim 28. A kit for measuring the presence or concentration of a TRAIL Compound in a biological fluid of a mammal, wherein said kit comprises a hollow casing comprising a multilayer filter system, and first and second porous carriers, wherein said second porous carrier is in communication with said first porous carrier, and said first porous carrier is in communication with said multilayer filter system, a portion of which is accessible from said casing; wherein: said first porous carrier contains a non-immobilized, labeled antibody that specifically binds said TRAIL Compound; and said second porous carrier contains an immobilized, unlabeled molecule that binds to a TRAIL Compound – antibody complex.

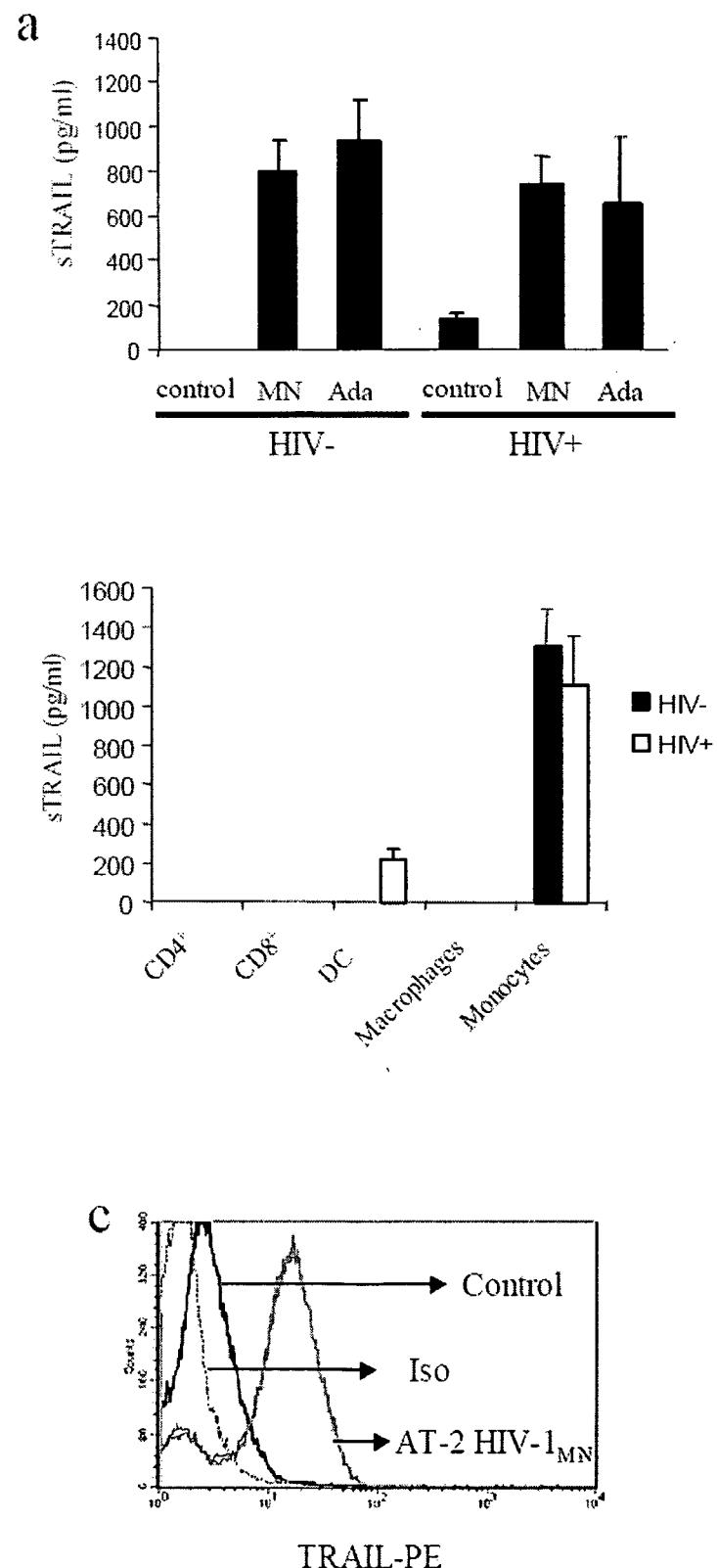
- Claim 29. The kit of claim 28, wherein said kit reveals the presence of said TRAIL Compound.
- Claim 30. The kit of claim 28, wherein said kit reveals the concentration of said TRAIL Compound.
- 5 Claim 31. The kit of claim 28, wherein said immobilized molecule capable of specific binding to said complex is an rsTRAIL molecule.
- Claim 32. The kit of claim 28, wherein said TRAIL Compound is sTRAIL, and said immobilized molecule capable of specific binding to said complex is an anti-sTRAIL antibody.
- 10 Claim 33. The kit of claim 28, wherein said kit is suitable for determining whether a mammal suffers from a disease or condition of involving immune system activation selected from the group consisting of HIV infection, AIDS, cancer, atherosclerosis, Alzheimer's disease, inflammation, autoimmune disorder, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant rejection, and graft vs host disease.
- 15 Claim 34. The immunoassay of claim 33, wherein said disease or condition of involving immune system activation is HIV infection or AIDS.

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Figure 1

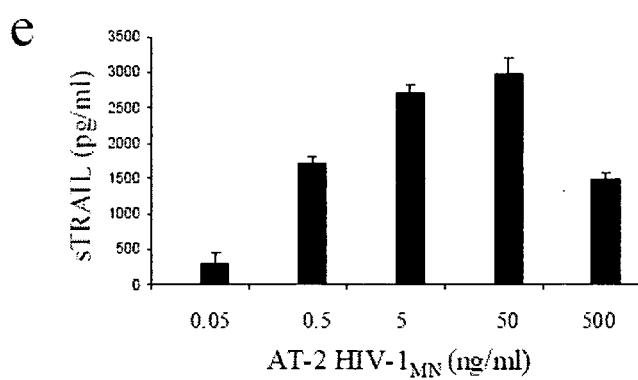
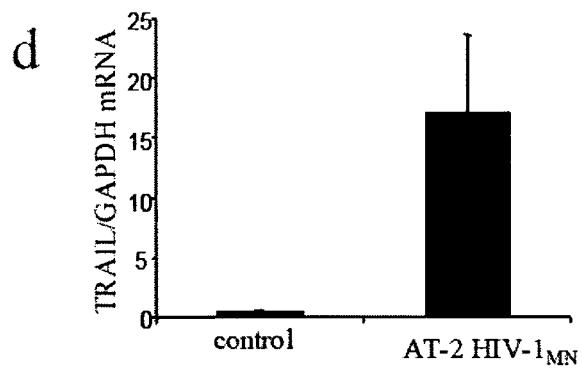
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Figure 2 (a-c)



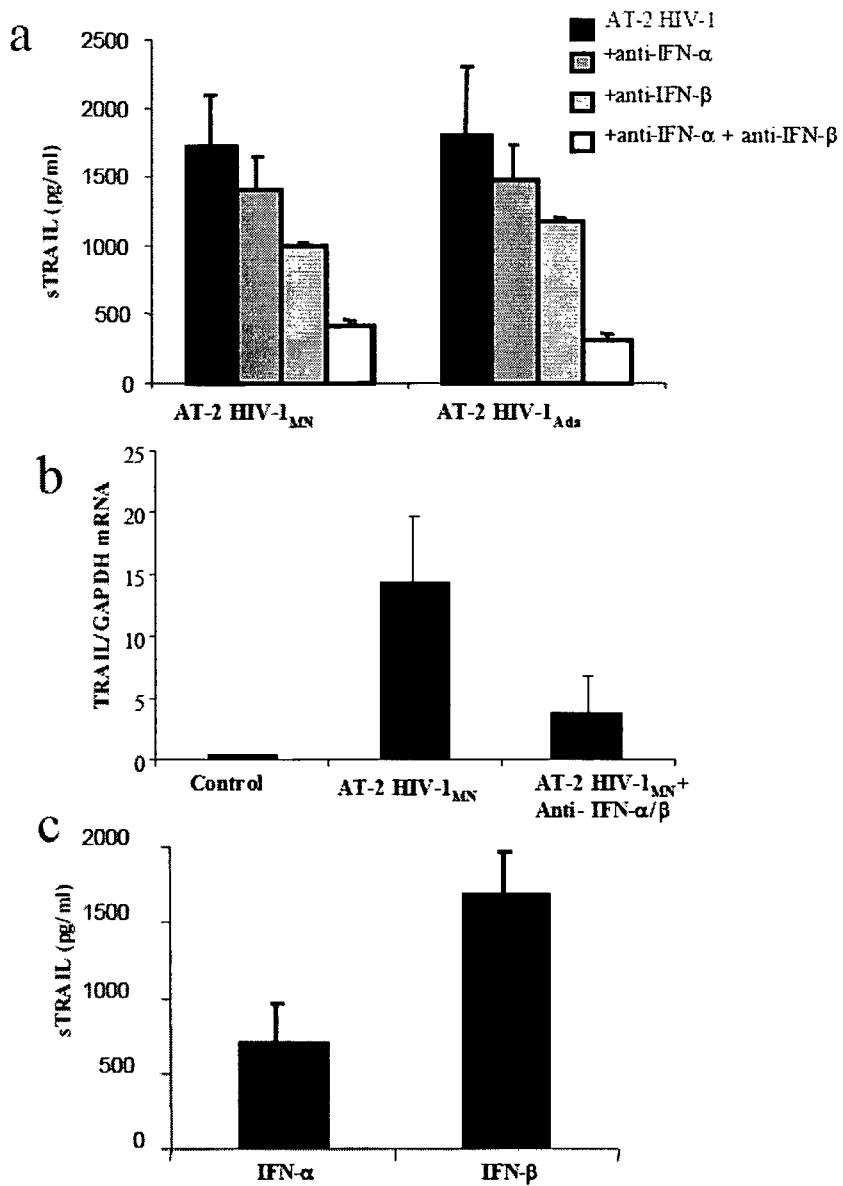
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Figure 2 (d-e)



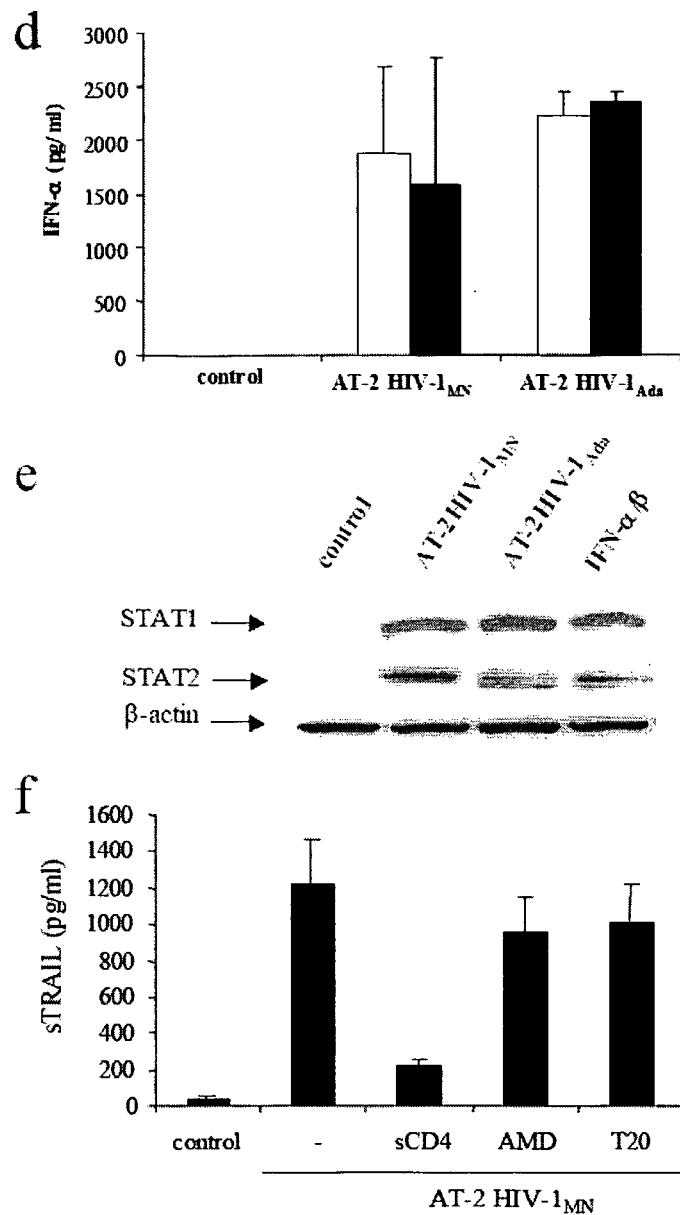
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Figure 3 (a-c)



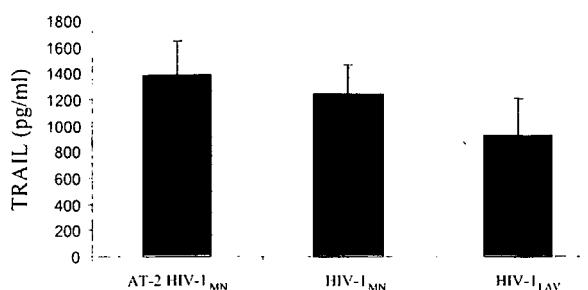
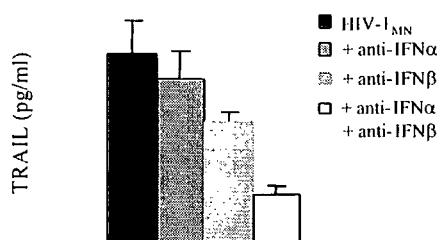
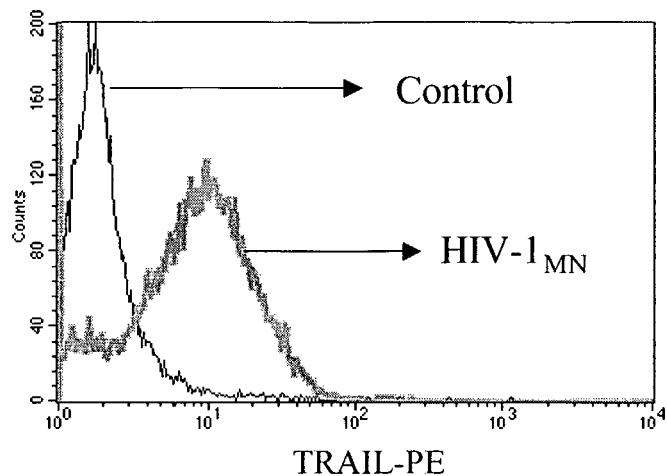
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Figure 3 (d-f)

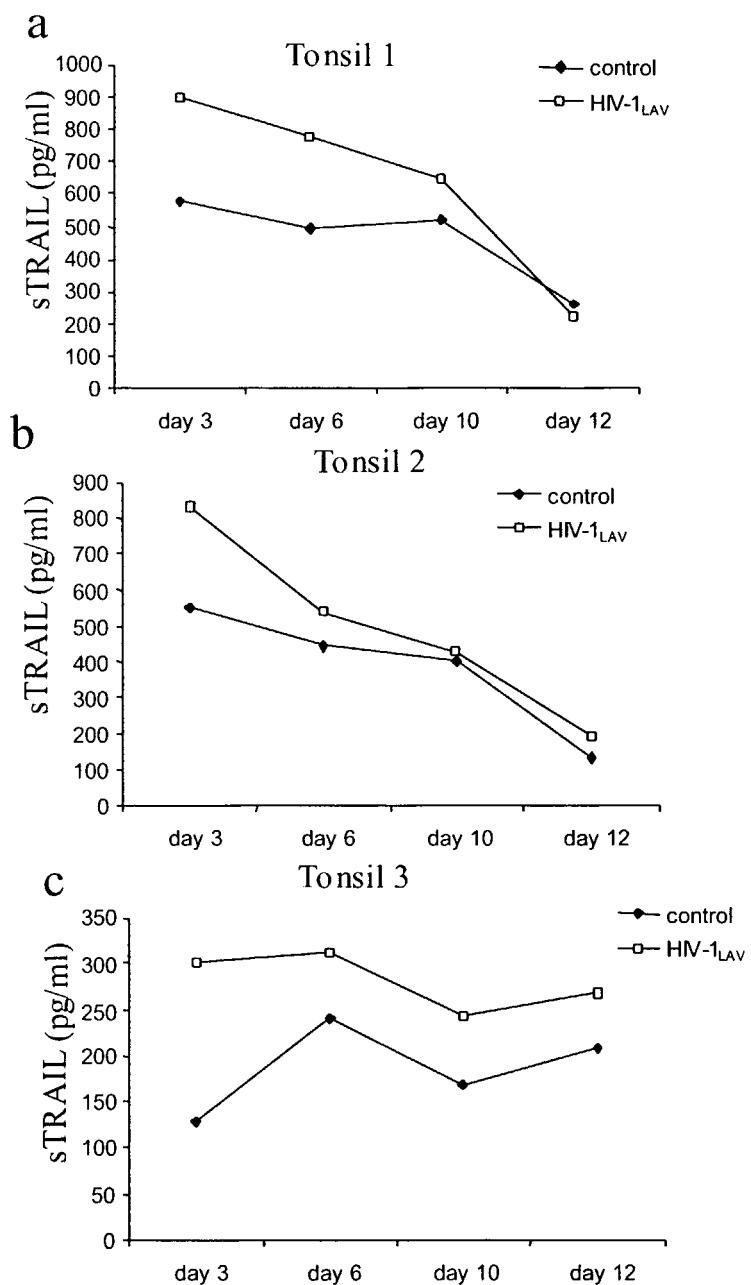


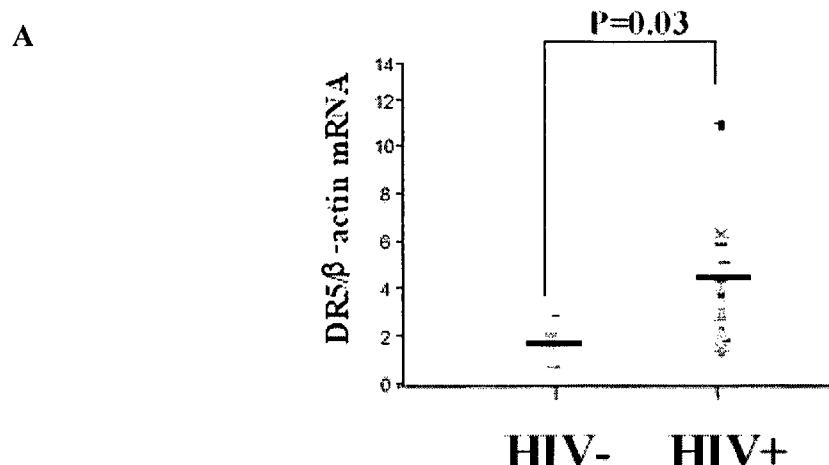
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Figure 4

a**b****c**

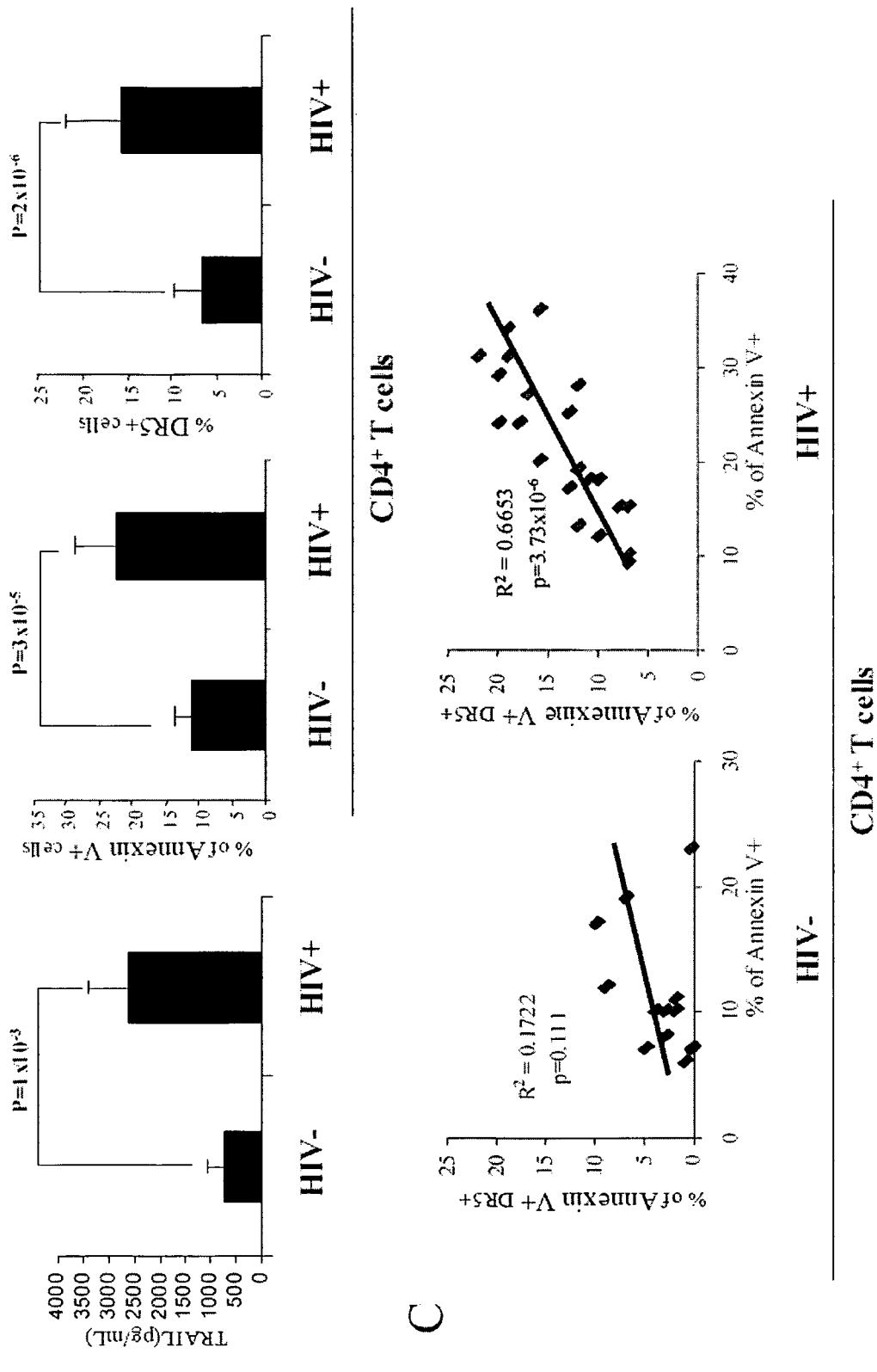
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Figure 5

8/15**Figure 6 (A)**

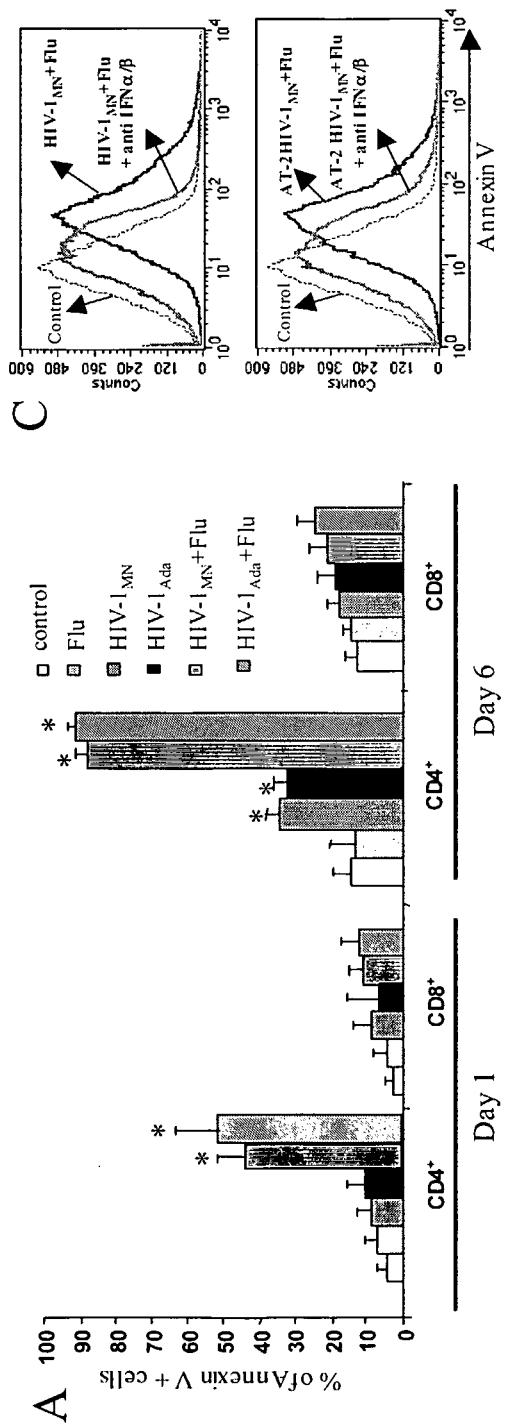
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Figure 6, (B-C)



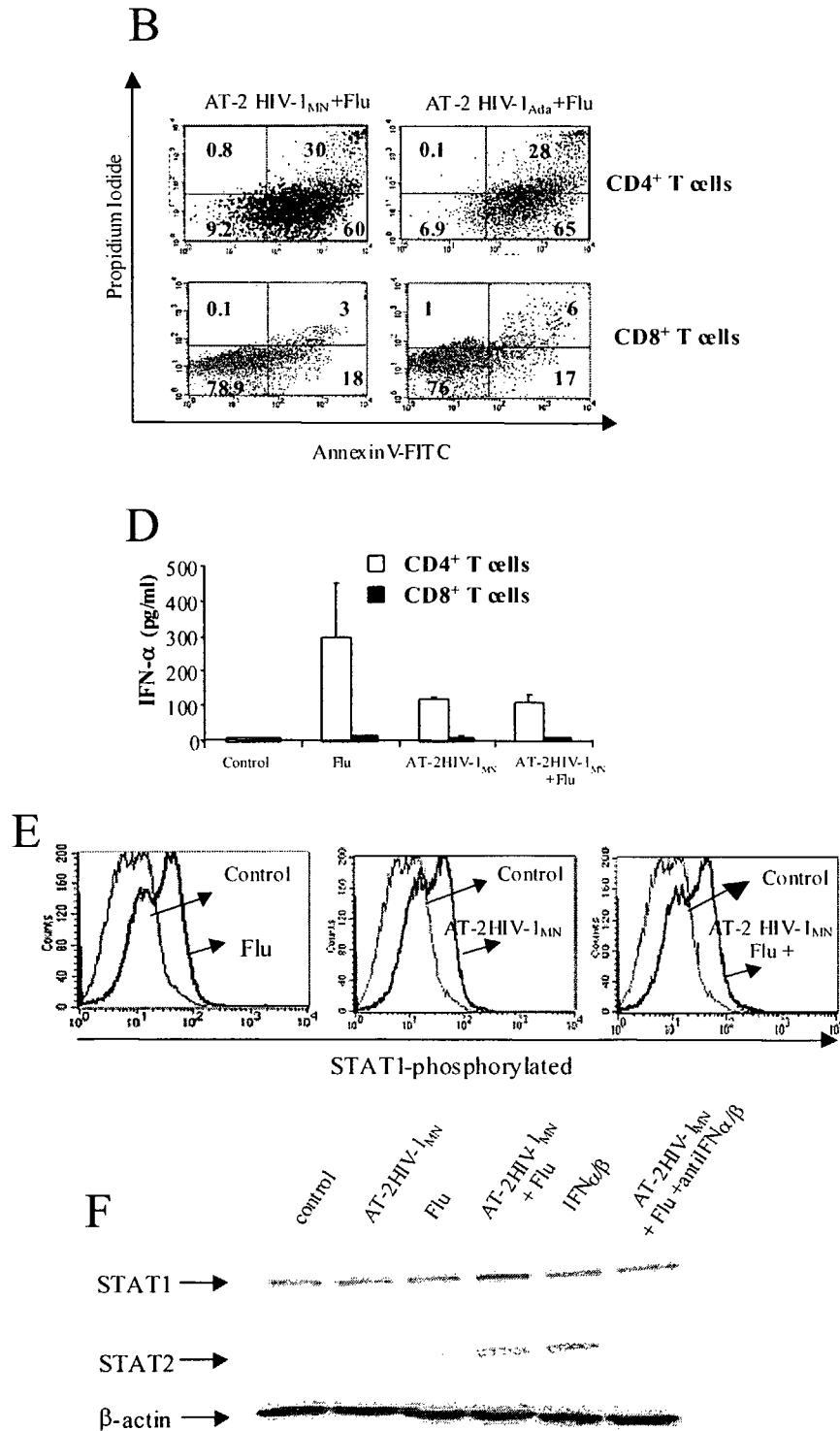
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Figure 7 (A, C)



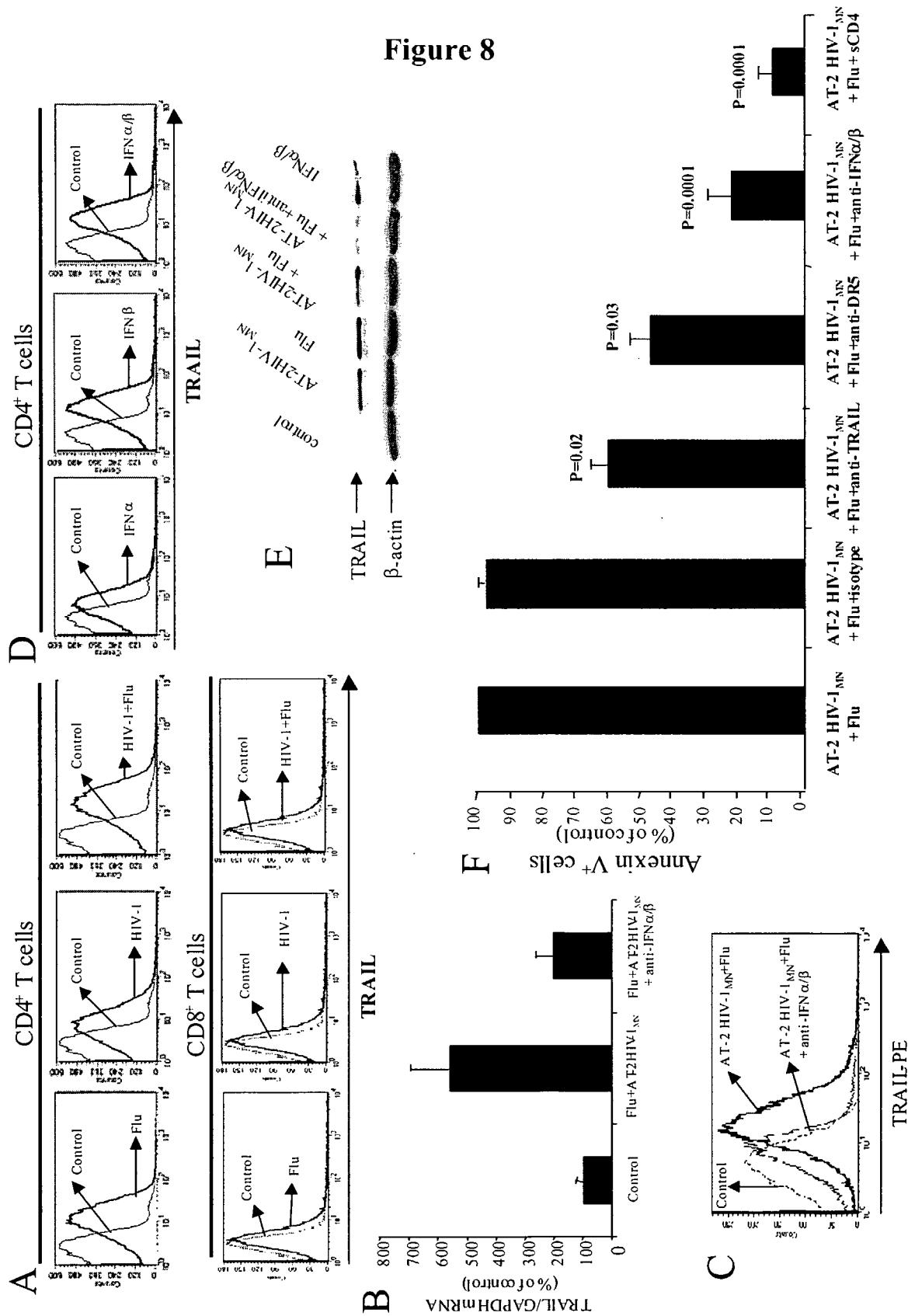
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Figure 7 (B, D-F)



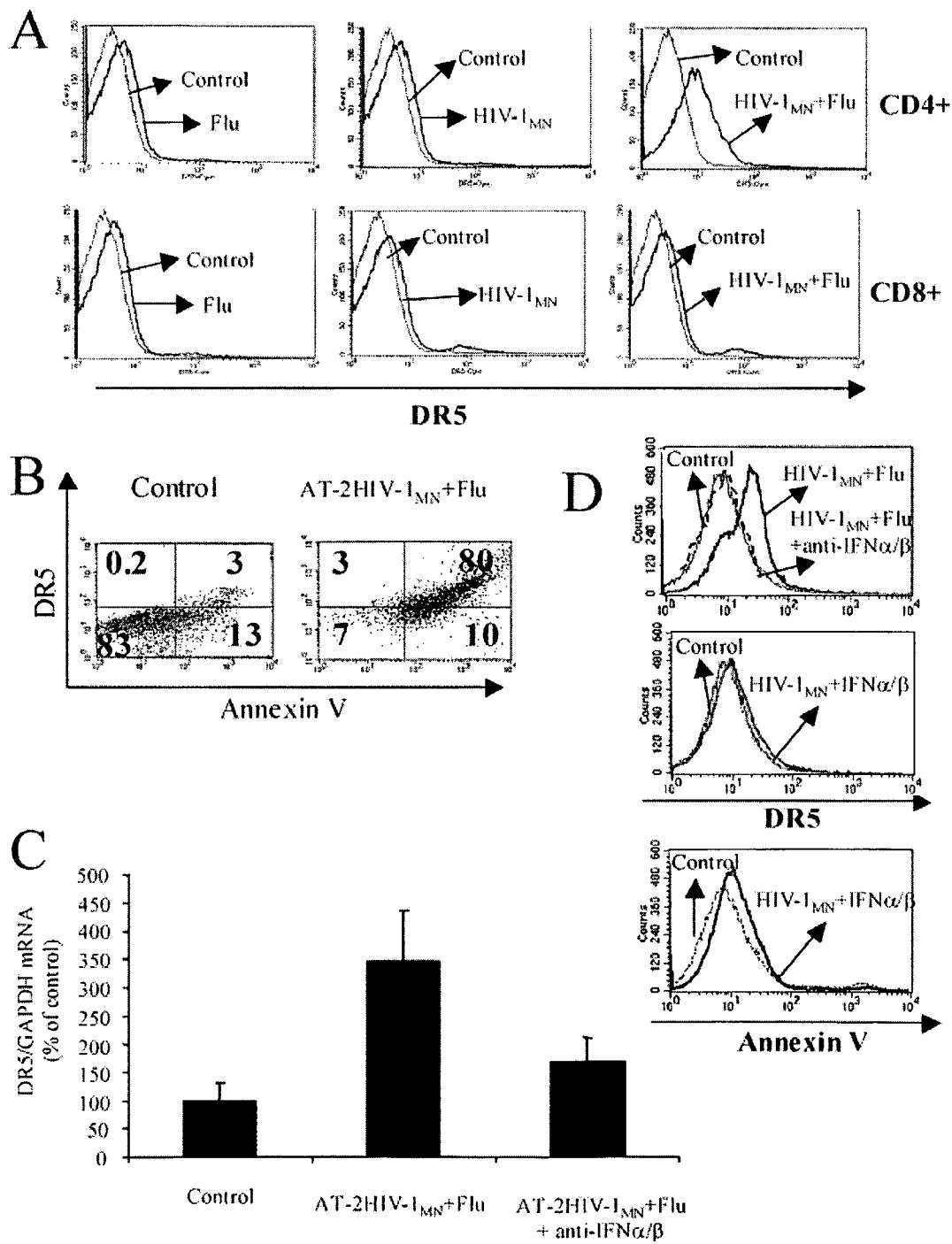
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Figure 8

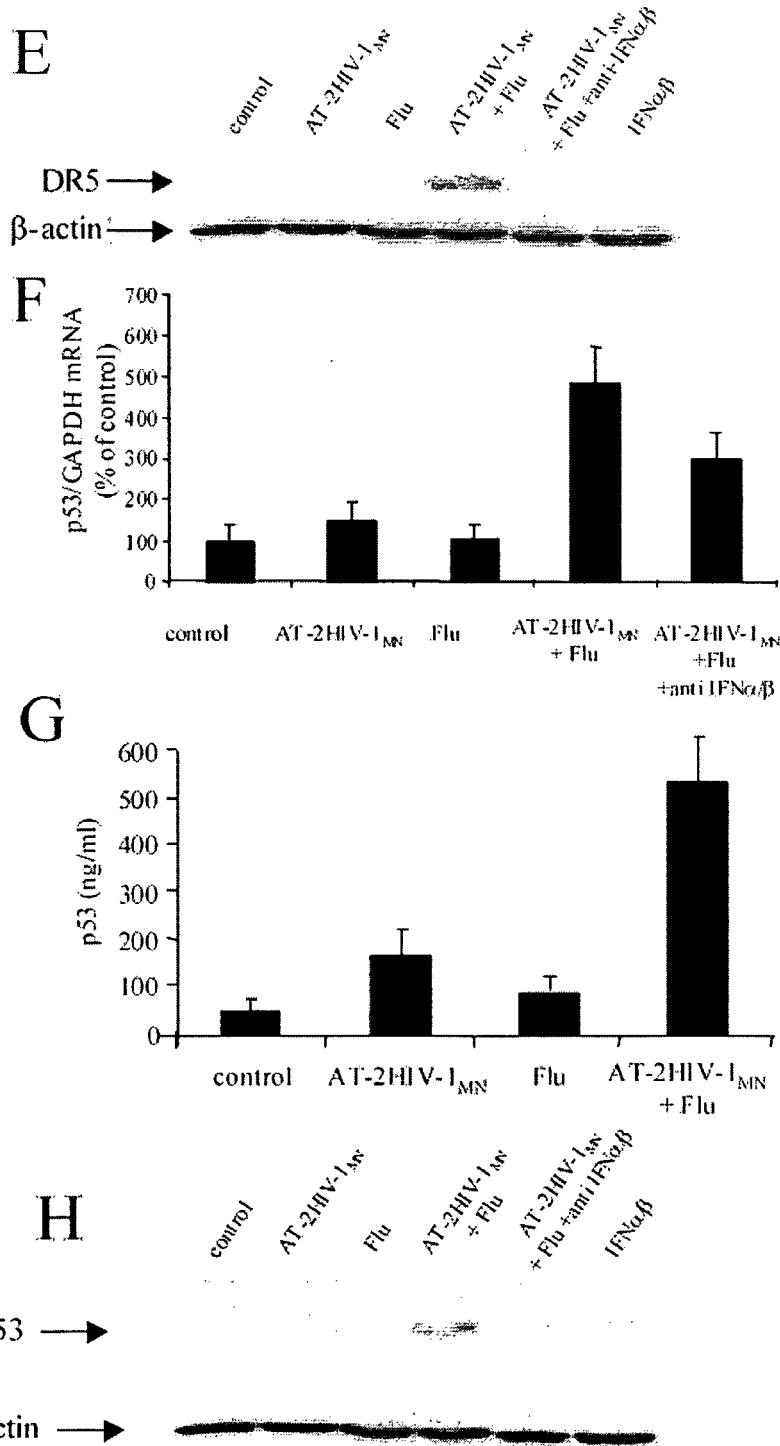


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Figure 9 (A-D)

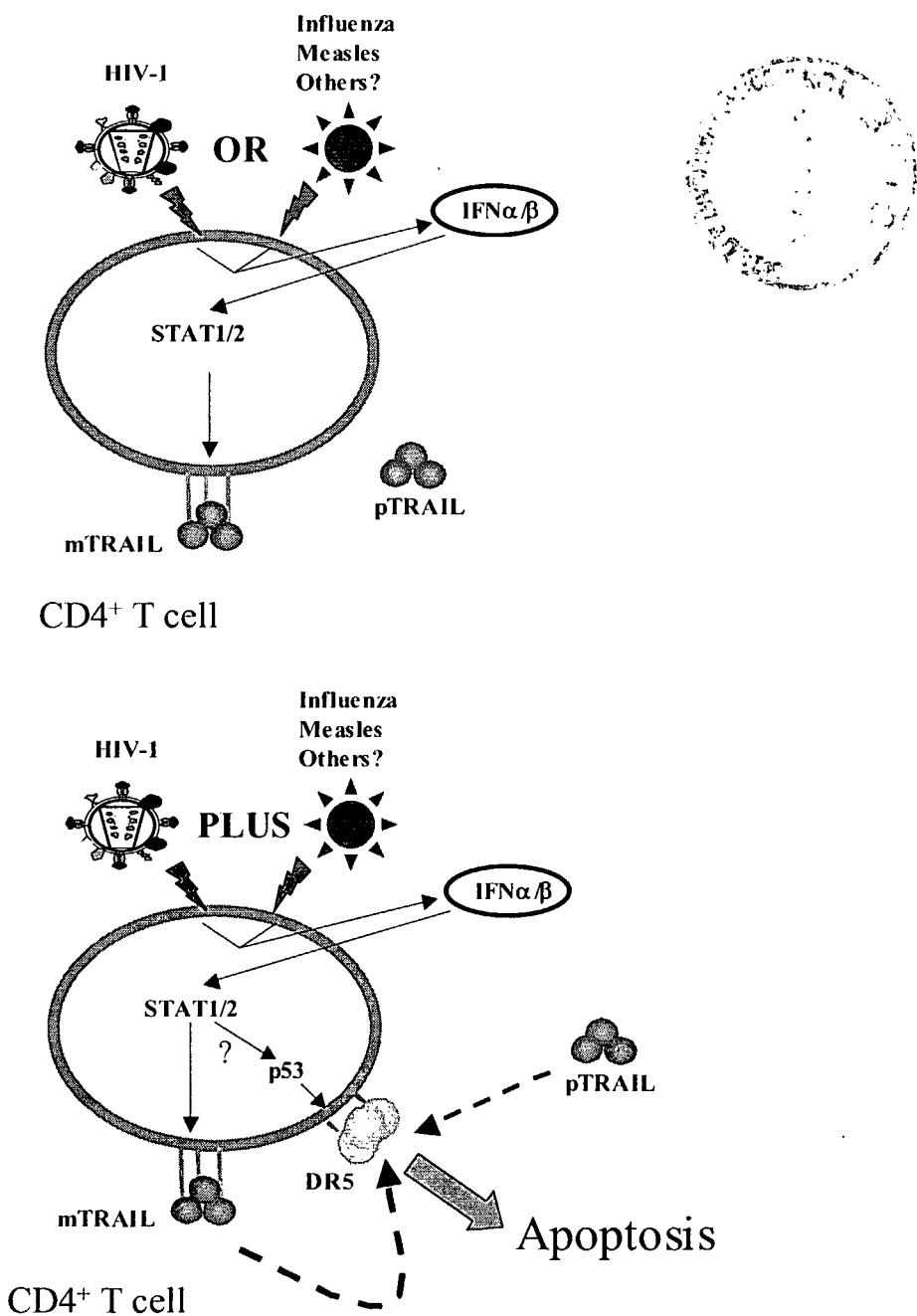


14/15

Figure 9 (E-H)

15/15

Figure 10



SEQUENCE LISTING

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Secretary of DHHS, NIH
5 Shearer, Gene M
Herbeuval , Jean P

<120> Methods and Compositions For Diagnosing AIDS And Other Diseases
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	Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp		
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Arg Asn Thr Phe Arg His Ser Val Val Val Pro Tyr Glu Pro Pro Glu
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Lys Pro Leu Asp Gly Glu Tyr Phe Thr Leu Gln Ile Arg Gly Arg Glu
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Arg Phe Glu Met Phe Arg Glu Leu Asn Ala Leu Glu Leu Lys Asp
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US 20050079518A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0079518 A1**

Baker et al.

(43) **Pub. Date:** **Apr. 14, 2005**

(54) **PREDICTION OF LIKELIHOOD OF CANCER
RECURRENCE**

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(21) Appl. No.: **10/872,063**

(22) Filed: **Jun. 17, 2004**

Related U.S. Application Data

(60) Provisional application No. 60/482,339, filed on Jun.
24, 2003.

Publication Classification

(51) **Int. Cl.⁷** **C12Q 1/68**
(52) **U.S. Cl.** **435/6**

(57) **ABSTRACT**

The present invention provides gene sets the expression of which is important in the diagnosis and/or prognosis of cancer, in particular of breast cancer.

PREDICTION OF LIKELIHOOD OF CANCER RECURRENCE

[0001] The present application claims the benefit under 35 U.S.C. 119(e) of the filing date of U.S. Application Ser. No. 60/482,339, filed on Jun. 24, 2003.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention provides gene sets the expression of which is important in the diagnosis and/or prognosis of cancer.

[0004] 2. Description of the Related Art

[0005] Oncologists have a number of treatment options available to them, including different combinations of chemotherapeutic drugs that are characterized as "standard of care," and a number of drugs that do not carry a label claim for particular cancer, but for which there is evidence of efficacy in that cancer. Best likelihood of good treatment outcome requires that patients be assigned to optimal available cancer treatment, and that this assignment be made as quickly as possible following diagnosis.

[0006] Currently, diagnostic tests used in clinical practice are single analyte, and therefore do not capture the potential value of knowing relationships between dozens of different markers. Moreover, diagnostic tests are frequently not quantitative, relying on immunohistochemistry. This method often yields different results in different laboratories, in part because the reagents are not standardized, and in part because the interpretations are subjective and cannot be easily quantified. RNA-based tests have not often been used because of the problem of RNA degradation over time and the fact that it is difficult to obtain fresh tissue samples from patients for analysis. Fixed paraffin-embedded tissue is more readily available and methods have been established to detect RNA in fixed tissue. However, these methods typically do not allow for the study of large numbers of genes (DNA or RNA) from small amounts of material. Thus, traditionally fixed tissue has been rarely used other than for immunohistochemistry detection of proteins.

[0007] In the past few years, several groups have published studies concerning the classification of various cancer types by microarray gene expression analysis (see, e.g. Golub et al., *Science* 286:531-537 (1999); Bhattacharjee et al., *Proc. Natl. Acad. Sci. USA* 98:13790-13795 (2001); Chen-Hsiang et al., *Bioinformatics* 17 (Suppl. 1):S316-S322 (2001); Ramaswamy et al., *Proc. Natl. Acad. Sci. USA* 98:15149-15154 (2001)). Certain classifications of human breast cancers based on gene expression patterns have also been reported (Martin et al., *Cancer Res.* 60:2232-2238 (2000); West et al., *Proc. Natl. Acad. Sci. USA* 98:11462-11467 (2001); Sorlie et al., *Proc. Natl. Acad. Sci. USA* 98:10869-10874 (2001); Yan et al., *Cancer Res.* 61:8375-8380 (2001)). However, these studies mostly focus on improving and refining the already established classification of various types of cancer, including breast cancer, and generally do not provide new insights into the relationships of the differentially expressed genes, and do not link the findings to treatment strategies in order to improve the clinical outcome of cancer therapy.

[0008] Although modern molecular biology and biochemistry have revealed hundreds of genes whose activities

influence the behavior of tumor cells, state of their differentiation, and their sensitivity or resistance to certain therapeutic drugs, with a few exceptions, the status of these genes has not been exploited for the purpose of routinely making clinical decisions about drug treatments. One notable exception is the use of estrogen receptor (ER) protein expression in breast carcinomas to select patients to treatment with anti-estrogen drugs, such as tamoxifen. Another exceptional example is the use of ErbB2 (Her2) protein expression in breast carcinomas to select patients with the Her2 antagonist drug Herceptin® (Genentech, Inc., South San Francisco, Calif.).

[0009] Despite recent advances, the challenge of cancer treatment remains to target specific treatment regimens to pathogenetically distinct tumor types, and ultimately personalize tumor treatment in order to maximize outcome. Hence, a need exists for tests that simultaneously provide predictive information about patient responses to the variety of treatment options. This is particularly true for breast cancer, the biology of which is poorly understood. It is clear that the classification of breast cancer into a few subgroups, such as ErbB2⁺ subgroup, and subgroups characterized by low to absent gene expression of the estrogen receptor (ER) and a few additional transcriptional factors (Perou et al., *Nature* 406:747-752 (2000)) does not reflect the cellular and molecular heterogeneity of breast cancer, and does not allow the design of treatment strategies maximizing patient response.

[0010] In particular, once a patient is diagnosed with cancer, such as breast or ovarian cancer, there is a strong need for methods that allow the physician to predict the expected course of disease, including the likelihood of cancer recurrence, long-term survival of the patient, and the like, and select the most appropriate treatment option accordingly.

SUMMARY OF THE INVENTION

[0011] The present invention provides a set of genes, the expression of which has prognostic value, specifically with respect to disease-free survival.

[0012] The present invention accommodates the use of archived paraffin-embedded biopsy material for assay of all markers in the set, and therefore is compatible with the most widely available type of biopsy material. It is also compatible with several different methods of tumor tissue harvest, for example, via core biopsy or fine needle aspiration. Further, for each member of the gene set, the invention specifies oligonucleotide sequences that can be used in the test.

[0013] In one aspect, the present invention concerns a method of predicting the likelihood of long-term survival of a cancer patient without the recurrence of cancer, comprising determining the expression level of one or more prognostic RNA transcripts or their expression products in a cancer cell obtained from the patient, normalized against the expression level of all RNA transcripts or their products in said cancer cell, or of a reference set of RNA transcripts or their expression products, wherein the prognostic RNA transcript is the transcript of one or more genes selected from the group consisting of B_Catenin; BAG1; BIN1; BUB1; C20_orf1; CCNB1; CCNE2; CDC20; CDH1; CEGP1; CLAP1; cMYC; CTSL2; DKFZp586M07; DR5;

EpCAM; EstR1; FOXM1; GRB7; GSTM1; GSTM3; HER2; HNRPAB; ID1; IGFR; ITGA7; Ki_67; KNSL2; LMNB1; MCM2; MELK; MMP12; MMP9; MYBL2; NEK2; NME1; NPD009; PCNA; PR; PREP; PTTG1; RPLPO; Src; STK15; STMY3; SURV; TFRC; TOP2A; and TS;

[0014] wherein expression of one or more of BUB1; C20_orf1; CCNB1; CCNE2; CDC20; CDH1; CTSL2; EpCAM; FOXM1; GRB7; HER2; HNRPAB; Ki_67; KNSL2; LMNB1; MCM2; MELK; MMP12; MMP9; MYBL2; NEK2; NME1; PCNA; PREP; PTTG1; Src; STK15; STMY3; SURV; TFRC; TOP2A; and TS indicates a decreased likelihood of long-term survival without cancer recurrence; and

[0015] the expression of one or more of BAG1; BCatenin; BIN1; CEGP1; CIAP1; cMYC; DKFZp586M07; DR5; EstR1; GSTM1; GSTM3; ID1; IGF1R; ITGA7; NPD009; PR; and RPLPO indicates an increased likelihood of long-term survival without cancer recurrence.

[0016] In various embodiments, the expression level of at least 2, or at least 5, or at least 10, or at least 15, or at least 20, or a least 25 prognostic RNA transcripts or their expression products is determined.

[0017] In another embodiment, the cancer is breast cancer or ovarian cancer.

[0018] In yet another embodiment, the cancer is node negative, ER positive breast cancer.

[0019] In a further embodiment, the RNA comprises intronic RNA.

[0020] In a still further embodiment, the expression level of one or more prognostic RNA transcripts or their expression products of one or more genes selected from the group consisting of MMP9, GSTM1, MELK, PR, DKFZp586M07, GSTM3, CDC20, CCNB1, STMY3, GRB7, MYBL2, CEGP1, SURV, LMNB1, CTSL2, PTTG1, BAG1, KNSL2, CIAP1, PREP, NEK2, EpCAM, PCNA, C20_orf1, ITGA7, ID1_B_Catenin, EstR1, CDH1, TS HER2, and cMYC is determined,

[0021] wherein expression of one or more of C20_orf1; CCNB1; CDC20; CDH1; CTSL2; EpCAM; GRB7; HER2; KNSL2; LMNB1; MCM2; MMP9; MYBL2; NEK2; PCNA; PREP; PTTG1; STMY3; SURV; TS; and MELK indicates a decreased likelihood of long-term survival without cancer recurrence; and

[0022] the expression of one or more of BAG1; BCatenin; CEGP1; CIAP1; cMYC; DKFZp586M07; EstR1; GSTM1; GSTM3; ID1; ITGA7; and PR indicates an increased likelihood of long-term survival without cancer recurrence.

[0023] In another embodiment, the expression level of one or more prognostic RNA transcripts or their expression products of one or more genes selected from the group consisting of GRB7, SURV, PR, LMNB1, MYBL2, HER2, GSTM1, MELK, S20_orf1, PTTG1, BUB1, CDC20, CCNB1, STMY3, KNSL2, CTSL2, MCM2, NEK2, DR5, Ki_67, CCNE2, TOP2A, PCNA, PREP, FOXM1, NME1, CEGP1, BAG1, STK15, HNRPAB, EstR1, MMP9,

DKFZp586M07, TS, Src, BIN1, NP009, RPLPO, GSTM3, MMP12, TFRC, and IGF1R is determined,

[0024] wherein expression of one or more of GRB7; SURV; LMNB1; MYBL2; HER2; MELK; C20_orf1; PTTG1; BUB1; CDC20; CCNB1; STMY3; KNSL2; CTSL2; MCM2; NEK2; Ki_67; CCNE2; TOP2A_4; PCNA; PREP; FOXM1; NME1; STK15; HNRPAB; MMP9; TS; Src; MMP12; and TFRC indicates a decreased likelihood of long-term survival without cancer recurrence; and

[0025] the expression of one or more of PR; GSTM1; DR5; CEGP1; BAG1; EstR1; DKFZp586M07; BIN1; NPD009; RPLPO; GSTM3; IGF1R indicates an increased likelihood of long-term survival without cancer recurrence.

[0026] In another aspect, the invention concerns a method of predicting the likelihood of long-term survival of a cancer patient without the recurrence of cancer, comprising determining the expression level of one or more prognostic RNA transcripts or their expression products in a cancer cell obtained from said patient, normalized against the expression level of all RNA transcripts or their products in the cancer cell, or of a reference set of RNA transcripts or their expression products, wherein the prognostic RNA transcript is the transcript of one or more genes selected from the group consisting of GRB7; LMNB1; ER; STMY3; KLK10; PR; KRT5; FGFR1; MCM6; SNRPF,

[0027] wherein expression of one or more of GRB7, LMNB1, STMY3, KLK10, FGFR1, and SNRPF indicates a decreased likelihood of long term survival without cancer recurrence; and the expression of one or more of ER, PR, KRT5 and MCM6 ER, PR, KRT5 and MCM6 indicates an increased likelihood of long-term survival without cancer recurrence.

[0028] In an embodiment of this method, the RNA is isolated from a fixed, wax-embedded breast cancer tissue specimen of the patient.

[0029] In another embodiment, the RNA is isolated from core biopsy tissue or fine needle aspirate cells.

[0030] In a different aspect, the invention concerns an array comprising polynucleotides hybridizing to two or more of the following genes: B_Catenin; BAG1; BIN1; BUB1; C20_orf1; CCNB1; CCNE2; CDC20; CDH1; CEGP1; CIAP1; cMYC; CTSL2; DKFZp586M07; DR5; EPCAM; EstR1; FOXM1; GRB7; GSTM1; GSTM3; HER2; HNRPAB; ID1; IGF1R; ITGA7; Ki_67; KNSL2; LMNB1; MCM2; MELK; MMP12; MMP9; MYBL2; NEK2; NME1; NPD009; PCNA; PR; PREP; PTTG1; RPLPO; Src; STK15; STMY3; SURV; TFRC; TOP2A; and TS, immobilized on a solid surface.

[0031] In an embodiment, the array comprises polynucleotides hybridizing to two or more of the following genes: MMP9, GSTM1, MELK, PR, DKFZp586M07, GSTM3, CDC20, CCNB1, STMY3, GRB7, MYBL2, CEGP1, SURV, LMNB1, CTSL2, PTTG1, BAG1, KNSL2, CIAP1, PREP, NEK2, EpCAM, PCNA, C20_orf1, ITGA7, ID1_B_Catenin, EstR1, CDH1, TS HER2, and cMYC.

[0032] In another embodiment, the array comprises polynucleotides hybridizing to two or more of the following

genes: GRB7, SURV, PR, LMNB1, MYBL2, HER2, GSTM1, MELK, S20_orf1, PTTG1, BUB1, CDC20, CCNB1, STMY3, KNSL2, CTSL2, MCM2, NEK2, DR5, Ki_67, CCNE2, TOP2A, PCNA, PREP, FOXM1, NME1, CEGP1, BAG1, STK15, HNRPAB, EstR1, MMP9, DKFZp586M07, TS, Src, BIN1, NP009, RPLPO, GSTM3, MMP12, TFRC, and IGF1R.

[0033] In a further embodiment, the arrays comprise polynucleotides hybridizing to at least 3, or at least 5, or at least 10, or at least 15, or at least 20, or at least 25 of the listed genes.

[0034] In a still further embodiment, the arrays comprise polynucleotides hybridizing to all of the listed genes.

[0035] In yet another embodiment, the arrays comprise more than one polynucleotide hybridizing to the same gene.

[0036] In an additional embodiment, the arrays comprise intron-based sequences.

[0037] In another embodiment, the polynucleotides are cDNAs, which can, for example, be about 500 to 5000 bases long.

[0038] In yet another embodiment, the polynucleotides are oligonucleotides, which can, for example, be about 20 to 80 bases long.

[0039] The arrays can, for example, be immobilized on glass, and can contain hundreds of thousand, e.g. 330,000 oligonucleotides.

[0040] In a further aspect, the invention concerns a method of predicting the likelihood of long-term survival of a patient diagnosed with invasive breast cancer, without the recurrence of breast cancer, comprising the steps of

[0041] (a) determining the expression levels of the RNA transcripts or the expression products of genes of a gene set selected from the group consisting of B_Catenin; BAG1; BIN1; BUB1; C20_orf1; CCNB1; CCNE2; CDC20; CDH1; CEGP1; CIAP1; cMYC; CTSL2; DKFZp586M07; DR5; EpCAM; EstR1; FOXM1; GRB7; GSTM1; GSTM3; HER2; HNRPAB; ID1; IGF1R; ITGA7; Ki_67; KNSL2; LMNB1; MCM2; MELK; MMP12; MMP9; MYBL2; NEK2; NME1; NP009; PCNA; PR; PREP; PTTG1; RPLPO; Src; STK15; STMY3; SURV; TFRC; TOP2A; and TS in a breast cancer cell obtained from the patient, normalized against the expression levels of all RNA transcripts or their expression products in said breast cancer cell, or of a reference set of RNA transcripts or their products;

[0042] (b) subjecting the data obtained in step (a) to statistical analysis; and;

[0043] (c) determining whether the likelihood of said long-term survival has increased or decreased.

[0044] In a still further aspect, the invention concerns a method of preparing a personalized genomics profile for a patient, comprising the steps of

[0045] (a) subjecting RNA extracted from a breast tissue obtained from the patient to gene expression analysis;

[0046] (b) determining the expression level in the tissue of one or more genes selected from the breast cancer gene set listed in any one of Tables 1 and 2, wherein the expression level is normalized against a control gene or genes and optionally is compared to the amount found in a breast cancer reference tissue set; and

[0047] (c) creating a report summarizing the data obtained by said gene expression analysis.

[0048] The breast tissue may comprise breast cancer cells.

[0049] In another embodiment, the breast tissue is obtained from a fixed, paraffin-embedded biopsy sample, in which the RNA may be fragmented.

[0050] The report may include prediction of the likelihood of long term survival of the patient and/or a recommendation for a treatment modality of said patient.

[0051] In a further aspect, the invention concerns a method for measuring levels of mRNA products of genes listed in Tables 1 and 2 by real time polymerase chain reaction (RT-PCR), by using an amplicon listed in Table 3 and a primer-probe set listed in Tables 4A-4D.

[0052] In a still further aspect, the invention concerns a PCR primer-probe set listed in Tables 4A-4D, and a PCR amplicon listed in Table 3.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0053] A. Definitions

[0054] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application.

[0055] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

[0056] The term "microarray" refers to an ordered arrangement of hybridizable array elements, preferably polynucleotide probes, on a substrate.

[0057] The term "polynucleotide," when used in singular or plural, generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as defined herein include, without limitation, single- and double-stranded DNA, DNA including single- and double-stranded regions, single- and double-stranded RNA, and RNA including single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or include single- and double-stranded regions. In addition, the term "polynucleotide" as used herein refers to triple-

stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. The term “polynucleotide” specifically includes cDNAs. The term includes DNAs (including cDNAs) and RNAs that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are “polynucleotides” as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritiated bases, are included within the term “polynucleotides” as defined herein. In general, the term “polynucleotide” embraces all chemically, enzymatically and/or metabolically modified forms of unmodified polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells.

[0058] The term “oligonucleotide” refers to a relatively short polynucleotide, including, without limitation, single-stranded deoxyribonucleotides, single- or double-stranded ribonucleotides, RNA:DNA hybrids and double-stranded DNAs. Oligonucleotides, such as single-stranded DNA probe oligonucleotides, are often synthesized by chemical methods, for example using automated oligonucleotide synthesizers that are commercially available. However, oligonucleotides can be made by a variety of other methods, including *in vitro* recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms.

[0059] The terms “differentially expressed gene,” “differential gene expression” and their synonyms, which are used interchangeably, refer to a gene whose expression is activated to a higher or lower level in a subject suffering from a disease, specifically cancer, such as breast cancer, relative to its expression in a normal or control subject. The terms also include genes whose expression is activated to a higher or lower level at different stages of the same disease. It is also understood that a differentially expressed gene may be either activated or inhibited at the nucleic acid level or protein level, or may be subject to alternative splicing to result in a different polypeptide product. Such differences may be evidenced by a change in mRNA levels, surface expression, secretion or other partitioning of a polypeptide, for example. Differential gene expression may include a comparison of expression between two or more genes or their gene products, or a comparison of the ratios of the expression between two or more genes or their gene products, or even a comparison of two differently processed products of the same gene, which differ between normal subjects and subjects suffering from a disease, specifically cancer, or between various stages of the same disease. Differential expression includes both quantitative, as well as qualitative, differences in the temporal or cellular expression pattern in a gene or its expression products among, for example, normal and diseased cells, or among cells which have undergone different disease events or disease stages. For the purpose of this invention, “differential gene expression” is considered to be present when there is at least an about two-fold, preferably at least about four-fold, more preferably at least about six-fold, most preferably at least about ten-fold difference between the expression of a given gene in normal and diseased subjects, or in various stages of disease development in a diseased subject.

[0060] The term “over-expression” with regard to an RNA transcript is used to refer to the level of the transcript determined by normalization to the level of reference mRNAs, which might be all measured transcripts in the specimen or a particular reference set of mRNAs.

[0061] The phrase “gene amplification” refers to a process by which multiple copies of a gene or gene fragment are formed in a particular cell or cell line. The duplicated region (a stretch of amplified DNA) is often referred to as “amplicon.” Usually, the amount of the messenger RNA (mRNA) produced, i.e., the level of gene expression, also increases in the proportion of the number of copies made of the particular gene expressed.

[0062] The term “prognosis” is used herein to refer to the prediction of the likelihood of cancer-attributable death or progression, including recurrence, metastatic spread, and drug resistance, of a neoplastic disease, such as breast cancer. The term “prediction” is used herein to refer to the likelihood that a patient will respond either favorably or unfavorably to a drug or set of drugs, and also the extent of those responses, or that a patient will survive, following surgical removal or the primary tumor and/or chemotherapy for a certain period of time without cancer recurrence. The predictive methods of the present invention can be used clinically to make treatment decisions by choosing the most appropriate treatment modalities for any particular patient. The predictive methods of the present invention are valuable tools in predicting if a patient is likely to respond favorably to a treatment regimen, such as surgical intervention, chemotherapy with a given drug or drug combination, and/or radiation therapy, or whether long-term survival of the patient, following surgery and/or termination of chemotherapy or other treatment modalities is likely.

[0063] The term “long-term” survival is used herein to refer to survival for at least 3 years, more preferably for at least 8 years, most preferably for at least 10 years following surgery or other treatment.

[0064] The term “tumor,” as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

[0065] The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, breast cancer, ovarian cancer, colon cancer, lung cancer, prostate cancer, hepatocellular cancer, gastric cancer, pancreatic cancer, cervical cancer, liver cancer, bladder cancer, cancer of the urinary tract, thyroid cancer, renal cancer, carcinoma, melanoma, and brain cancer.

[0066] The “pathology” of cancer includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of inflammatory or immunological response, neoplasia, pre-malignancy, malignancy, invasion of surrounding or distant tissues or organs, such as lymph nodes, etc.

[0067] “Stringency” of hybridization reactions is readily determinable by one of ordinary skill in the art, and gener-

ally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, (1995).

[0068] "Stringent conditions" or "high stringency conditions", as defined herein, typically: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50° C.; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42° C.; or (3) employ 50% formamide, 5×SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5× Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42° C., with washes at 42° C. in 0.2×SSC (sodium chloride/sodium citrate) and 50% formamide at 55° C., followed by a high-stringency wash consisting of 0.1×SSC containing EDTA at 55° C.

[0069] "Moderately stringent conditions" may be identified as described by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and % SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37° C. in a solution comprising: 20% formamide, 5×SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5× Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1×SSC at about 37-50° C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0070] In the context of the present invention, reference to "at least one," "at least two," "at least five," etc. of the genes listed in any particular gene set means any one or any and all combinations of the genes listed.

[0071] The term "node negative" cancer, such as "node negative" breast cancer, is used herein to refer to cancer that has not spread to the lymph nodes.

[0072] The terms "splicing" and "RNA splicing" are used interchangeably and refer to RNA processing that removes introns and joins exons to produce mature mRNA with continuous coding sequence that moves into the cytoplasm of an eukaryotic cell.

[0073] In theory, the term "exon" refers to any segment of an interrupted gene that is represented in the mature RNA

product (B. Lewin. *Genes IV* Cell Press, Cambridge Mass. 1990). In theory the term "intron" refers to any segment of DNA that is transcribed but removed from within the transcript by splicing together the exons on either side of it. Operationally, exon sequences occur in the mRNA sequence of a gene as defined by Ref. SEQ ID numbers. Operationally, intron sequences are the intervening sequences within the genomic DNA of a gene, bracketed by exon sequences and having GT and AG splice consensus sequences at their 5' and 3' boundaries.

[0074] B. Detailed Description

[0075] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, and biochemistry, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", 2nd edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Animal Cell Culture" (R. I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Handbook of Experimental Immunology", 4th edition (D. M. Weir & C. C. Blackwell, eds., Blackwell Science Inc., 1987); "Gene Transfer Vectors for Mammalian Cells" (J. M. Miller & M. P. Calos, eds., 1987); "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987); and "PCR: The Polymerase Chain Reaction", (Mullis et al., eds., 1994).

[0076] 1. Gene Expression Profiling

[0077] Methods of gene expression profiling include methods based on hybridization analysis of polynucleotides, methods based on sequencing of polynucleotides, and proteomics-based methods. The most commonly used methods known in the art for the quantification of mRNA expression in a sample include northern blotting and in situ hybridization (Parker & Barnes, Methods in Molecular Biology 106:247-283 (1999)); RNase protection assays (Hod, Biotechniques 13:852-854 (1992)); and PCR-based methods, such as reverse transcription polymerase chain reaction (RT-PCR) (Weis et al., Trends in Genetics 8:263-264 (1992)). Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. Representative methods for sequencing-based gene expression analysis include Serial Analysis of Gene Expression (SAGE), and gene expression analysis by massively parallel signature sequencing (MPSS).

[0078] 2. PCR-based Gene Expression Profiling Methods

[0079] a. Reverse Transcriptase PCR (RT-PCR)

[0080] Of the techniques listed above, the most sensitive and most flexible quantitative method is RT-PCR, which can be used to compare mRNA levels in different sample populations, in normal and tumor tissues, with or without drug treatment, to characterize patterns of gene expression, to discriminate between closely related mRNAs, and to analyze RNA structure.

[0081] The first step is the isolation of mRNA from a target sample. The starting material is typically total RNA isolated from human tumors or tumor cell lines, and corresponding normal tissues or cell lines, respectively. Thus RNA can be isolated from a variety of primary tumors,

including breast, lung, colon, prostate, brain, liver, kidney, pancreas, spleen, thymus, testis, ovary, uterus, etc., tumor, or tumor cell lines, with pooled DNA from healthy donors. If the source of mRNA is a primary tumor, mRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) tissue samples.

[0082] General methods for mRNA extraction are well known in the art and are disclosed in standard textbooks of molecular biology, including Ausubel et al., *Current Protocols of Molecular Biology*, John Wiley and Sons (1997). Methods for RNA extraction from paraffin embedded tissues are disclosed, for example, in Rupp and Locker, *Lab Invest.* 56:A67 (1987), and De Andrés et al., *BioTechniques* 18:42044 (1995). In particular, RNA isolation can be performed using purification kit, buffer set and protease from commercial manufacturers, such as Qiagen, according to the manufacturer's instructions. For example, total RNA from cells in culture can be isolated using Qiagen RNeasy mini-columns. Other commercially available RNA isolation kits include MasterPure™ Complete DNA and RNA Purification Kit (EPICENTRE®, Madison, Wis.), and Paraffin Block RNA Isolation Kit (Ambion, Inc.). Total RNA from tissue samples can be isolated using RNA Stat-60 (Tel-Test). RNA prepared from tumor can be isolated, for example, by cesium chloride density gradient centrifugation.

[0083] As RNA cannot serve as a template for PCR, the first step in gene expression profiling by RT-PCR is the reverse transcription of the RNA template into cDNA, followed by its exponential amplification in a PCR reaction. The two most commonly used reverse transcriptases are avilo myeloblastosis virus reverse transcriptase (AMV-RT) and Moloney murine leukemia virus reverse transcriptase (MMLV-RT). The reverse transcription step is typically primed using specific primers, random hexamers, or oligo-dT primers, depending on the circumstances and the goal of expression profiling. For example, extracted RNA can be reverse-transcribed using a GeneAmp RNA PCR kit (Perkin Elmer, Calif., USA), following the manufacturer's instructions. The derived cDNA can then be used as a template in the subsequent PCR reaction.

[0084] Although the PCR step can use a variety of thermostable DNA-dependent DNA polymerases, it typically employs the Taq DNA polymerase, which has a 5'-3' nuclease activity but lacks a 3'-5' proofreading endonuclease activity. Thus, TaqMan® PCR typically utilizes the 5'-nuclease activity of Taq or Tth polymerase to hydrolyze a hybridization probe bound to its target amplicon, but any enzyme with equivalent 5' nuclease activity can be used. Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liber-

ated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

[0085] TaqMan® RT-PCR can be performed using commercially available equipment, such as, for example, ABI PRISM 7700™ Sequence Detection System™ (Perkin-Elmer-Applied Biosystems, Foster City, Calif., USA), or Lightcycler (Roche Molecular Biochemicals, Mannheim, Germany). In a preferred embodiment, the 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI PRISM 7700™ Sequence Detection System™. The system consists of a thermocycler, laser, charge-coupled device (CCD), camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

[0086] 5'-Nuclease assay data are initially expressed as Ct, or the threshold cycle. As discussed above, fluorescence values are recorded during every cycle and represent the amount of product amplified to that point in the amplification reaction. The point when the fluorescent signal is first recorded as statistically significant is the threshold cycle (C_t).

[0087] To minimize errors and the effect of sample-to-sample variation, RT-PCR is usually performed using an internal standard. The ideal internal standard is expressed at a constant level among different tissues, and is unaffected by the experimental treatment. RNAs most frequently used to normalize patterns of gene expression are mRNAs for the housekeeping genes glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and β-actin.

[0088] A more recent variation of the RT-PCR technique is the real time quantitative PCR, which measures PCR product accumulation through a dual-labeled fluorogenic probe (i.e., TaqMan® probe). Real time PCR is compatible both with quantitative competitive PCR, where internal competitor for each target sequence is used for normalization, and with quantitative comparative PCR using a normalization gene contained within the sample, or a house-keeping gene for RT-PCR. For further details see, e.g. Held et al., *Genome Research* 6:986-994 (1996).

[0089] The steps of a representative protocol for profiling gene expression using fixed, paraffin-embedded tissues as the RNA source, including mRNA isolation, purification, primer extension and amplification are given in various published journal articles {for example: T. E. Godfrey et al. *J. Molec. Diagnostics* 2: 84-91 [2000]; K. Specht et al., *Am. J. Pathol.* 158: 419-29 [2001]}. Briefly, a representative process starts with cutting about 10 μm thick sections of paraffin-embedded tumor tissue samples. The RNA is then extracted, and protein and DNA are removed. After analysis of the RNA concentration, RNA repair and/or amplification steps may be included, if necessary, and RNA is reverse transcribed using gene specific promoters followed by RT-PCR.

[0090] b. MassARRAY System

[0091] In the MassARRAY-based gene expression profiling method, developed by Sequenom, Inc. (San Diego, Calif.) following the isolation of RNA and reverse transcrip-

tion, the obtained cDNA is spiked with a synthetic DNA molecule (competitor), which matches the targeted cDNA region in all positions, except a single base, and serves as an internal standard. The cDNA/competitor mixture is PCR amplified and is subjected to a post-PCR shrimp alkaline phosphatase (SAP) enzyme treatment, which results in the dephosphorylation of the remaining nucleotides. After inactivation of the alkaline phosphatase, the PCR products from the competitor and cDNA are subjected to primer extension, which generates distinct mass signals for the competitor- and cDNA-derives PCR products. After purification, these products are dispensed on a chip array, which is pre-loaded with components needed for analysis with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis. The cDNA present in the reaction is then quantified by analyzing the ratios of the peak areas in the mass spectrum generated. For further details see, e.g. Ding and Cantor, Proc. Natl. Acad. Sci. USA 100:3059-3064 (2003).

[0092] c. Other PCR-Based Methods

[0093] Further PCR-based techniques include, for example, differential display (Liang and Pardee, Science 257:967-971 (1992)); amplified fragment length polymorphism (iAFLP) (Kawamoto et al., Genome Res. 12:1305-1312 (1999)); BeadArray™ technology (Illumina, San Diego, Calif.; Olyphant et al., Discovery of Markers for Disease (Supplement to Biotechniques), June 2002; Ferguson et al., Analytical Chemistry 72:5618 (2000)); BeadsArray for Detection of Gene Expression (BADGE), using the commercially available Luminex100 LabMAP system and multiple color-coded microspheres (Luminex Corp., Austin, Tex.) in a rapid assay for gene expression (Yang et al., Genome Res. 11:1888-1898 (2001)); and high coverage expression profiling (HiCEP) analysis (Fukumura et al., Nucl. Acids. Res. 31(16) e94 (2003)).

[0094] 3. Microarrays

[0095] Differential gene expression can also be identified, or confirmed using the microarray technique. Thus, the expression profile of breast cancer-associated genes can be measured in either fresh or paraffin-embedded tumor tissue, using microarray technology. In this method, polynucleotide sequences of interest (including cDNAs and oligonucleotides) are plated, or arrayed, on a microchip substrate. The arrayed sequences are then hybridized with specific DNA probes from cells or tissues of interest. Just as in the RT-PCR method, the source of mRNA typically is total RNA isolated from human tumors or tumor cell lines, and corresponding normal tissues or cell lines. Thus RNA can be isolated from a variety of primary tumors or tumor cell lines. If the source of mRNA is a primary tumor, mRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) tissue samples, which are routinely prepared and preserved in everyday clinical practice.

[0096] In a specific embodiment of the microarray technique, PCR amplified inserts of cDNA clones are applied to a substrate in a dense array. Preferably at least 10,000 nucleotide sequences are applied to the substrate. The microarrayed genes, immobilized on the microchip at 10,000 elements each, are suitable for hybridization under stringent conditions. Fluorescently labeled cDNA probes may be generated through incorporation of fluorescent nucleotides by reverse transcription of RNA extracted from

tissues of interest. Labeled cDNA probes applied to the chip hybridize with specificity to each spot of DNA on the array. After stringent washing to remove non-specifically bound probes, the chip is scanned by confocal laser microscopy or by another detection method, such as a CCD camera. Quantitation of hybridization of each arrayed element allows for assessment of corresponding mRNA abundance. With dual color fluorescence, separately labeled cDNA probes generated from two sources of RNA are hybridized pairwise to the array. The relative abundance of the transcripts from the two sources corresponding to each specified gene is thus determined simultaneously. The miniaturized scale of the hybridization affords a convenient and rapid evaluation of the expression pattern for large numbers of genes. Such methods have been shown to have the sensitivity required to detect rare transcripts, which are expressed at a few copies per cell, and to reproducibly detect at least approximately two-fold differences in the expression levels (Schena et al., Proc. Natl. Acad. Sci. USA 93(2):106-149 (1996)). Microarray analysis can be performed by commercially available equipment, following manufacturer's protocols, such as by using the Affymetrix GenChip technology, or Incyte's microarray technology.

[0097] The development of microarray methods for large-scale analysis of gene expression makes it possible to search systematically for molecular markers of cancer classification and outcome prediction in a variety of tumor types.

[0098] 4. Serial Analysis of Gene Expression (SAGE)

[0099] Serial analysis of gene expression (SAGE) is a method that allows the simultaneous and quantitative analysis of a large number of gene transcripts, without the need of providing an individual hybridization probe for each transcript. First, a short sequence tag (about 10-14 bp) is generated that contains sufficient information to uniquely identify a transcript, provided that the tag is obtained from a unique position within each transcript. Then, many transcripts are linked together to form long serial molecules, that can be sequenced, revealing the identity of the multiple tags simultaneously. The expression pattern of any population of transcripts can be quantitatively evaluated by determining the abundance of individual tags, and identifying the gene corresponding to each tag. For more details see, e.g. Velculescu et al., Science 270:484-487 (1995); and Velculescu et al., Cell 88:243-51 (1997).

[0100] 5. Gene Expression Analysis by Massively Parallel Signature Sequencing (MPSS)

[0101] This method, described by Brenner et al., *Nature Biotechnology* 18:630-634 (2000), is a sequencing approach that combines non-gel-based signature sequencing with in vitro cloning of millions of templates on separate 5 μm diameter microbeads. First, a microbead library of DNA templates is constructed by in vitro cloning. This is followed by the assembly of a planar array of the template-containing microbeads in a flow cell at a high density (typically greater than 3×10⁶ microbeads/cm²). The free ends of the cloned templates on each microbead are analyzed simultaneously, using a fluorescence-based signature sequencing method that does not require DNA fragment separation. This method has been shown to simultaneously and accurately provide, in a single operation, hundreds of thousands of gene signature sequences from a yeast cDNA library.

[0102] 6. Immunohistochemistry

[0103] Immunohistochemistry methods are also suitable for detecting the expression levels of the prognostic markers of the present invention. Thus, antibodies or antisera, preferably polyclonal antisera, and most preferably monoclonal antibodies specific for each marker are used to detect expression. The antibodies can be detected by direct labeling of the antibodies themselves, for example, with radioactive labels, fluorescent labels, hapten labels such as, biotin, or an enzyme such as horse radish peroxidase or alkaline phosphatase. Alternatively, unlabeled primary antibody is used in conjunction with a labeled secondary antibody, comprising antisera, polyclonal antisera or a monoclonal antibody specific for the primary antibody. Immunohistochemistry protocols and kits are well known in the art and are commercially available.

[0104] 7. Proteomics

[0105] The term "proteome" is defined as the totality of the proteins present in a sample (e.g. tissue, organism, or cell culture) at a certain point of time. Proteomics includes, among other things, study of the global changes of protein expression in a sample (also referred to as "expression proteomics"). Proteomics typically includes the following steps: (1) separation of individual proteins in a sample by 2-D gel electrophoresis (2-D PAGE); (2) identification of the individual proteins recovered from the gel, e.g. by mass spectrometry or N-terminal sequencing, and (3) analysis of the data using bioinformatics. Proteomics methods are valuable supplements to other methods of gene expression profiling, and can be used, alone or in combination with other methods, to detect the products of the prognostic markers of the present invention.

[0106] 8. General Description of the mRNA Isolation, Purification and Amplification

[0107] The steps of a representative protocol for profiling gene expression using fixed, paraffin-embedded tissues as the RNA source, including mRNA isolation, purification, primer extension and amplification are provided in various published journal articles (for example: T. E. Godfrey et al., *J Molec. Diagnostics* 2: 84-91 [2000]; K. Specht et al., *Am. J. Pathol.* 158: 419-29 [2001]). Briefly, a representative process starts with cutting about 10 μm thick sections of paraffin-embedded tumor tissue samples. The RNA is then extracted, and protein and DNA are removed. After analysis of the RNA concentration, RNA repair and/or amplification steps may be included, if necessary, and RNA is reverse transcribed using gene specific promoters followed by RT-PCR. Finally, the data are analyzed to identify the best treatment option(s) available to the patient on the basis of the characteristic gene expression pattern identified in the tumor sample examined, dependent on the predicted likelihood of cancer recurrence.

[0108] 9. Breast Cancer Gene Set, Assayed Gene Subsequences, and Clinical Application of Gene Expression Data

[0109] An important aspect of the present invention is to use the measured expression of certain genes by breast cancer tissue to provide prognostic information. For this purpose it is necessary to correct for (normalize away) both differences in the amount of RNA assayed and variability in the quality of the RNA used. Therefore, the assay typically measures and incorporates the expression of certain normal-

izing genes, including well known housekeeping genes, such as GAPDH and Cyp1. Alternatively, normalization can be based on the mean or median signal (C_t) of all of the assayed genes or a large subset thereof (global normalization approach). On a gene-by-gene basis, measured normalized amount of a patient tumor mRNA is compared to the amount found in a breast cancer tissue reference set. The number (N) of breast cancer tissues in this reference set should be sufficiently high to ensure that different reference sets (as a whole) behave essentially the same way. If this condition is met, the identity of the individual breast cancer tissues present in a particular set will have no significant impact on the relative amounts of the genes assayed. Usually, the breast cancer tissue reference set consists of at least about 30, preferably at least about 40 different FPE breast cancer tissue specimens. Unless noted otherwise, normalized expression levels for each mRNA/tested tumor/patient will be expressed as a percentage of the expression level measured in the reference set. More specifically, the reference set of a sufficiently high number (e.g. 40) of tumors yields a distribution of normalized levels of each mRNA species. The level measured in a particular tumor sample to be analyzed falls at some percentile within this range, which can be determined by methods well known in the art. Below, unless noted otherwise, reference to expression levels of a gene assume normalized expression relative to the reference set although this is not always explicitly stated.

[0110] 10. Design of Intron-Based PCR Primers and Probes

[0111] According to one aspect of the present invention, PCR primers and probes are designed based upon intron sequences present in the gene to be amplified. Accordingly, the first step in the primer/probe design is the delineation of intron sequences within the genes. This can be done by publicly available software, such as the DNA BLAT software developed by Kent, W. J., *Genome Res.* 12(4):656-64 (2002), or by the BLAST software including its variations. Subsequent steps follow well established methods of PCR primer and probe design.

[0112] In order to avoid non-specific signals, it is important to mask repetitive sequences within the introns when designing the primers and probes. This can be easily accomplished by using the Repeat Masker program available on-line through the Baylor College of Medicine, which screens DNA sequences against a library of repetitive elements and returns a query sequence in which the repetitive elements are masked. The masked intron sequences can then be used to design primer and probe sequences using any commercially or otherwise publicly available primer/probe design packages, such as Primer Express (Applied Biosystems); MGB assay-by-design (Applied Biosystems); Primer3 (Steve Rozen and Helen J. Skaletsky (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Humana Press, Totowa, N.J., pp 365-386)

[0113] The most important factors considered in PCR primer design include primer length, melting temperature (T_m), and G/C content, specificity, complementary primer sequences, and 3'-end sequence. In general, optimal PCR primers are generally 17-30 bases in length, and contain

about 20-80%, such as, for example, about 50-60% G+C bases. Tm's between 50 and 80° C., e.g. about 50 to 70° C. are typically preferred.

[0114] For further guidelines for PCR primer and probe design see, e.g. Dieffenbach, C. W. et al., "General Concepts for PCR Primer Design" in: *PCR Primer; A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1995, pp. 133-155; Innis and Gelfand, "Optimization of PCRs" in: *PCR Protocols, A Guide to Methods and Applications*, CRC Press, London, 1994, pp. 5-11; and Plasterer, T. N. Primerselect: Primer and probe design. *Methods Mol. Biol.* 70:520-527 (1997), the entire disclosures of which are hereby expressly incorporated by reference.

[0115] Further details of the invention will be described in the following non-limiting Example.

EXAMPLE

[0116] A Phase II Study of Gene Expression in 242 Malignant Breast Tumors

[0117] A gene expression study was designed and conducted with the primary goal to molecularly characterize gene expression in paraffin-embedded, fixed tissue samples of invasive breast ductal carcinoma, and to explore the correlation between such molecular profiles and disease-free survival.

[0118] Study Design

[0119] Molecular assays were performed on paraffin-embedded, formalin-fixed primary breast tumor tissues obtained from 252 individual patients diagnosed with invasive breast cancer. All patients were lymph node-negative, ER-positive, and treated with Tamoxifen. Mean age was 52 years, and mean clinical tumor size was 2 cm. Median follow-up was 10.9 years. As of Jan. 1, 2003, 41 patients had local or distant disease recurrence or breast cancer death. Patients were included in the study only if histopathologic assessment, performed as described in the Materials and Methods section, indicated adequate amounts of tumor tissue and homogeneous pathology.

[0120] Materials and Methods

[0121] Each representative tumor block was characterized by standard histopathology for diagnosis, semi-quantitative assessment of amount of tumor, and tumor grade. When tumor area was less than 70% of the section, the tumor area was grossly dissected and tissue was taken from 6 (10 micron) sections. Otherwise, a total of 3 sections (also 10 microns in thickness each) were prepared. Sections were placed in two Costar Brand Microcentrifuge Tubes (Polypropylene, 1.7 mL tubes, clear). If more than one tumor block was obtained as part of the surgical procedure, the block most representative of the pathology was used for analysis.

[0122] Gene Expression Analysis

[0123] mRNA was extracted and purified from fixed, paraffin-embedded tissue samples, and prepared for gene expression analysis as described in chapter 6 above.

[0124] Molecular assays of quantitative gene expression were performed by RT-PCR, using the ABI PRISM 7900TM Sequence Detection SystemTM (Perkin-Elmer-Applied Biosystems, Foster City, Calif., USA). ABI PRISM 7900TM

consists of a thermocycler, laser, charge-coupled device (CCD), camera and computer. The system amplifies samples in a 384-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 384 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

[0125] Analysis and Results

[0126] Tumor tissue was analyzed for 187 cancer-related genes and 5 reference genes. Adequate RT-PCR profiles were obtained from 242 of the 252 patients. The threshold cycle (CT) values for each patient were normalized based on the median of the 7 reference genes for that particular patient. Clinical outcome data were available for all patients from a review of registry data and selected patient charts. Outcomes were classified as:

[0127] Event: Alive with local, regional or distant breast cancer recurrence or death due to breast cancer.

[0128] No Event: Alive without local, regional or distant breast cancer recurrence or alive with contralateral breast cancer recurrence or alive with non-breast second primary cancer or died prior to breast cancer recurrence.

[0129] Analysis was performed by:

[0130] A. determination of the relationship between normalized gene expression and the binary outcomes of 0 or 1;

[0131] B. Analysis of the relationship between normalized gene expression and the time to outcome (0 or 1 as defined above) where patients who were alive without breast cancer recurrence or who died due to a cause other than breast cancer were censored. This approach was used to evaluate the prognostic impact of individual genes and also sets of multiple genes.

[0132] Analysis of Patients with Invasive Breast Carcinoma by Binary Approach

[0133] In the first (binary) approach, analysis was performed on all 242 patients with invasive breast carcinoma. A t test was performed on the groups of patients classified as either no recurrence and no breast cancer related death at 10 years, versus recurrence, or breast cancer-related death at 10 years, and the p-values for the differences between the groups for each gene were calculated.

[0134] Table 1 lists the 33 genes for which the p-value for the differences between the groups was <0.05. The first column of mean expression values pertains to patients who had a metastatic recurrence or nor died from breast cancer. The second column of mean expression values pertains to patients who neither had a metastatic recurrence of nor died from breast cancer.

TABLE 1

Gene	Mean group A Event	Mean group B No event	T statistic	P value
MMP9	-3.15	-4.27	3.75	0.00
GSTM1	-5.02	-4.03	-3.56	0.00
MELK	-3.89	-4.66	3.34	0.00
PE	-4.56	-3.18	-3.27	0.00

TABLE 1-continued

Gene	Mean group	Mean group	T statistic	P value
	A	B		
	Event	No event		
DKFZp586M07	-3.83	-2.94	-3.09	0.00
GSTM3	-2.56	-1.69	-3.06	0.00
MCM2	-3.51	-4.08	3.03	0.00
CDC20	-3.01	-3.75	3.01	0.00
CCNB1	-4.48	-5.17	3.02	0.00
STMY3	-0.58	-1.20	2.95	0.00
GRB7	-1.93	-3.01	2.98	0.00
MYBL2	-3.91	-4.78	2.91	0.01
CEGP1	-3.00	-1.85	-2.89	0.01
SURV	-4.23	-5.06	2.88	0.01
LMNB1	-2.40	-2.91	2.81	0.01
CTSL2	-5.74	-6.39	2.83	0.01
PTTG1	-3.49	-4.14	2.72	0.01
BAG1	-1.76	-1.30	-2.58	0.01
KNSL2	-3.35	-4.06	2.60	0.01
CLAP1	-4.44	-4.02	-2.58	0.01
PREP	-3.34	-3.74	2.56	0.01
NEK2	-5.25	-5.80	2.53	0.01
EpcAM	-1.95	-2.31	2.50	0.01
PCNA	-2.79	-3.13	2.42	0.02
C20_orf1	-2.48	-3.09	2.39	0.02
ITGA7	-4.53	-3.87	-2.37	0.02
ID1	-2.58	-2.17	-2.30	0.02
B_Catenin	-1.32	-1.08	-2.28	0.03
EstR1	-0.78	-0.12	-2.28	0.03
CDH1	-2.76	-3.27	2.20	0.03
TS	-2.86	-3.29	2.18	0.03
HER2	0.53	-0.22	2.18	0.03
cMYC	-3.22	-2.85	-2.16	0.04

[0135] In the foregoing Table 1, negative t-values indicate higher expression, associated with better outcomes, and, inversely, higher (positive) t-values indicate higher expression associated with worse outcomes. Thus, for example, elevated expression of the CCNB1 gene (t-value=3.02; CT mean alive<CT mean deceased) indicates a reduced likelihood of disease free survival. Similarly, elevated expression of the GSTM1 gene (t-value=-3.56; CT mean alive>CT mean deceased) indicates an increased likelihood of disease free survival.

[0136] Thus, based on the data set forth in Table 1, the expression of any of the following genes in breast cancer indicates a reduced likelihood of survival without cancer recurrence: C20_orf1; CCNB1; CDC20; CDH1; CTS12; EpcAM; GRB7; HER2; KNSL2; LMNB1; MCM2; MMP9; MYBL2; NEK2; PCNA; PREP; PTTG1; STMY3; SURV; TS; MELK.

[0137] Based on the data set forth in Table 1, the expression of any of the following genes in breast cancer indicates a better prognosis for survival without cancer recurrence: BAG1; BCatenin; CEGP1; CIAP1; cMYC; DKFZp586M07; EstR1; GSTM1; GSTM3; ID1; ITGA7; PR.

[0138] Analysis of Multiple Genes and Indicators of Outcome

[0139] Two approaches were taken in order to determine whether using multiple genes would provide better discrimination between outcomes. First, a discrimination analysis was performed using a forward stepwise approach. Models were generated that classified outcome with greater discrimination than was obtained with any single gene alone.;

According to a second approach (time-to-event approach), for each gene a Cox Proportional Hazards model (see, e.g. Cox, D. R., and Oakes, D. (1984), *Analysis of Survival Data*, Chapman and Hall, London, N.Y.) was defined with time to recurrence or death as the dependent variable, and the expression level of the gene as the independent variable. The genes that have a p-value<0.05 in the Cox model were identified. For each gene, the Cox model provides the relative risk (RR) of recurrence or death for a unit change in the expression of the gene. One can choose to partition the patients into subgroups at any threshold value of the measured expression (on the CT scale), where all patients with expression values above the threshold have higher risk, and all patients with expression values below the threshold have lower risk, or vice versa, depending on whether the gene is an indicator of bad (RR>1.01) or good (RR<1.01) prognosis. Thus, any threshold value will define subgroups of patients with respectively increased or decreased risk. The results are summarized in Table 2, which lists the 42 genes for which the p-value for the differences between the groups was <0.05.

TABLE 2

Gene	Relative Risk	p-value
GRB7	1.52	0.000011
SURV	1.57	0.000090
PR	0.74	0.000129
LMNB1	1.92	0.000227
MYBL2	1.46	0.000264
HER2	1.46	0.000505
GSTM1	0.68	0.000543
MELK	1.59	0.000684
C20_orf1	1.59	0.000735
PTTG1	1.63	0.001135
BUB1	1.58	0.001425
CDC20	1.54	0.001443
CCNB1	1.60	0.001975
STMY3	1.47	0.002337
KNSL2	1.48	0.002910
CTSL2	1.43	0.003877
MCM2	1.59	0.005203
NEK2	1.48	0.006533
DR5	0.62	0.006660
Ki_67	1.46	0.008188
CCNE2	1.38	0.009505
TOP2A	1.38	0.009551
PCNA	1.67	0.010237
PREP	1.69	0.012308
FOXM1	1.52	0.012837
NME1	1.46	0.013622
CEGP1	0.84	0.013754
BAG1	0.68	0.015422
STK15	1.46	0.017013
HNRPAB	1.96	0.017942
EstR1	0.80	0.018877
MMP9	1.19	0.019591
DKFZp586M07	0.79	0.020073
TS	1.44	0.025186
Src	1.70	0.037398
BIN1	0.75	0.038979
NPD009	0.80	0.039020
RPLPO	0.52	0.041575
GSTM3	0.84	0.041848
MMP12	1.27	0.042074
TFRC	1.57	0.046145
IGF1R	0.78	0.046745

[0140] Based on the data set forth in Table 2, the expression of any of the following genes in breast cancer indicates a reduced likelihood of survival without cancer recurrence:

GRB7; SURV; LMNB1; MYBL2; HER2; MELK; C20_orf1; PTIG1; BUB1; CDC20; CCNB1; STMY3; KNSL2; CTSL2; MCM2; NEK2; Ki_67; CCNE2; TOP2A-4; PCNA; PREP; FOXM1; NME1; STK15; HNRPAB; MMP9; TS; Src; MMP12; TFRC.

[0141] Based on the data set forth in Table 2, the expression of any of the following genes in breast cancer indicates a better prognosis for survival without cancer recurrence: PR; GSTM1; DRS; CEGP1; BAG1; EstR1; DKFZp586M07; BIN1; NPD009; RPLPO; GSTM3; IGF1R.

[0142] The binary and time-to-event analyses, with few exceptions, identified the same genes as prognostic markers. For example, comparison of Tables 1 and 2 shows that 10 genes were represented in the top 15 genes in both lists. Furthermore, when both analyses identified the same gene at [p<0.10], which happened for 26 genes, they were always concordant with respect to the direction (positive or negative sign) of the correlation with survival/recurrence. Overall, these results strengthen the conclusion that the identified markers have significant prognostic value.

[0143] Multivariate Gene Analysis of 242 Patients with Invasive Breast Carcinoma

[0144] For Cox models comprising more than two genes (multivariate models), stepwise entry of each individual gene into the model is performed, where the first gene entered is pre-selected from among those genes having significant univariate p-values, and the gene selected for entry into the model at each subsequent step is the gene that best improves the fit of the model to the data. This analysis can be performed with any total number of genes. In the analysis the results of which are shown below, stepwise entry was performed for up to 10 genes.

[0145] Multivariate analysis was performed using the following equation:

$$RR = \exp[\text{coef(geneA)} \times Ct(\text{geneA}) + \text{coef(geneB)} \times Ct(\text{geneB}) + \text{coef(geneC)} \times Ct(\text{geneC}) + \dots]$$

[0146] In this equation, coefficients for genes that are predictors of beneficial outcome are positive numbers and

coefficients for genes that are predictors of unfavorable outcome are negative numbers. The "Ct" values in the equation are ΔCt s, i.e. reflect the difference between the average normalized Ct value for a population and the normalized Ct measured for the patient in question. The convention used in the present analysis has been that ΔCt s below and above the population average have positive signs and negative signs, respectively (reflecting greater or lesser mRNA abundance). The relative risk (RR) calculated by solving this equation will indicate if the patient has an enhanced or reduced chance of long-term survival without cancer recurrence.

[0147] A multivariate stepwise analysis, using the Cox Proportional Hazards Model, was performed on the gene expression data obtained for all 242 patients with invasive breast carcinoma. The following ten-gene set has been identified by this analysis as having particularly strong predictive value of patient survival: GRB7; LMNB1; ER; STMY3; KLK10; PR; KRT5; FGFR1; MCM6; SNRPF. In this gene set ER, PR, KRT5 and MCM6 contribute to good prognosis, while GRB7, LMNB1, STMY3, KLK10, FGFR1, and SNRPF contribute to poor prognosis.

[0148] While the present invention has been described with reference to what are considered to be the specific embodiments, it is to be understood that the invention is not limited to such embodiments. To the contrary, the invention is intended to cover various modifications and equivalents included within the spirit and scope of the appended claims. For example, while the disclosure focuses on the identification of various breast cancer associated genes and gene sets, and on the personalized prognosis of breast cancer, similar genes, gene sets and methods concerning other types of cancer are specifically within the scope herein. In particular, the present gene sets or variants thereof can be used as prognostic markers to predict the likelihood of long-term survival or cancer recurrence in the case of ovarian cancer.

[0149] All references cited throughout the disclosure are hereby expressly incorporated by reference.

TABLE 3

Gene	Accession	Start	Stop	SEQ ID NO.	Sequence
B-Catenin	NM_001904	1549	1629	SEQ ID NO: 1	GGCTCTTGTCGCTACTGTCCTTCCGGTGGACAGGGAAAGACATCACTGAGCTGCGCATCTTGCTCTTCGTCATCTGA
BAG1	NM_004323	673	754	SEQ ID NO: 2	CGTGTCTAGGACTTGAAATGAGTTGGGGCATGTTAATGGAAAAAGAACAGTCACAGGAAGAGGTGAAC
BTNL1	NM_004305	866	942	SEQ ID NO: 3	CCITGAAAGGGAAACAAAGGCCCTTCGCCTCAGATGGCTCCGGGAGATAGAGTCACAC
BUB1	NM_004336	1002	1070	SEQ ID NO: 4	CCGAGGTTAATCCAGCACGTATGGGCCAAGTGAGGCTCCACAGGAACATGAGCCATGCTT
C20 orf1	NM_012112	2675	2740	SEQ ID NO: 5	TCAAGTTGTGAGCTGGGATACCGCCGGCAATGGACCTGCTUTAACCTCAAACCTTAGGACCT
CCNB1	NM_031966	823	907	SEQ ID NO: 6	TTCAAGGGTTGTGAGGACCATGTAATGACTGTCCTTGTGAAATAATGTTGATCGGTCAATGAGGAAAGATG
CCNE2	NM_057749	2026	2108	SEQ ID NO: 7	ATGCTGGCTCCCTTAACCTGGCTTCTTGACATGTTAGGTGCTGTTGTAATAACCTTTGTATATCACAAATTGGGT
CDC20	NM_001255	679	747	SEQ ID NO: 8	TGGATTGGAGTCTGGGATGTACTGGCGTGGCACTGGACAACAGTGTACCTGGAGTCAGC
CDH1	NM_004360	2499	2580	SEQ ID NO: 9	TGAGTTGTCGGCGTATCTCCCGGATGAAATTGGAAATTGATGAAATCTGAAGGGCTG
CEGP1	NM_020974	563	640	SEQ ID NO: 10	TGACAAATCAGAACACTCATTACCGCTCGAAGAGGGCTGAAGCTGATGAATAAGGATCAGGCTGTAGTCACA
CIAP1	NM_001166	1822	1894	SEQ ID NO: 11	TGCCCTGTGGGGAAAGTCAGTAATGGGAAACAAAGGATGATCTATGTCAGAACACGGGAGCATTTCC
cMYC	NM_002467	1494	1578	SEQ ID NO: 12	TCCCCTCACTCGGAAAGCACTATCTGCTGCAAGTGGACACTGTCAGCTGAGCTCTGAGACATCAGCAACACCG
CTSL2	NM_001333	671	738	SEQ ID NO: 13	TGTTCTACTGAGCGAGAAATCTGTTGGGACTCTAAGGCATTAAGGCTGTGCAAGTGGCAAGGAACTGCA
DKEZp586	AL050227	559	633	SEQ ID NO: 14	TCCCATTTCTACCTGTGAACTCATTGTGCAAGGGCCCTGGAAGAAAGGAAACCGACTGCA
DR5	NM_003842	1127	1211	SEQ ID NO: 15	CTCTGAGACAGTGCTGATGACTTGCAGACTTGTCGCTTGTGAGCTGGGCTCATGAGGAAGTGGGCCTCATGG
Epcam	NM_002354	435	510	SEQ ID NO: 16	GGGCCCTCCAGAACATGATGGCTTATGATCCCTGACTGCGATGAGGGCTCTTTAAGCCAAGCAGTGC
EstR1	NM_000125	1956	2024	SEQ ID NO: 17	CCTGGTGGCCCTCTATGACCTGCTGGAGATGCTGGACGCCACCGCTACATGGGCCACTAGGCC
FGFR1	NM_023109	2685	2759	SEQ ID NO: 18	CACGGGACATTCACCACATCGACTACTATAAAAAGAACCCAAAGGGCCGACTGCCCTGTGAAGTGGGAC
FOXM1	NM_021953	1898	1980	SEQ ID NO: 19	CCACCCGAGCAAATCTGCTCCAGAACCCCTGAATCTGGGGCTCACGCCAAAGTAGGGGACTGGATT
GRB7	NM_005310	1275	1342	SEQ ID NO: 20	CCATCTGCACTCATCTGCTGGCTCCCCACCCCTGAGAACGTCCTGATATACTCTGGGGCC
GSTM1	NM_000561	93	179	SEQ ID NO: 21	AAGCTATGAGGAAAGTACACGATGGGGACGCTCTGATATGACAGAAAGCCAGTGGCTGAATGAAAAATTCAAGCTGGCC

TABLE 3-continued

TABLE 3-continued

Gene	Accession	Start	Stop	SEQ ID NO.	Sequence
NME1	NM_000269	365	439	SEQ ID NO:	40 CCACCCCTGGAGACTCAAGCCCTGGAGACATCGTGGAGACTTGATCAGGTTGGCAGGAACTATACT
NPD009	NM_020286	589	662	SEQ ID NO:	41 GGCTCTGGCTAGGCCATGGCTGTAGCACCTCTGCTGGAGTGAGACACTCTGGGAACTTGACCTCGAATGTC
PCNA	NM_002592	157	228	SEQ ID NO:	42 GAAGGTTGGAGGACTCAAGGAACTCATCAACGAGGCCTCATTAAGGGCATATGGCTGGGATATTAGCTCCAGGGTGTAAACC
PR	NM_000926	1895	1980	SEQ ID NO:	43 GCATCAGGCTGTCATATGGTGTCCCTAACCTGTGGAGCTGAGCTCCAACTATCGCAACATTGACTTCTGGGATCCCTG
PREP	NM_002726	889	965	SEQ ID NO:	44 GGGACGGGTGTCATTCAGAGGAATCGCCAGTCTCCAACTATCGCTGGATCAACATTGACTTCTGGGATCCCTG
PTTG1	NM_004219	48	122	SEQ ID NO:	45 GGCTACTCTGATCTATGTGATAAGAAAATGGGAAACCAGGGACCCGGTAGGTGCTTAAGGATGGCTGAAGGC
RPLPO	NM_001002	791	866	SEQ ID NO:	46 CCATTCATCATCAACGGGTACAAACGAGTCCTGGCTTGTCTGGAGACGGATTACACCTTCCACTTGTGA
SNRPF	NM_003095	71	150	SEQ ID NO:	47 GGCTGGTGGCAGAGAGTAGCCCTGCCAACATTCGGCCGGTGGTTAACATGAGTTACCCCTCAATCCAAACCTTCTCA
Src	NM_004383	979	1043	SEQ ID NO:	48 CCTGAAACATGAGGGAGTGAAGCTGCTGCAAGACCATGGGAACGGGAGTTCGGAGACGTGATGATG
STK15	NM_003600	1101	1170	SEQ ID NO:	49 CATCTTCCAGGAGGCCACTCTGTGGCACCCCTGGACTACCTGGCCCTGAAATGATTGAAAGTCGGA
STMY3	NM_005940	2090	2180	SEQ ID NO:	50 CCTGGGGCTGCAACATACTCAATCTGTCCCAGGGGATCTCCCTGAAGCCCTTTTCGGAACACTGCTATCCCTCCAAAGGCCATTGTA
SURV	NM_001168	737	817	SEQ ID NO:	51 TGTGATTCGGCTTACAGGGTGAAGTGAGGGAGAAGGGAGAAGTGGCTCCCTTTGCTAGGCTGACAGCTG
TFRC	NM_003234	2110	2178	SEQ ID NO:	52 GCCAAACTGGCTTCATTTGTGGGGATCTGAACCAATAAGGAAATGGGCTGAGT
TOP2A	NM_001067	4505	4577	SEQ ID NO:	53 AATCCAAGGGGAGATGATGACTTCATGGACTTGACTCACTGCTCTGGCAAAATCTGTAC
TS	NM_001071	764	829	SEQ ID NO:	54 GccTcGGGTTGCTTCAACATCGCAGCTAGCCTGCTCACCTGACATGATTGGCACATCAC

[0150]

TABLE 4A

Gene	Accession Name	SEQ ID NO	Sequence	
B-Catenin	NM_001904 S2150/B-Cate.f3	SEQ ID NO:55	GGCTCTTGTGCGTACTGTCCTT	22
B-Catenin	NM_001904 S2151/B-Cate.r3	SEQ ID NO:56	TCAGATGACGAAGAGCACAGATG	23
B-Catenin	NM_001904 S50461B-Cate.p3	SEQ ID NO:57	AGGCTCAGTGATGTCCTCCCTGTCACCAG	29
BAG1	NM_004323 S1386/BAG1.f2	SEQ ID NO:58	CGTTGTCAGCACTTGGAAATACAA	23
BAG1	NM_004323 S1387/BAG1.r2	SEQ ID NO:59	GTTCAACCTCTTCCTGTGGACTGT	24
BAG1	NM_004323 S4731/BAG1.p2	SEQ ID NO:60	CCCAATTAACATGACCCGGCAACCAT	26
BIN1	NM_004305 S2651/BIN1.f3	SEQ ID NO:61	CCTGCAAAGGAAACAAGAG	20
BIN1	NM_004305 S2652/BIN1.r3	SEQ ID NO:62	CGTGGTTGACTCTGATCTCG	20
BIN1	NM_004305 S4954/BIN1.p3	SEQ ID NO:63	CTTCGCCTCCAGATGGCTCCC	21
BUB1	NM_004336 S4294/BUB1.f1	SEQ ID NO:64	CCGAGGTTAACCCAGCACGTA	21
BUB1	NM_004336 S4295/BUB1.r1	SEQ ID NO:65	AAGACATGGCCTCTCAGTTC	21
BUB1	NM_004336 S4296/BUB1.p1	SEQ ID NO:66	TGCTGGGAGCCTACACTTGGCCC	23
C20 orf1	NM_012112 S3560/C20 or.f1	SEQ ID NO:67	TCAGCTGTGAGCTGCGGATA	20
C20 orf1	NM_012112 S3561/C20 or.r1	SEQ ID NO:68	ACGGTCCTAGGTTGAGGTTAAGA	24
C20 orf1	NM_012112 S3562/C20 or.p1	SEQ ID NO:69	CAGGTCCCATTGCCGGCG	19
CCNB1	NM_031966 S1720/CCNB1.f2	SEQ ID NO:70	TTCAGGTTGTTGCAGGAGAC	20
CCNB1	NM_031966 S1721/CCNB1.r2	SEQ ID NO:71	CATCTTCTTGGCACACAAT	20
CCNB1	NM_031966 S4733/CCNB1.p2	SEQ ID NO:72	TGTCTCCATTATTGATCGGTTCATGCA	27
CCNE2	NM_057749 S1458/CCNE2.f2	SEQ ID NO:73	ATGCTGTGGCTCTTCCTAACT	22
CCNE2	NM_057749 S1459/CCNE2.r2	SEQ ID NO:74	ACCCAAATTGTGATATACAAAAAGTT	27
CCNE2	NM_057749 S4945/CCNE2.p2	SEQ ID NO:75	TACCAAGCAACCTACATGTCAAGAAAGCCC	30
CDC20	NM_001255 S4447/CDC20.f1	SEQ ID NO:76	TGGATTGGAGTTCTGGGAATG	21
CDC20	NM_001255 S4448/CDC20.r1	SEQ ID NO:77	GCTTGCACTCCACAGGTACACA	22
CDC20	NM_001255 S4449/CDC20.p1	SEQ ID NO:78	ACTGGCCGTGGCACTGGACAACA	23
CDH1	NM_004360 S0073/CDH1.f3	SEQ ID NO:79	TGAGTGTCCCCGGTATCTTC	21
CDH1	NM_004360 S0075/CDH1.r3	SEQ ID NO:80	CAGCCGCTTCAGATTTCAT	21
CDH1	NM_004360 S4990/CDH1.p3	SEQ ID NO:81	TGCCAATCCCAGTGAATTGGAAATT	27
CEGP1	NM_020974 S1494/CEGP1.f2	SEQ ID NO:82	TGACAATCAGCACACCTGCAT	21
CEGP1	NM_020974 S1495/CEGP1.r2	SEQ ID NO:83	TGTGACTACAGCGTGTACCTTA	23
CEGP1	NM_020974 S4735/CEGP1.p2	SEQ ID NO:84	CAGGCCCTCTCCGAGCGGT	20
CIAP1	NM_001166 S0764/CIAP1.f2	SEQ ID NO:85	TGCCTGTGGTGGGAAGCT	18
CIAP1	NM_001166 S0765/CIAP1.r2	SEQ ID NO:86	GGAAAATGCCTCCGGTGT	19
CIAP1	NM_001166 S4802/CIAP1.p2	SEQ ID NO:87	TGACATAGCATCATCCTTGGTCCCAGTT	30
cMYC	NM_002467 S0085/cMYC.f3	SEQ ID NO:88	TCCCTCACTCGGAAGGACTA	21
cMYC	NM_002467 S0087/cMYC.r3	SEQ ID NO:89	CGGTTGTTGCTGATCTGTCTCA	22
cMYC	NM_002467 S4994/cMYC.p3	SEQ ID NO:90	TCTGACACTGTCCAACTTGACCCCTTT	27

TABLE 4A-continued

Gene	Accession Name	SEQ ID NO	Sequence	
CTSL2	NM_001333 S4354/CTSL2.f1	SEQ ID NO:91	TGTCTCACTGAGCGAGCAGAA	21
CTSL2	NM_001333 S4355/CTSL2.r1	SEQ ID NO:92	ACCATTGCAGCCCTGATTG	19
CTSL2	NM_001333 S4356/CTSL2.p1	SEQ ID NO:93	CTTGAGGACGCCAACAGTCCACCA	24
DKFZp586M0723 AL050227	S4396/DKFZp5.f1	SEQ ID NO:94	TCCATTTCTACCTGTTAACCTTCATC	27
DKFZp586M0723 AL050227	S4397/DKFZp5.r1	SEQ ID NO:95	ATGCAGTCGGCCCTTCCT	19
DKFZp586M0723 AL050227	S4398/DKFZp5.p1	SEQ ID NO:96	TTGCTTCCAGGGCTGCACAAAA	23
DR5	NM_003842 S2551/DR5.f2	SEQ ID NO:97	CTCTGAGACAGTGCCTCGATGACT	24
DR5	NM_003842 S2552/DR5.r2	SEQ ID NO:98	CCATGAGGCCAACTTCC	19
DR5	NM_003842 S4979/DR5.p2	SEQ ID NO:99	CAGACTTGGTGCCTTTGACTCC	23
EpCAM	NM_002354 S1807/EpCAM.f1	SEQ ID NO:100	GGGCCCTCCAGAACATAATGAT	20

[0151]

TABLE 4B

EpcAM	NM_002354 S1808/EpcAM.r1	SEQ ID NO:101	TGCACTGCTTGGCCTTAAAGA	21
EpcAM	NM_002354 S4984/EpcAM.p1	SEQ ID NO:102	CCGCTCTCATCGCAGTCAGGATCAT	25
EstR1	NM_000125 S0115/EstR1.f1	SEQ ID NO:103	CGTGGTCCCCCTCTATGAC	19
EstR1	NM_000125 S0117/EstR1.r1	SEQ ID NO:104	GGCTAGTGGGCGATGTAG	19
EstR1	NM_000125 S4737/EstR1.p1	SEQ ID NO:105	CTGGAGATGCTGGACCCCC	19
FGFR1	NM_023109 S0818/FGFR1.f3	SEQ ID NO:106	CACGGGACATTCAACCACATC	20
FGFR1	NM_023109 S0819/FGFR1.r3	SEQ ID NO:107	GGGTGCCATCCACTTCACA	19
FGFR1	NM_023109 S4816/FGFR1.p3	SEQ ID NO:108	ATAAAAAGACAACCAACGGCGACTGC	27
FOXM1	NM_021953 S2006/FOXM1.f1	SEQ ID NO:109	CCACCCGAGCAAATCTGT	19
FOXM1	NM_021953 S2007/FOXM1.r1	SEQ ID NO:110	AAATCCAGTCCCCCTACTTTGG	22
FOXM1	NM_021953 S4757/FOXM1.p1	SEQ ID NO:111	CCTGAATCCTGGAGGCTCACGCC	23
GRB7	NM_005310 S0130/GRB7.f2	SEQ ID NO:112	ccatctgcatccatcftgft	20
GRB7	NM_005310 S0132/GRB7.r2	SEQ ID NO:113	ggccaccaggatttatctg	20
GRB7	NM_005310 S4726/GRB7.p2	SEQ ID NO:114	cgtccccacccttgagaagtgcct	23
GSTM1	NM_000561 S2026/GSTM1.r1	SEQ ID NO:115	GGCCCAGCTTGAATTTC	20
GSTM1	NM_000561 S2027/GSTM1.f1	SEQ ID NO:116	AAGCTATGAGGAAAAGAAGTACACGAT	27
GSTM1	NM_000561 S4739/GSTM1.p1	SEQ ID NO:117	TCAGCCACTGGCTCTGTCTAACAGGAG	30
GSTM3	NM_000849 S2038/GSTM3.f2	SEQ ID NO:118	CAATGCCATCTTGCCTACAT	21
GSTM3	NM_000849 S2039/GSTM3.r2	SEQ ID NO:119	GTCCACTCGAATCTTCTTCA	25
GSTM3	NM_000849 S5064/GSTM3.p2	SEQ ID NO:120	CTCGCAAGCACACATGTGTGGTGAGA	27
HER2	NM_004448 S0142/HER2.f3	SEQ ID NO:121	CGGTGTGAGAAGTGCAGCAA	20
HER2	NM_004448 S0144/HER2.r3	SEQ ID NO:122	CCTCTCGCAAGTGCCTCCAT	19
HER2	NM_004448 S4729/HER2.p3	SEQ ID NO:123	CCAGACCAGACACTCGGGCAC	24

TABLE 4B-continued

HNRPAB	NM_004499	S4510/HNRPAB.f3	SEQ ID NO:124CAAGGGAGCGACCAACTGA	19
HNRPAB	NM_004499	S4511/HNRPAB.r3	SEQ ID NO:125GTTTGCCAAGTTAAATTGGTACATAAT	28
HNRPAB	NM_004499	S4512/HNRPAB.p3	SEQ ID NO:126CTCCATATCCAAACAAAGCATGTGTGCC	28
ID1	NM_002165	S0820/ID1.f1	SEQ ID NO:127AGAACCCCAAGGTGAGCAA	19
ID1	NM_002165	S0821/ID1.r1	SEQ ID NO:128TCCAAGTGAAGGTCCCTGATG	21
ID1	NM_002165	S4832/ID1.p1	SEQ ID NO:129TGGAGATTCTCCAGCACGTACATGAC	26
IGF1R	NM_000875	S1249/IGF1R.f3	SEQ ID NO:130GCATGGTAGCCGAAGATTCA	21
IGF1R	NM_000875	S1250/IGF1R.r3	SEQ ID NO:131TTTCCGGTAATAGTCTGTCTCATAGATATC	30
IGF1R	NM_000875	S4895/IGF1R.p3	SEQ ID NO:132CGCGTCATACCAAAATCTCGATTGAA	28
ITGA7	NM_002206	S0859/ITGA7.f1	SEQ ID NO:133GATATGATTGGTCGCTGCTTGT	22
ITGA7	NM_002206	S0920/ITGA7.r1	SEQ ID NO:134AGAACTTCCATTCCCCACCAT	21
ITGA7	NM_002206	S4795/ITGA7.p1	SEQ ID NO:135CAGCCAGGGACCTGGCCATCCG	21
KI-67	NM_002417	S0436/Ki-67.f2	SEQ ID NO:136CGGACTTTGGGTGCGACTT	19
Ki-67	NM_002417	S0437/Ki-67.r2	SEQ ID NO:137TTACAACCTTCCACTGGGACGAT	24
Ki-67	NM_002417	S4741/Ki-67.p2	SEQ ID NO:138CCACTTGTGCGAACACCACGCTCGT	23
KLK10	NM_002776	S2624/KLK10.f3	SEQ ID NO:139GCCAGAGGCTCCATCGT	18
KLK10	NM_002776	S2625/KLK10.r3	SEQ ID NO:140CAGAGGTTGAAACAGTGCAGACA	23
KLK10	NM_002776	S4978/KLK10.p3	SEQ ID NO:141CCTCTCCTCCCCAGTCGGCTGA	23
KNSL2	BC000712	S4432/KNSL2.f2	SEQ ID NO:142CCACCTCGCCATGATTTTC	20
KNSL2	BC000712	S4433/KNSL2.r2	SEQ ID NO:143GCAATCTTCAAACACTTCATCCT	25
KNSL2	BC000712	S4434/KNSL2.p2	SEQ ID NO:144TTTGACCGGGTATTCCCACCAGGAA	25
KRT5	NM_000424	S0175/KRT5.f3	SEQ ID NO:145tcagtggagaaggagttgga	20
KRT5	NM_000424	S0177/KRT5.r3	SEQ ID NO:146tgccatatccagaggaaaca	20
KRT5	NM_000424	S5015/KRT5.p3	SEQ ID NO:147ccagtcaacatctgtgtcacaagca	28
LMNB1	NM_005573	S4477/LMNB1.f1	SEQ ID NO:148TGCAAACGCTGGTGTACAA	19

[0152]

TABLE 4C

LMNB1	NM_005573	S4478/LMNB1.r1	SEQ ID NO:149CCCCACGAGTTCTGGTTCTTC	21
LMNB1	NM_005573	S4479/LMNB1.p1	SEQ ID NO:150CAGCCCCCAACTGACCTCATC	22
MCM2	NM_004526	S1602/MCM2.f2	SEQ ID NO:151GACTTTGCCGCTACCTTTC	21
MCM2	NM_004526	S1603/MCM2.r2	SEQ ID NO:152GCCACTAATGCTTCAGTATGAAGAG	26
MCM2	NM_004526	S4900/MCM2.p2	SEQ ID NO:153ACAGCTCATTGTTGTCACGCCGGA	24
MCM6	NM_005915	S1704/MCM6.f3	SEQ ID NO:154TGATGGCCTATGTTGTCACATTCA	24
MCM6	NM_005915	S1705/MCM6.r3	SEQ ID NO:155TGGGACAGGAAACACACCAA	20
MCM6	NM_005915	S4919/MCM6.p3	SEQ ID NO:156CAGGTTCATACCAACACAGGCTTCAGCAC	30
MELK	NM_014791	S4318/MELK.f1	SEQ ID NO:157AACCCGGCGATCGAAAAG	18

TABLE 4C-continued

MELK	NM_014791	S4319/MELK.r1	SEQ ID NO:158GGGCCTGCTGTCCTGAGA	18
MELK	NM_014791	S4320/MELK.p1	SEQ ID NO:159TCTTAGAACGCCGTACCAGCCGC	24
MMP12	NM_002426	S4381/MMP12.f2	SEQ ID NO:160CCAACGCTTGCCAAATCCT	19
MMP12	NM_002426	S4382/MMP12.r2	SEQ ID NO:161ACGGTAGTGACAGCATAAAAC	24
MMP12	NM_002426	S4383/MMP12.p2	SEQ ID NO:162AACCAGCTCTGTGACCCCCATT	24
MMP9	NM_004994	S0656/MMP9.f1	SEQ ID NO:163GAGAACCAATCTCACCGACA	20
MMP9	NM_004994	S0657/MMP9.r1	SEQ ID NO:164CACCCGAGTGTAACCATAGC	20
MMP9	NM_004994	S4760/MMP9.p1	SEQ ID NO:165ACAGGTATTCCCTGTGCCAGCTGCC	24
MYBL2	NM_002466	S3270/MYBL2.f1	SEQ ID NO:166GCCGAGATCGCCAAGATG	18
MYBL2	NM_002466	S3271/MYBL2.r1	SEQ ID NO:167CTTTGATGGTAGAGTCCAGTGATT	27
MYBL2	NM_002466	S4742/MYBL2.p1	SEQ ID NO:168CAGCATTGTCTGTCCTCCCTGGCA	24
NEK2	NM_002497	S4327/NEK2.f1	SEQ ID NO:169GTGAGGCAGCGCGACTCT	18
NEK2	NM_002497	S4328/NEK2.r1	SEQ ID NO:170TGGCAATGGGTACAAACACTTCA	23
NEK2	NM_002497	S4329/NEK2.p1	SEQ ID NO:171TGCCTTCCCAGGCTGAGGACT	21
NME1	NM_000269	S2526/NME1.f3	SEQ ID NO:172CCAACCCTGCAGACTCCAA	19
NME1	NM_000269	S2527/NME1.r3	SEQ ID NO:173ATGTATAATGTTCTGCCAATTGTAT	28
NME1	NM_000269	S4949/NME1.p3	SEQ ID NO:174CCTGGACCATCGTGAGACTTCT	25
NPD009	NM_020686	S4474/NPD009.f3	SEQ ID NO:175GGCTGTGGCTGAGGCTGTAG	20
NPD009	NM_020686	S4475/NPD009.r3	SEQ ID NO:176GGAGCATTGAGGTCAAATCA	21
NPD009	NM_020686	S4476/NPD009.p3	SEQ ID NO:177TTCCAGAGTGTCTCACCTCCAGCAGAG	28
PCNA	NM_002592	S0447/PCNA.f2	SEQ ID NO:178GAAGGTGTTGGAGGCACTCAAG	22
PCNA	NM_002592	S0448/PCNA.r2	SEQ ID NO:179GGTTACACCCTGGAGCTAA	21
PCNA	NM_002592	S4784/PCNA.p2	SEQ ID NO:180ATCCCAGCAGGCCCTCGTTGATGAG	24
PR	NM_000926	S1336/PR.f6	SEQ ID NO:181GCATCAGGCTGTCAATTATGG	20
PR	NM_000926	S1337/PR.r6	SEQ ID NO:182AGTAGTTGTGCTGCCCTTCC	20
PR	NM_000926	S4743/PR.p6	SEQ ID NO:183TGTCTTACCTGTGGAGCTGTAAGGTC	28
PREP	NM_002726	S1771/PREP.f1	SEQ ID NO:184GGGACGGTGTTCACATTCAAG	21
PREP	NM_002726	S1772/PREP.r1	SEQ ID NO:185CAGGATCCCAGAAGTCATGTT	23
PREP	NM_002726	S4929/PREP.p1	SEQ ID NO:186TCGCCAGTCTCCCAACTATCGCGT	24
PTTG1	NM_004219	S4525/PTTG1.f2	SEQ ID NO:187GGCTACTCTGATCTATGTTGATAAGGAA	28
PTTG1	NM_004219	S4526/PTTG1.r2	SEQ ID NO:188GCTTCAGCCCATCCTTAGCA	20
PTTG1	NM_004219	S4527/PTTG1.p2	SEQ ID NO:189CACACGGGTGCCTGGTTCTCCA	22
RPLPO	NM_001002	S0256/RPLPO.f2	SEQ ID NO:190CCATTCTATCATCAACGGGTACAA	24
RPLPO	NM_001002	S0258/RPLPO.r2	SEQ ID NO:191TCAGCAAGTGGGAAGGTGTAATC	23
RPLPO	NM_001002	S4744/RPLPO.p2	SEQ ID NO:192TCTCCACAGACAAGGCCAGGACTCG	25
SNRPF	NM_003095	S4489/SNRPF.f2	SEQ ID NO:193GGCTGGTCGGCAGAGAGTAG	20
SNRPF	NM_003095	S4490/SNRPF.r2	SEQ ID NO:194TGAGGAAAGGTTGGGATTGA	21

TABLE 4C-continued

SNRPF	NM_003095	S4491/SNRPF.p2	SEQ ID NO:195AAACTCATGTAAACCACGGCCGAATGTTG	29
Src	NM_004383	S1820/Src.f2	SEQ ID NO:196CCTGAACATGAAGGAGCTGA	20

[0153]

TABLE 4D

Src	NM_004383	S1821/Src.r2	SEQ ID NO:197CATCACGTCCTCGAACTCC	19
Src	NM_004383	S5034/Src.p2	SEQ ID NO:198TCCCGATGGTCTGCAGCAGCT	21
STK15	NM_003600	S0794/STK15.f2	SEQ ID NO:199CATCTTCAGGAGGACCACT	20
STK15	NM_003600	S0795/STK15.r2	SEQ ID NO:200TCCGACCTTCAAATCATTCA	20
STK15	NM_003600	S4745/STK15.p2	SEQ ID NO:201CTCTGTGGCACCCCTGGACTACCTG	24
STMY3	NM_005940	S2067/STMY3.f3	SEQ ID NO:202CCTGGAGGCTGCAACATACC	20
STMY3	NM_005940	S2068/STMY3.r3	SEQ ID NO:203TACAATGGCTTGGAGGATAGCA	23
STMY3	NM_005940	S4746/STMY3.p3	SEQ ID NO:204ATCCTCCTGAAGCCCTTCGAGC	25
SURV	NM_001168	S02591SURV.f2	SEQ ID NO:205TGTTTGATTCCCCGGGCTTA	20
SURV	NM_001168	S0261/SURV.r2	SEQ ID NO:206CAAAGCTGTCAGCTTAGCAAAAG	24
SURV	NM_001168	S4747/SURV.p2	SEQ ID NO:207TGCCTTCTTCCTCCCTCAGTCTCACCT	28
TFRC	NM_003234	S1352/TFRC.f3	SEQ ID NO:208GCCAACTGCTTCATTGTG	20
TFRC	NM_003234	S1353/TFRC.r3	SEQ ID NO:209ACTCAGGCCATTTCCTTA	20
TFRC	NM_003234	S4748/TFRC.p3	SEQ ID NO:210AGGGATCTGAACCAATACAGAGCAGACA	28
TOP2A	NM_001067	S0271/TOP2A.f4	SEQ ID NO:211AATCCAAGGGGGAGAGTGAT	20
TOP2A	NM_001067	S0273/TOP2A.r4	SEQ ID NO:212GTACAGATTTGCCCGAGGA	20
TOP2A	NM_001067	S4777/TOP2A.p4	SEQ ID NO:213CATATGGACTTGACTCAGCTGTGGC	26
TS	NM_001071	S0280/TS.f1	SEQ ID NO:214GCCTCGGTGTGCCATTCA	18
TS	NM_001071	S0282/TS.r1	SEQ ID NO:215CGTGATGTGCGCAATCATG	19
TS	NM_001071	S4780/TS.p1	SEQ ID NO:216CATGCCAGCTACGCCCTGCTC	22

[0154]

SEQUENCE LISTING

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<223> OTHER INFORMATION: PCR Amplicon

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83

<210> SEQ ID NO 27

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<223> OTHER INFORMATION: PCR Amplicon

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<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 28

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80

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<210> SEQ ID NO 29
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<220> FEATURE:
<223> OTHER INFORMATION: PCR Amplicon

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<223> OTHER INFORMATION: PCR Amplicon

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aaccgcgcga tcgaaaatgt tcttaggaac gccgttaccag ccgcgttctt caggacagca	60
ggccc	65

<210> SEQ ID NO 36

<211> LENGTH: 78

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 36

ccaacgcgtt ccaaattctt acaatttgc accagctctc tgtgacccca attttttttt	60
tgtatgttgc actaccgt	78

<210> SEQ ID NO 37

<211> LENGTH: 67

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 37

gagaaccaat ctcaccgaca ggcagctggc agagaaatac ctgtaccgct atggttacac	60
tccgggtt	67

<210> SEQ ID NO 38

<211> LENGTH: 74

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 38

gccgagatcg ccaagatgtt gccaggagg acagacaatg ctgtgaagaa tcactggAAC	60
tctaccatca aaag	74

<210> SEQ ID NO 39

<211> LENGTH: 79

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 39

gtgaggcgcg cgactctgg cgactggccg gccatgcctt cccgggtga ggactatgaa	60
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gtgttgtaca ccattggca	79
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ccaaccctgc agactccaa cctgggacca tccgtggaga cttctgcata caagttggca	60
ggaacattat acat	74
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ggctgtggct gaggctgttag catctctgct ggaggtgaga cactctggga actgatttga	60
cctcgaatgc tcc	73
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gaagggtttt gaggcactca aggacctcat caacgaggcc tgctggata ttagctccag	60
cggtgtaaac c	71
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gcatcaggct gtcattatgg tgtccttacc tgtggagct gtaaggctt cttaagagg	60
gcaatggaag ggcagcacaa ctact	85
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gggacggtgt tcacattcaa gacgaatcgc cagtctccca actatcgct gatcaacatt	60
gacttctggg atcctg	76
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 45

ggctactctg atctatgttg ataaggaaaa tggagaacca ggcacccgtg tggttgctaa 60
ggatgggctg aagc 74

<210> SEQ ID NO 46
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 46

ccattctatac atcaacgggt acaaaccgagt cctggccttg tctgtggaga cggttacac 60
cttcccactt gctga 75

<210> SEQ ID NO 47
<211> LENGTH: 79
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 47

ggctggtcgg cagagagtag cctgcaacat tcggccgtgg tttacatgag tttaccctc 60
aatcccaaac ctttcctca 79

<210> SEQ ID NO 48
<211> LENGTH: 64
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 48

cctgaacatg aaggagctga agctgtgca gaccatcgaa aagggggagt tcggagacgt 60
gatg 64

<210> SEQ ID NO 49
<211> LENGTH: 69
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 49

catttccag gaggaccact ctctgtggca ccctggacta cctgccccct gaaatgattg 60
aaggtcgaa 69

<210> SEQ ID NO 50
<211> LENGTH: 90
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Amplicon

<400> SEQUENCE: 50

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cctggaggct gcaacatacc tcaatcctgt cccaggccgg atcctcctga agccctttc	60
gcagcactgc tatcctccaa agccattgtta	90
<210> SEQ ID NO 51	
<211> LENGTH: 79	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: PCR Amplicon	
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tgttttgatt cccgggctta ccaggtgaga agtgagggag gaagaaggca gtgtccctt	60
tgcttagagct gacagcttg	79
<210> SEQ ID NO 52	
<211> LENGTH: 68	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: PCR Amplicon	
<400> SEQUENCE: 52	
gcacaactgct ttcattttgtt agggatctga accaatacag agcagacata aaggaaatgg	60
gcctgagt	68
<210> SEQ ID NO 53	
<211> LENGTH: 72	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: PCR Amplicon	
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aatccaagg ggagagtat gacttcata tggactttga ctcaactgtt gctcctcggg	60
caaaaatctgt ac	72
<210> SEQ ID NO 54	
<211> LENGTH: 65	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: PCR Amplicon	
<400> SEQUENCE: 54	
gcctcggtgt gcctttcaac atcgccagct acggccctgtt cacgtacatg attgcgcaca	60
tcacg	65
<210> SEQ ID NO 55	
<211> LENGTH: 22	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<223> OTHER INFORMATION: PCR primer-probe	
<400> SEQUENCE: 55	
ggcttctgtt cgtactgtcc tt	22
<210> SEQ ID NO 56	
<211> LENGTH: 23	
<212> TYPE: DNA	
<213> ORGANISM: Artificial Sequence	

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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 56

tcagatgacg aagagcacag atg

23

<210> SEQ ID NO 57
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 57

aggctcagtg atgtcttccc tgtcaccag

29

<210> SEQ ID NO 58
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 58

cgttgtcagc acttggata caa

23

<210> SEQ ID NO 59
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 59

gttcaacctc ttcctgtgga ctgt

24

<210> SEQ ID NO 60
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 60

cccaattaac atgacccggc aaccat

26

<210> SEQ ID NO 61
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 61

cctgc当地 ggaacaagag

20

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 62

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cgtggttgac tctgatctcg 20

<210> SEQ ID NO 63
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 63

cttcgcctcc agatggctcc c 21

<210> SEQ ID NO 64
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 64

ccgaggtaa tccagcacgt a 21

<210> SEQ ID NO 65
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 65

aagacatggc gctctcagtt c 21

<210> SEQ ID NO 66
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 66

tgctgggagc ctacacttgg ccc 23

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 67

tcaagtgtga gctgcggata 20

<210> SEQ ID NO 68
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 68

acgggtcctag gtttgaggtt aaga 24

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<210> SEQ ID NO 69
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 69
caggtcccat tgccggcg 19

<210> SEQ ID NO 70
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 70
ttcagggtgt tgcaggagac 20

<210> SEQ ID NO 71
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 71
catcttcttg ggcacacaat 20

<210> SEQ ID NO 72
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 72
tgtctccatt attgatcggt tcatgca 27

<210> SEQ ID NO 73
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 73
atgctgtggc tccttcctaa ct 22

<210> SEQ ID NO 74
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 74
acccaaatttg tgatatacaa aaagggtt 27

<210> SEQ ID NO 75
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 75

taccaagcaa cctacatgtc aagaaaagccc

30

<210> SEQ ID NO 76
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 76

tggattggag ttctggaaat g

21

<210> SEQ ID NO 77
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 77

gcttgcactc cacaggtaca ca

22

<210> SEQ ID NO 78
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 78

actggccgtg gcactggaca aca

23

<210> SEQ ID NO 79
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 79

tgagtgtccc ccggtatctt c

21

<210> SEQ ID NO 80
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 80

cagccgcttt cagatttca t

21

<210> SEQ ID NO 81
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 81

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tgc~~ccaa~~atccc gatgaaattt gaaattt

27

<210> SEQ ID NO 82
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 82

tgac~~aat~~atcg cacacctgca t

21

<210> SEQ ID NO 83
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 83

tgtgactaca gccgtgatcc tta

23

<210> SEQ ID NO 84
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 84

caggccctct tccgagcggt

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<210> SEQ ID NO 85
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 85

tgc~~cct~~gtggt gggaa~~g~~ct

18

<210> SEQ ID NO 86
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 86

ggaaaatgcc tccgg~~t~~ttt

19

<210> SEQ ID NO 87
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 87

tgacatagca tcatcc~~t~~ttt gttcccagtt

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<210> SEQ ID NO 88
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 88

tccctccact cggaggact a

21

<210> SEQ ID NO 89
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 89

cgtttgtgc tgatctgtct ca

22

<210> SEQ ID NO 90
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 90

tctgacactg tccaacttga ccctctt

27

<210> SEQ ID NO 91
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 91

tgtctcaactg agcgagcaga a

21

<210> SEQ ID NO 92
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 92

accattgcag ccctgattg

19

<210> SEQ ID NO 93
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 93

cttgaggacg cgaacagtcc acca

24

<210> SEQ ID NO 94
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 94

tccattttctt acctgttaac cttcatc

27

<210> SEQ ID NO 95
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 95

atgcagtcgg tcccttcct

19

<210> SEQ ID NO 96
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 96

ttgcttccag ggcctgcaca aaa

23

<210> SEQ ID NO 97
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 97

ctctgagaca gtgcttcgat gact

24

<210> SEQ ID NO 98
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 98

ccatgaggcc caacttcct

19

<210> SEQ ID NO 99
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 99

cagacttggt gccctttgac tcc

23

<210> SEQ ID NO 100
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 100

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ggccctcca gaacaatgat 20

<210> SEQ ID NO 101
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 101

tgcactgctt ggccttaaag a 21

<210> SEQ ID NO 102
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 102

ccgctctcat cgcaagtcaagg atcat 25

<210> SEQ ID NO 103
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 103

cgtggtgccc ctctatgac 19

<210> SEQ ID NO 104
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 104

ggcttagtggg cgcattgttag 19

<210> SEQ ID NO 105
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 105

ctggagatgc tggacgccc 19

<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 106

cacgggacat tcaccacatc 20

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<210> SEQ ID NO 107
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 107

gggtgccatc cacttcaca

19

<210> SEQ ID NO 108
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 108

ataaaaagac aaccaacggc cgactgc

27

<210> SEQ ID NO 109
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 109

ccaccccgag caaatctgt

19

<210> SEQ ID NO 110
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 110

aaatccagtc cccctacttt gg

22

<210> SEQ ID NO 111
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 111

cctgaatcct ggaggctcac gcc

23

<210> SEQ ID NO 112
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 112

ccatctgcat ccatcttggtt

20

<210> SEQ ID NO 113
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 113

ggccaccagg gtattatctg

20

<210> SEQ ID NO 114
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 114

ctccccaccc ttgagaagt cct

23

<210> SEQ ID NO 115
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 115

ggcccagctt gaatttttca

20

<210> SEQ ID NO 116
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 116

aagctatgag gaaaagaagt acacgat

27

<210> SEQ ID NO 117
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 117

tcagccactg gcttctgtca taatcaggag

30

<210> SEQ ID NO 118
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 118

caatgccatc ttgcgcatac t

21

<210> SEQ ID NO 119
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 119

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gtccactcgatcttttcttcttca 25

<210> SEQ ID NO 120
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 120

ctcgcaagca caacatgtgttgtgaga 27

<210> SEQ ID NO 121
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 121

cggtgtgaga agtgacgcaa 20

<210> SEQ ID NO 122
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 122

cctctcgaa gtgtccat 19

<210> SEQ ID NO 123
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 123

ccagaccata gcacactcgg gcac 24

<210> SEQ ID NO 124
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 124

caaggggagcg accaactga 19

<210> SEQ ID NO 125
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 125

gtttgccaag ttaaatgg tacataat 28

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<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 126
ctccatatcc aaacaaagca tgtgtgcg                                28

<210> SEQ ID NO 127
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 127
agaaccgcaa ggtgagcaa                                         19

<210> SEQ ID NO 128
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 128
tccaaactgaa ggtccctgat g                                         21

<210> SEQ ID NO 129
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 129
tggagattct ccagcacgtc atcgac                                26

<210> SEQ ID NO 130
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 130
gcatggtagc cgaagatttc a                                         21

<210> SEQ ID NO 131
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 131
tttccggtaa tagtctgtct catagatatac                            30

<210> SEQ ID NO 132
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 132

cgcgtcatac caaaatctcc gattttga

28

<210> SEQ ID NO 133
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 133

gatatgattt gtcgctgctt tg

22

<210> SEQ ID NO 134
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 134

agaacttcca ttccccacca t

21

<210> SEQ ID NO 135
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 135

cagccaggac ctggccatcc g

21

<210> SEQ ID NO 136
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 136

cggactttgg gtgcgactt

19

<210> SEQ ID NO 137
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 137

ttacaactct tccactggga cgat

24

<210> SEQ ID NO 138
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 138

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ccacttgcg aaccaccgct cgt 23

<210> SEQ ID NO 139
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 139

gccccagaggc tccatcg 18

<210> SEQ ID NO 140
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 140

cagaggtttg aacagtgcag aca 23

<210> SEQ ID NO 141
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 141

cctcttcctc cccagtcggc tga 23

<210> SEQ ID NO 142
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 142

ccacacctcgcc atgatttttc 20

<210> SEQ ID NO 143
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 143

gcaatctctt caaacacttc atcct 25

<210> SEQ ID NO 144
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 144

tttgaccggg tattcccccacc aggaa 25

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<210> SEQ ID NO 145
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 145

tca gtggaga agg aggtt gga

20

<210> SEQ ID NO 146
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 146

tgc catatcc agaggaaaca

20

<210> SEQ ID NO 147
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 147

cc agtcaaca tctctgttgt cacaagca

28

<210> SEQ ID NO 148
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 148

tgcaa acgct ggtgtcaca

19

<210> SEQ ID NO 149
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 149

ccccacgagt tctggttctt c

21

<210> SEQ ID NO 150
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 150

cagcccccca actgacacctca tc

22

<210> SEQ ID NO 151
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 151

gactttgcc cgctacctt c

21

<210> SEQ ID NO 152
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 152

gcacactaact gcttcagtagt gaagag

26

<210> SEQ ID NO 153
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 153

acaggtcatt gttgtcacgc cgga

24

<210> SEQ ID NO 154
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 154

tgatggtcct atgtgtcaca ttca

24

<210> SEQ ID NO 155
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 155

tgggacagga aacacaccaa

20

<210> SEQ ID NO 156
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 156

cagggttcat accaacacag gtttcagcac

30

<210> SEQ ID NO 157
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 157

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aaccggcgatcgaaaag 18

<210> SEQ ID NO 158
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 158

gggcctgctgtcctgaga 18

<210> SEQ ID NO 159
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 159

tcttaggaac gccgttaccaggccgc 24

<210> SEQ ID NO 160
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 160

ccaacgcgttg ccaaattcct 19

<210> SEQ ID NO 161
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 161

acggtagtga cagcatcaaa actc 24

<210> SEQ ID NO 162
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 162

aaccagctct ctgtgacccc aatt 24

<210> SEQ ID NO 163
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 163

gagaaccaat ctcaccgaca 20

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<210> SEQ ID NO 164
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 164

cacccgagtg taaccatagc

20

<210> SEQ ID NO 165
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 165

acaggttattc ctctgccagc tgcc

24

<210> SEQ ID NO 166
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 166

gccgagatcg ccaagatg

18

<210> SEQ ID NO 167
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 167

cttttgcatgg tagagttcca gtgattc

27

<210> SEQ ID NO 168
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 168

cagcattgtc tgtcctccct ggca

24

<210> SEQ ID NO 169
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 169

gtgaggcagc gcgactct

18

<210> SEQ ID NO 170
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 170

tgc~~ccaa~~ttgggt gtacaacact tca

23

<210> SEQ ID NO 171
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 171

tgc~~c~~cttccccg ggctgaggac t

21

<210> SEQ ID NO 172
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 172

ccaaccctgc agactccaa

19

<210> SEQ ID NO 173
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 173

atgtataatg ttcc~~tgc~~caa cttgtatg

28

<210> SEQ ID NO 174
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 174

cct~~gg~~gacca tccgtggaga cttct

25

<210> SEQ ID NO 175
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 175

ggctgtggct gaggctgtag

20

<210> SEQ ID NO 176
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 176

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ggagcattcg aggtcaaatac a 21

<210> SEQ ID NO 177
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 177

tccccagagt gtctcacac tc cagcagag 28

<210> SEQ ID NO 178
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 178

gaagggtttt gaggcactca ag 22

<210> SEQ ID NO 179
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 179

ggtttacacc gctggagcta a 21

<210> SEQ ID NO 180
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 180

atcccaagcag gcctcggttg a tgag 24

<210> SEQ ID NO 181
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 181

gcattcaggct gtcattatgg 20

<210> SEQ ID NO 182
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 182

agtagtttgt ctgcccttcc 20

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<210> SEQ ID NO 183
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 183

tgccttacc tgtggagct gtaaggc

28

<210> SEQ ID NO 184
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 184

gggacggtgt tcacattcaa g

21

<210> SEQ ID NO 185
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 185

caggatcca gaagtcaatg ttg

23

<210> SEQ ID NO 186
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 186

tgcgcagtct cccaaactatc gcgt

24

<210> SEQ ID NO 187
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 187

ggctactctg atctatgtt ataaggaa

28

<210> SEQ ID NO 188
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 188

gtttcagccc atccttagca

20

<210> SEQ ID NO 189
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 189

cacacgggtg cctgggttctc ca

22

<210> SEQ ID NO 190
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 190

ccattctatc atcaacgggtt acaa

24

<210> SEQ ID NO 191
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 191

ttagcaagtg ggaagggtta atc

23

<210> SEQ ID NO 192
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 192

tctccacaga caaggccagg actcg

25

<210> SEQ ID NO 193
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 193

ggctggtcgg cagagagtag

20

<210> SEQ ID NO 194
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 194

tgaggaaagg tttgggattg a

21

<210> SEQ ID NO 195
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 195

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aaactcatgt aaaccacggc cgaatggt 29

<210> SEQ ID NO 196
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 196

cctgaacatg aaggagctga 20

<210> SEQ ID NO 197
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 197

catcacgtct ccgaactcc 19

<210> SEQ ID NO 198
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 198

tcccgatggt ctgcagcagc t 21

<210> SEQ ID NO 199
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 199

catcttccag gaggaccact 20

<210> SEQ ID NO 200
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 200

tccgaccttc aatcattca 20

<210> SEQ ID NO 201
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 201

ctctgtggca ccctggacta cctg 24

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<210> SEQ ID NO 202
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 202

cctggaggct gcaacatacc

20

<210> SEQ ID NO 203
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 203

tacaatggct ttggaggata gca

23

<210> SEQ ID NO 204
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 204

atccttcctga agcccttttc gcagc

25

<210> SEQ ID NO 205
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 205

tgttttgatt cccgggctta

20

<210> SEQ ID NO 206
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 206

caaagctgtc agctctagca aaag

24

<210> SEQ ID NO 207
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 207

tgccttcttc ctcccact tctcacct

28

<210> SEQ ID NO 208
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 208

gccaactgct ttcatttgtg

20

<210> SEQ ID NO 209
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 209

actcaggccc atttccttta

20

<210> SEQ ID NO 210
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 210

agggatctga accaatacag agcagaca

28

<210> SEQ ID NO 211
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 211

aatccaaggg ggagagtat

20

<210> SEQ ID NO 212
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 212

gtacagattt tgcccgagga

20

<210> SEQ ID NO 213
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 213

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26

<210> SEQ ID NO 214
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

<400> SEQUENCE: 214

-continued

gcctcggtgt gccttca

18

```

<210> SEQ ID NO 215
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

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<400> SEQUENCE: 215

cgtgatgtgc gcaatcatg

19

```

<210> SEQ ID NO 216
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer-probe

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<400> SEQUENCE: 216

catcgccagc tacgcccctgc tc

22

What is claimed is:

1. A method of predicting the likelihood of long-term survival of a cancer patient without the recurrence of cancer, comprising determining the expression level of one or more prognostic RNA transcripts or their expression products in a cancer cell obtained from said patient, normalized against the expression level of all RNA transcripts or their products in said cancer cell, or of a reference set of RNA transcripts or their expression products, wherein the prognostic RNA transcript is the transcript of one or more genes selected from the group consisting of B Catenin; BAG1; BIN1; BUB1; C20_orf1; CCNB1; CCNE2; CDC20; CDH1; CEGP1; CIAP1; cMYC; CTSL2; DKFZp586M07; DR5; EpCAM; EstR1; FOXM1; GRB7; GSTM1; GSTM3; HER2; HNRPAB; ID1; IGF1R; ITGA7; Ki_67; KNSL2; LMNB1; MCM2; MELK; MMP12; MMP9; MYBL2; NEK2; NME1; NPD009; PCNA; PR; PREP; PTTG1; RPLPO; Src; STK15; STMY3; SURV; TFRC; TOP2A; and TS;

wherein expression of one or more of BUB1; C20_orf1; CCNB1; CCNE2; CDC20; CDH1; CTSL2; EPCAM; FOXM1; GRB7; HER2; HNRPAB; Ki_67; KNSL2; LMNB1; MCM2; MELK; MMP12; MMP9; MYBL2; NEK2; NME1; PCNA; PREP; PTTG1; Src; STK15; STMY3; SURV; TFRC; TOP2A; and TS indicates a decreased likelihood of long-term survival without cancer recurrence; and

the expression of one or more of BAG1; BCatenin; BIN1; CEGP1; CIAP1; cMYC; DKFZp586M07; DR5; EstR1; GSTM1; GSTM3; ID1; IGF1R; ITGA7; NPD009; PR; and RPLPO indicates an increased likelihood of long-term survival without cancer recurrence.

2. The method of claim 1 comprising determining the expression level of at least two of said prognostic RNA transcripts or their expression products.

3. The method of claim 1 comprising determining the expression level of at least 5 of said prognostic RNA transcripts or their expression products.

4. The method of claim 1 comprising determining the expression level of at least 10 of said prognostic RNA transcripts or their expression products.

5. The method of claim 1 comprising determining the expression level of at least 15 of said prognostic transcripts or their expression products.

6. The method of claim 1 wherein said cancer is breast cancer.

7. The method of claim 6 wherein said cancer is node negative, ER positive breast cancer.

8. The method of claim 1 wherein said cancer is ovarian cancer.

9. The method of claim 1 wherein the expression level of one or more prognostic RNA transcripts is determined.

10. The method of claim 1 wherein said RNA comprises intronic RNA.

11. The method of claim 1 comprising determining the expression level of one or more prognostic RNA transcripts or their expression products of one or more genes selected from the group consisting of MMP9, GSTM1, MELK, PR, DKFZp586M07, GSTM3, CDC20, CCNB1, STMY3, GRB7, MYBL2, CEGP1, SURV, LMNB1, CTSL2, PTTG1, BAG1, KNSL2, CIAP1, PREP, NEK2, EpcAM, PCNA, C20_orf1, ITGA7, ID1 B_Catenin, EstR1, CDH1, TS HER2, and cMYC,

wherein expression of one or more of C20_orf1; CCNB1; CDC20; CDH1; CTSL2; EpcAM; GRB7; HER2; KNSL2; LMNB1; MCM2; MMP9; MYBL2; NEK2; PCNA; PREP; PTTG1; STMY3; SURV; TS; and MELK indicates a decreased likelihood of long-term survival without cancer recurrence; and

expression of one or more of BAG1; BCatenin; CEGP1; CIAP1; cMYC; DKFZp586M07; EstR1; GSTM1; GSTM3; ID1; ITGA7; and PR indicates an increased likelihood of long-term survival without cancer recurrence.

12. The method of claim 1 comprising determining the expression level of one or more prognostic RNA transcripts or their expression products of one or more genes selected from the group consisting of GRB7, SURV, PR, LMNB1, MYBL2, HER2, GSTM1, MELK, S20_orf1, PTTG1, BUB1, CDC20, CCNB1, STMY3, KNSL2, CTS2, MCM2, NEK2, DR5, Ki_67, CCNE2, TOP2A, PCNA, PREP, FOXM1, NME1, CEGP1, BAG1, STK15, HNRPAB, EstR1, MMP9, DKFZp586M07, TS, Src, BIN1, NPD009, RPLPO, GSTM3, MMP12, TFRC, and IGF1R,

wherein expression of one or more of GRB7; SURV; LMNB1; MYBL2; HER2; MELK; C20_orf1; PTTG1; BUB1; CDC20; CCNB1; STMY3; KNSL2; CTS2; MCM2; NEK2 Ki_67; CCNE2; TOP2A_4; PCNA; PREP; FOXM1; NME1; STK15; HNRPAB; MMP9; TS; Src; MMP12; and TFRC indicates a decreased likelihood of long-term survival without cancer recurrence; and

the expression of one or more of PR; GSTM1; DR5; CEGP1; BAG1; EstR1; DKFZp586M07; BIN1; NPD009; RPLPO; GSTM3; IGF1R indicates an increased likelihood of long-term survival without cancer recurrence.

13. A method of predicting the likelihood of long-term survival of a cancer patient without the recurrence of cancer, comprising determining the expression level of one or more prognostic RNA transcripts or their expression products in a cancer cell obtained from said patient, normalized against the expression level of all RNA transcripts or their products in said cancer cell, or of a reference set of RNA transcripts or their expression products, wherein the prognostic RNA transcript is the transcript of one or more genes selected from the group consisting of GRB7; LMNB1; ER; STMY3; KLK10; PR; KRT5; FGFR1; MCM6; SNRPF,

wherein expression of one or more of GRB7, LMNB1, STMY3, KLK10, FGFR1, and SNRPF indicates a decreased likelihood of long term survival without cancer recurrence; and the expression of one or more of ER, PR, KRT5 and MCM6 indicates an increased likelihood of long-term survival without cancer recurrence.

14. The method of claim 6 wherein said RNA is isolated from a fixed, wax-embedded breast cancer tissue specimen of said patient.

15. The method of claim 6 wherein said RNA is isolated from core biopsy tissue or fine needle aspirate cells.

16. An array comprising polynucleotides hybridizing to two or more of the following genes: B_Catenin; BAG1; BIN1; BUB1; C20_orf1; CCNB1; CCNE2; CDC20; CDH1; CEGP1; CIAP1; cMYC; CTS2; DKFZp586M07; DR5; EpCAM; EstR1; FOXM1; GRB7; GSTM1; GSTM3; HER2; HNRPAB; ID1; IGF1R; ITGA7; Ki_67; KNSL2; LMNB1; MCM2; MELK; MMP12; MMP9; MYBL2; NEK2; NME1; NPD009; PCNA; PR; PREP; PTTG1; RPLPO; Src; STK15; STMY3; SURV; TFRC; TOP2A; and TS, immobilized on a solid surface.

17. The array of claim 16 comprising polynucleotides hybridizing to two or more of the following genes: MMP9, GSTM1, MELK, PR, DKFZp586M07, GSTM3, CDC20, CCNB1, STMY3, GRB7, MYBL2, CEGP1, SURV, LMNB1, CTS2, PTTG1, BAG1, KNSL2, CIAP1, PREP, NEK2, EpCAM, PCNA, C20_orf1, ITGA7, ID1 B_Catenin, EstR1, CDH1, TS HER2, and cMYC.

18. The array of claim 16 comprising polynucleotides hybridizing to two or more of the following genes: GRB7, SURV, PR, LMNB1, MYBL2, HER2, GSTM1, MELK, S20_orf1, PTTG1, BUB1, CDC20, CCNB1, STMY3, KNSL2, CTS2, MCM2, NEK2, DR5, Ki_67, CCNE2, TOP2A, PCNA, PREP, FOXM1, NME1, CEGP1, BAG1, STK15, HNRPAB, EstR1, MMP9, DKFZp586M07, TS, Src, BIN1, NPD009, RPLPO, GSTM3, MMP12, TFRC, and IGF1R.

19. The array of claim 16 comprising polynucleotides hybridizing to at least 3 of said genes.

20. The array of claim 16 comprising polynucleotides hybridizing to at least 5 of said genes.

21. The array of claim 16 comprising polynucleotides hybridizing to at least 10 of said genes.

22. The array of claim 16 comprising polynucleotides hybridizing to all of said genes.

23. The array of claim 16 comprising more than one polynucleotide hybridizing to the same gene.

24. The array of claim 16 wherein said polynucleotides are cDNAs.

25. The array of claim 24 wherein said cDNAs are about 500 to 5000 bases long.

26. The array of claim 16 wherein said polynucleotides are oligonucleotides.

27. The array of claim 26 wherein said oligonucleotides are about 20 to 80 bases long.

28. The array of claim 16 wherein the solid surface is glass.

29. The array of claim 16 which comprises about 330,000 oligonucleotides.

30. The method of claim 16 wherein said array comprises intron-based polynucleotide sequences.

31. A method of predicting the likelihood of long-term survival of a patient diagnosed with invasive breast cancer, without the recurrence of breast cancer, comprising the steps of

(a) determining the expression levels of the RNA transcripts or the expression products of genes of a gene set selected from the group consisting of B_Catenin; BAG1; BIN1; BUB1; C20_orf1; CCNB1; CCNE2; CDC20; CDH1; CEGP1; CIAP1; cMYC; CTS2; DKFZp586M07; DR5; EpCAM; EstR1; FOXM1; GRB7; GSTM1; GSTM3; HER2; HNRPAB; ID1; IGF1R; ITGA7; Ki_67; KNSL2; LMNB1; MCM2; MELK; MMP12; MMP9; MYBL2; NEK2; NME1; NPD009; PCNA; PR; PREP; PTTG1; RPLPO; Src; STK15; STMY3; SURV; TFRC; TOP2A; and TS in a breast cancer cell obtained from said patient, normalized against the expression levels of all RNA transcripts or their expression products in said breast cancer cell, or of a reference set of RNA transcripts or their products;

(b) subjecting the data obtained in step (a) to statistical analysis; and;

(c) determining whether the likelihood of said long-term survival has increased or decreased.

32. A method of preparing a personalized genomics profile for a patient, comprising the steps of

(a) subjecting RNA extracted from a breast tissue obtained from the patient to gene expression analysis;

- (b) determining the expression level in the tissue of one or more genes selected from the breast cancer gene set listed in any one of Tables 1 and 2, wherein the expression level is normalized against a control gene or genes and optionally is compared to the amount found in a breast cancer reference tissue set; and
 - (c) creating a report summarizing the data obtained by said gene expression analysis.
- 33.** The method of claim 32 wherein said breast tissue comprises breast cancer cells.
- 34.** The method of claim 33 wherein said breast tissue is obtained from a fixed, paraffin-embedded biopsy sample.
- 35.** The method of claim 34 wherein said RNA is fragmented.

36. The method of claim 32 wherein said report includes prediction of the likelihood of long term survival of the patient.

37. The method of claim 32 wherein said report includes recommendation for a treatment modality of said patient.

38. A method for amplification of a gene listed in Tables 1 and 2 by polymerase chain reaction (PCR), comprising performing said PCR by using an amplicon listed in Table 3 and a primer-probe set listed in Tables 4A-4D.

39. A PCR primer-probe set listed in Tables 4A-4D.

40. A PCR amplicon listed in Table 3.

* * * * *



US 20050233958A1

(19) **United States**

(12) **Patent Application Publication**

Ni et al.

(10) **Pub. No.: US 2005/0233958 A1**

(43) **Pub. Date: Oct. 20, 2005**

(54) **DEATH DOMAIN CONTAINING RECEPTOR
5**

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(21) Appl. No.: **10/979,831**

(22) Filed: **Nov. 3, 2004**

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/648,825, filed on Aug. 27, 2003.
Continuation-in-part of application No. 09/565,009, filed on May 4, 2000, now Pat. No. 6,872,568.
Continuation-in-part of application No. 09/042,583, filed on Mar. 17, 1998.

(60) Provisional application No. 60/551,811, filed on Mar. 11, 2004. Provisional application No. 60/608,429, filed on Sep. 10, 2004. Provisional application No. 60/413,747, filed on Sep. 27, 2002. Provisional application No. 60/406,307, filed on Aug. 28, 2002. Provisional application No. 60/148,939, filed on Aug. 13, 1999. Provisional application No. 60/133,238, filed on May 7, 1999. Provisional application No. 60/132,498, filed on May 4, 1999. Provisional application No. 60/054,021, filed on Jul. 29, 1997. Provisional application No. 60/040,846, filed on Mar. 17, 1997.

Publication Classification

(51) **Int. Cl.⁷ A61K 38/17; C07K 14/705;
C07K 16/28**

(52) **U.S. Cl. 514/12; 530/350; 530/388.22**

ABSTRACT

The present invention relates to novel Death Domain Containing Receptor-5 (DR5) proteins which are members of the tumor necrosis factor (TNF) receptor family, and have now been shown to bind TRAIL. In particular, isolated nucleic acid molecules are provided encoding the human DR5 proteins. DR5 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying antagonists and agonists of DR5 activity. The invention also relates to the treatment of diseases associated with reduced or increased levels of apoptosis using antibodies specific for DR5, which may be agonists and/or antagonists of DR5 activity.

10	30	50
CACGCGTCCGGCGCGGCCGGAGAACCCCGCAATCTTGCGCCCACAAAATACACCGA		
70	90	110
CGATGCCGATCTACTTTAAGGGCTGAAACCCACGGGCTGAGAGACTATAAGAGCGTTC		
130	150	170
CCTACCGCCATGGAACAACGGGACAGAACGCCCGGCGCTTCGGGGCCCCGGAAAAGG		
<u>M E O R G Q N A P A A S G A R K R</u>		
190	210	230
CACGGCCCAGGACCCAGGGAGGCAGGGCCTGGGCCCCGGGTCCCCAAGACC		
H G P G P R E A R G A R P G P R V P K T		
250	270	290
CTTGTGCTCGTTGTCGCCCGGTCCTGCTGTTGGTCTCAGCTGAGTCTGCTCTGATCACC		
<u>L V L V V A A V L L L V S A E S A L I T</u>		
310	330	350
CAACAAAGACCTAGCTCCCCAGCAGAGAGCGGGCCCCACAACAAAAGAGGTCCAGCCCCCTCA		
Q Q D L A P Q Q R A A P Q Q K R S S P S		
370	390	410
GAGGGATTGTGTCACCTGGACACCATATCTCAGAAGACGGTAGAGATTGCATCTCCTGC		
E G L C P P G H H I S E D G R D C I S C		
430	450	470
AAATATGGACAGGACTATAGCACTCACTGGAATGACCTCCTTTCTGCTTGCCTGCACC		
K Y G Q D Y S T H W N D L L F C L R C T		
490	510	530
AGGTGTGATTCAAGGTGAAGTGGAGCTAACGTCCCTGCACACGACCAGAAACACAGTGTGT		
R C D S G E V E L S P C T T T R N T V C		
550	570	590
CAGTGCAGAAGAAGGCACCTTCCGGAAAGAAGATTCTCCTGAGATGTGCCGGAGTGCCGC		
Q C E E G T F R E E D S P E M C R K C R		
610	630	650
ACAGGGTGTCCCAGAGGGATGGTCAAGGTGGTATTGTACACCTGGAGTGACATCGAA		
T G C P R G M V K V G D C T P W S D I E		
670	690	710
TGTGTCCACAAAGAACAGGCATCATAGGAGTCACAGTTGCAGCCGTAGTCTTGATT		
C V H K E S G I I G V T V A A V V L I		
730	750	770
GTGGCTGTGTTGTTGCAAGTCTTACTGTGGAAGAAAGTCCTCCTAACCTGAAAGGC		
<u>V A V F V C K S L L W K K V L P Y L K G</u>		
790	810	830
ATCTGCTCAGGTGGTGGTGGGGACCTGAGCGTGTGGACAGAAGCTCACAAACGACCTGGG		
I C S G G G D P E R V D R S S Q R P G		

FIG.1A

850	870	890
GCTGAGGACAATGTCCTCAATGAGATCGTAGTATCTGCAGCCCACCCAGGTCCCTGAG		
A E D N V L N E I V S I L Q P T Q V P E		
910	930	950
CAGGAAATGGAAGTCCAGGAGCCAGCAGAGCCAACAGGTGTCAACATGTTGTCCCCGGG		
Q E M E V Q E P A E P T G V N M L S P G		
970	990	1010
GAGTCAGAGCATCTGCTGGAACCGGCAGAACGCTGAAAGGTCTCAGAGGAGGGCTGCTG		
E S E H L L E P A E A E R S Q R R R L L		
1030	1050	1070
GTTCCAGCAAATGAAGGTGATCCCAC TGAGACTCTGAGACAGTGCTTCGATGACTTGCA		
V P A N E G D P T E T L R Q C F D D F A		
1090	1110	1130
GACTTGGTGCCTTGTACTCCTGGAGGCCCTCATGAGGAAGTTGGGCCTCATGGACAAT		
D L V P F D S W E P L M R K L G L M D N		
1150	1170	1190
GAGATAAAGGTGGCTAAAGCTGAGGCAGCGGGCACAGGGACACCTTGTACACGATGCTG		
E I K V A K A E A A G H R D T L Y T M L		
1210	1230	1250
ATAAAGTGGTCAACAAAACCGGGCGAGATGCCCTGTCCACACCCCTGCTGGATGCCCTG		
I K W V N K T G R D A S V H T L L D A L		
1270	1290	1310
GAGACGCTGGGAGAGAGACTTGCCAAGCAGAACATTGAGGACCACTTGTGAGCTCTGGA		
E T L G E R L A K Q K I E D H L L S S G		
1330	1350	1370
AAGTTCATGTATCTAGAAGGTAATGCAGACTCTGCCATGCTCTAAAGTGTGATTCTCTCA		
K F M Y L E G N A D S A M S *		
1390	1410	1430
GGAAGTGAGACCTTCCCTGGTTACCTTTCTGGAAAAAGCCCAACTGGACTCCAGTC		
1450	1470	1490
AGTAGGAAAGTGCCACAATTGTCACATGACCGGTACTGGAAGAAACTCTCCCATCCAACA		
1510	1530	1550
TCACCCAGTGGATGGAACATCCTGTAACTTTCACTGCACTTGGCATTATTTTATAAGC		
1570	1590	
TGAATGTGATAATAAGGACACTATGGAAAAAAAAAAAAAA		

FIG.1B

FIG.2A

149 - - - - - C E H G I I - - - K E C - - - - - T L T S N T K C K E - - - h Fas protein
161 K Q N T V C T C H A G F F L R E N E C V S C S N C K K S L E C T K L C L P Q I E h TNFR I Protein
158 R D T D C G T C L P G F Y E H G D G C V S C P T S T L G - S C P E R C A A V C G DR3 protein
163 G M V K V G D C T P - - - W S D I E C V - - - - - H K E S G I I I G HLYBX88XXprotein

168 - - - - - E G S R S N L G W - - - - - L C L L - L L P I P L I V - - - - - W h Fas protein
201 N V K G T E D S G T T V L L P L V I F F G L C L L S L L F I G L M Y R Y Q R - W h TNFR I Protein
197 W R Q - - - - - M F W V V Q V L L A G L V V P L L G A T L T Y T Y R H C W DR3 protein
189 - - - - - V T V A A V V L I V A V F - - V C K S L L W K K V L P Y L K G I C S HLYBX88XXprotein

190 V K R K E V Q K T C R K H R K E N Q G S H E S - - - - - - - - - h Fas protein
240 - K S K L Y S I V C G K S T P E K E G E L E G T T T K P L A P N P S F S P T P G h TNFR I Protein
229 - P H K P L - V T A D E A G M E A L T P P P A T H L S P L D S A H T L L A P P D DR3 protein
221 - - - - - G G G G D P E R V D R S S Q R P G A E D N V L N E I V S I L Q P T Q HLYBX88XXprotein

213 - h Fas protein
279 F T P T L G F S P V P S S T F T S S S T Y T P G D - C P N F A A P R R E V A P P h TNFR I Protein
267 S S E K I C T V Q L V G N S W T P G Y P E T Q E A L C P Q V T W S W D Q L - - P DR3 protein
255 V P E Q E M E V Q E P A E - - - - P T G V N M L S P G - - E S E H L - - HLYBX88XXprotein

213 - - - - - P T L N P E T V A I N L - - - S D V D L S K Y I T T I A G V M h Fas protein
318 Y Q G A D P I L A T A L A S D P I P N P L Q K W E D S A H K P Q S L D T D D P A h TNFR I Protein
305 S R A L G P A A A P T L S P - - - - - E S P A G S P A M M L Q P G P Q DR3 protein
283 - - - - - L E P A E A E R S Q R R R L L V P A N E G D P T E T L R Q HLYBX88XXprotein

FIG.2B

241 T L S Q V - - - - - K G F V R K N G V N E A K I D E I K N D N V Q D T A h Fas protein
358 T L Y A V V E N V P P L R W K E F V R R L G L S D H E I D R L E L Q N G R C L R h TNFR I Protein
335 - L Y D V M D A V P A R R W K E F V R T L G L R E A E I E A V E V E I G R - F R DR3 protein
312 C F D D F A D L V P F D S W E P L M R K L G L M D N E I - K V A K A E A A G H R HLYBX88XXprotein

272 E Q K V Q L L R N W H Q L H G K K E A - Y D T L I K D L K K A N L C T L A E K I h Fas protein
398 E A Q Y S M L A T W R R R T P R R E A T L E L L G R V L R D M D L L G C L E D I h TNFR I Protein
373 D Q Q Y E M L K R W R Q Q Q P - - - A G L G A V Y A A L E R M G L D G C V E D L DR3 protein
351 D T L Y T M L I K W V N K T G R - D A S V H T L L D A L E T L G E R L A K Q K I HLYBX88XXprotein

311 Q T I I L K D I T S D S E N S N F R N E I Q S L V h Fas protein
438 E E A L - - - - - C G P A A L P P A P S L L R h TNFR I Protein
410 - - - - - - - R S R L Q R G P DR3 protein
390 E D H L L S S G K F M Y L E G N - - A D S A M S HLYBX88XXprotein

FIG.2C

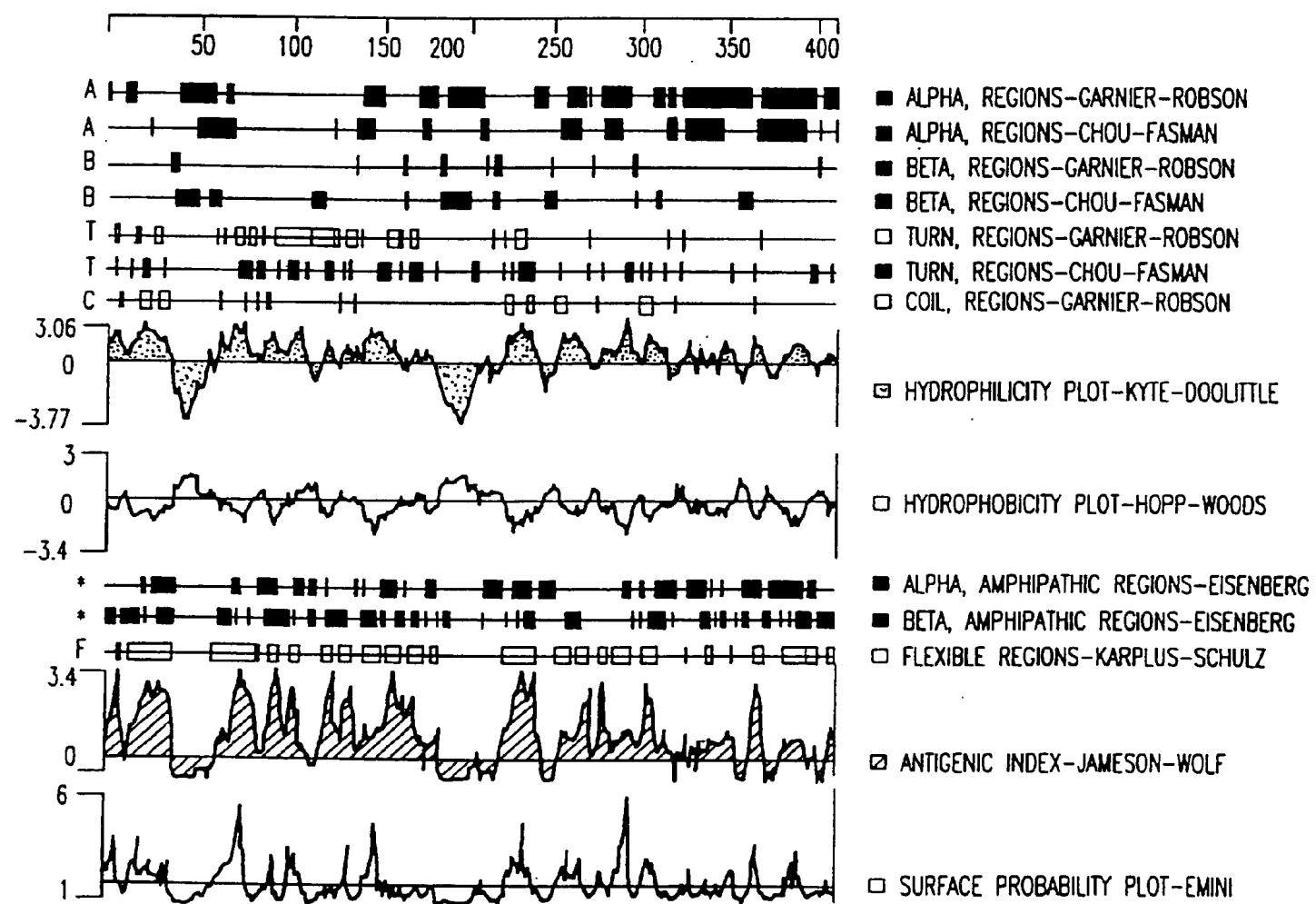


FIG.3

HAPBU13R

1 AATTGGCAC AGCTCTTCAG GAAGTCAGAC CTTCCCTGGT TTACCTTTT
51 TCTGGAAAAA GCCCAACTGG GACTCCAGTC AGTAGGAAAG TGCCACAATT
101 GTCACATGAC CGGTACTGGA AGAAAACTCTC CCATCCAACA TCACCCAGTG
151 GNATGGGAAC ACTGATGAAC TTTTCACTGC ACTTGGCATT ATTTTGTNA
201 AGCTGAATGT GATAATAAGG GCACTGATGG AAATGTCTGG ATCATCCGG
251 TTGTGCGTAC TTTGAGATTT GNGTTGGGG ATGTNCATTG TGTTTGACAG
301 CACTTTTTN ATCCCTAATG TNAAATGCNT NATTGATTG TGANTTGGGG
351 GTNAACATTG GTNAAGGNTN CCCNTNTGAC ACAGTAGNTG GTNCCCGACT
401 TANAATNGNN GAANANGATG NATNANGAAC CTTTTTTGG GTGGGGGGT
451 NNCGGGGCAG TNNAANGNNG NCTCCCCAGG TTTGGNGTNG CAATNGNGGA
501 ANNNTGG

HSBBU76R

1 TTTTTTTGT AGATGGATCT TACAATGTAG CCCAAATAAA TAAATAAAGC
51 ATTTACATTA GGATAAAAAA GTGCTGTGAA AACAAATGACA TCCCAAACCA
101 AATCTCAAAG TACGCACAAA CGGAATGATC CAGACATTTC CATAGNGTCC
151 TTATTATCAC ATTCAAGCTTA TAAAANTAAT GCCAAGTGCA GTGAAAAGTT
201 ACAGGATGTT CCATCCACTG GGTGGATT

FIG.4

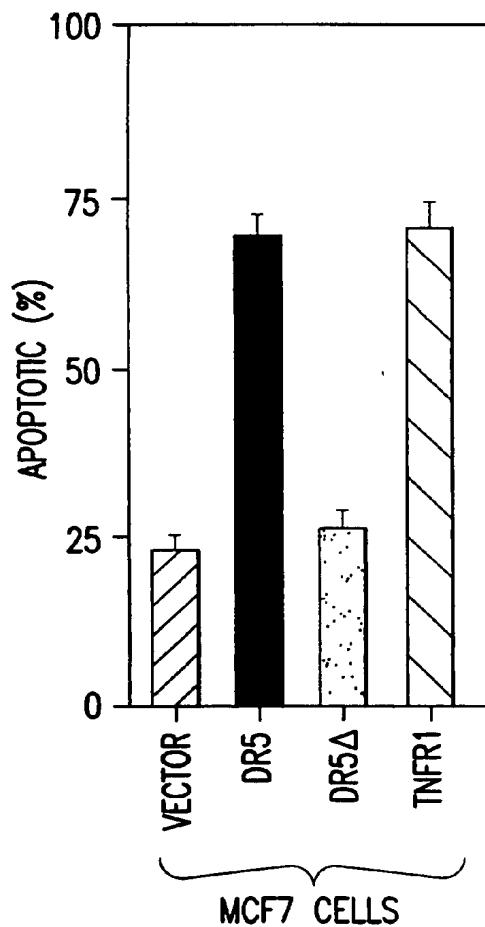


FIG. 5A

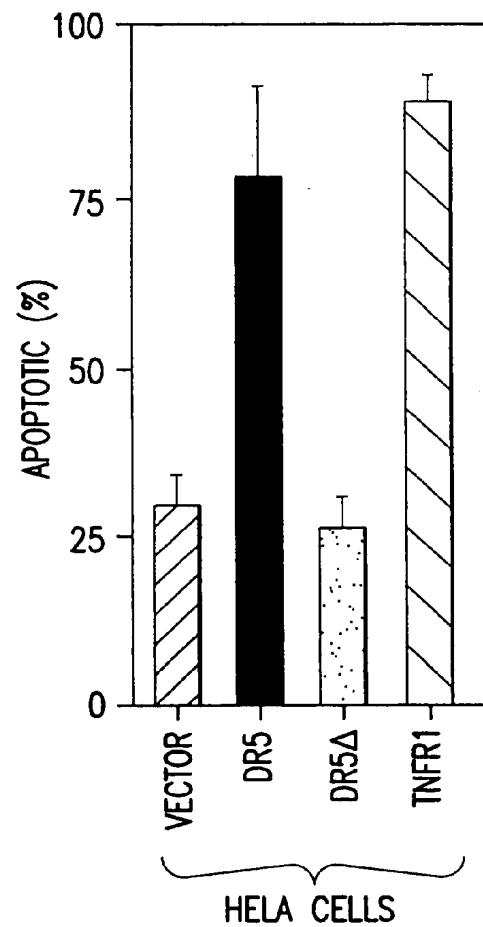


FIG. 5B

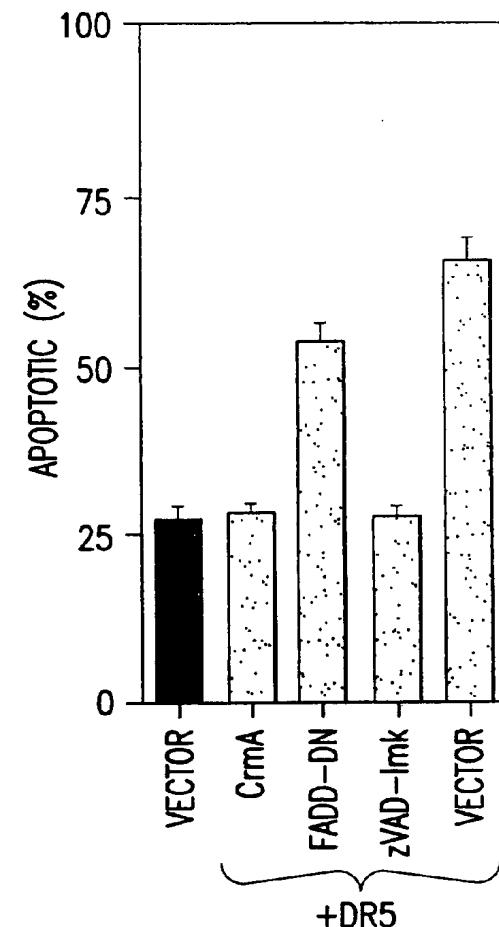


FIG. 5C

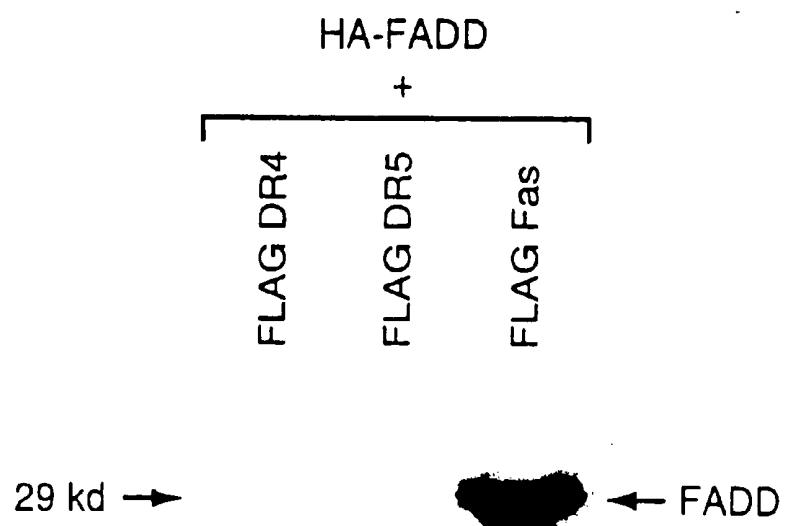
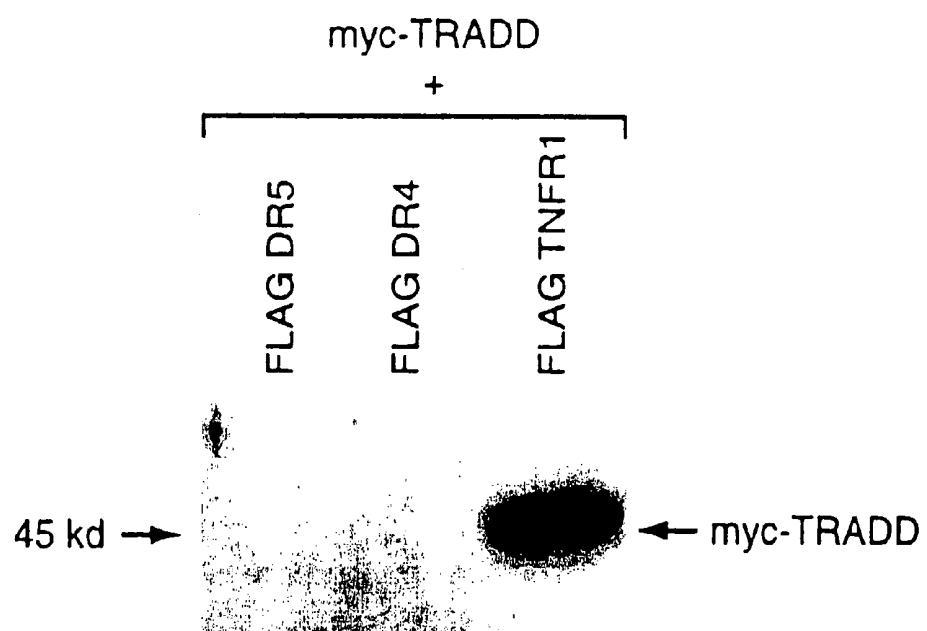


FIG.5D



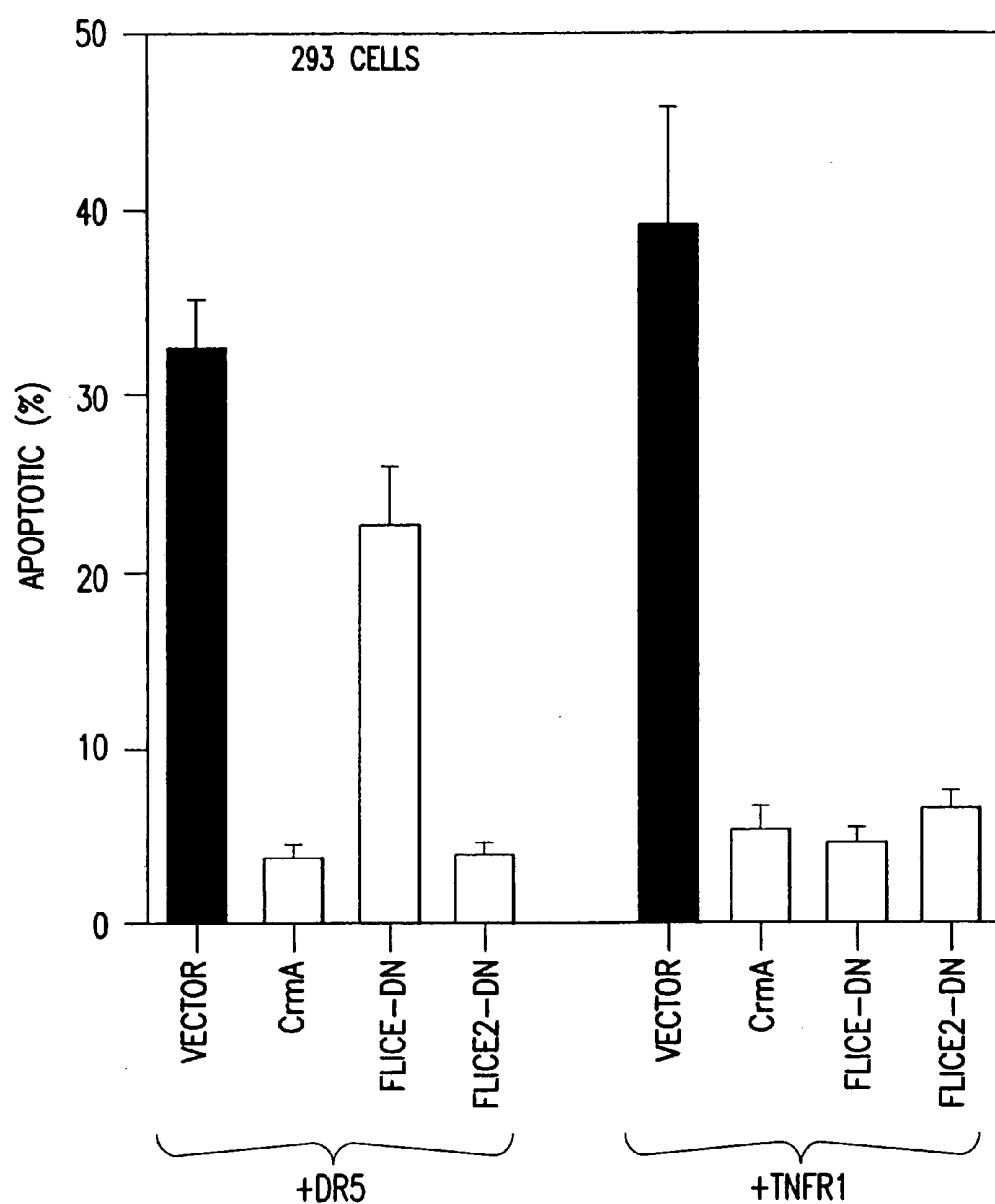


FIG. 5E

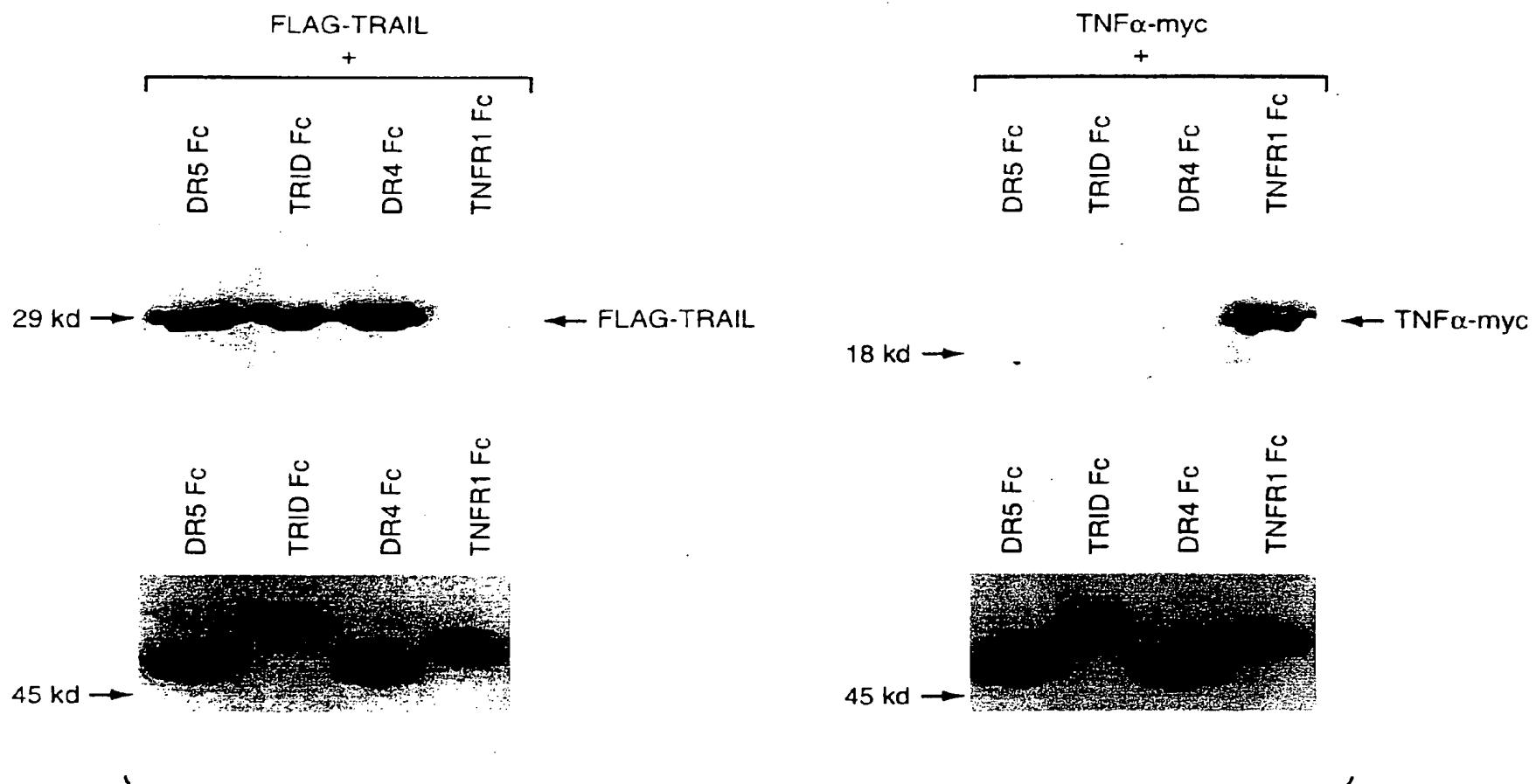


FIG. 6A

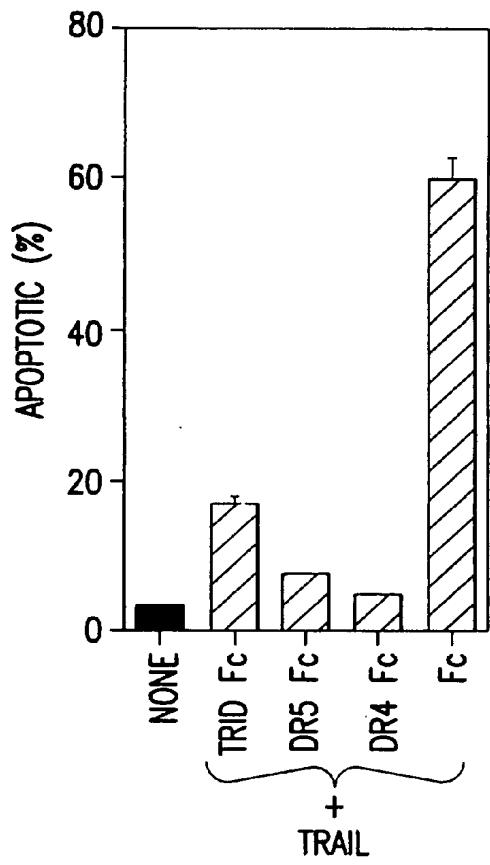


FIG. 6B

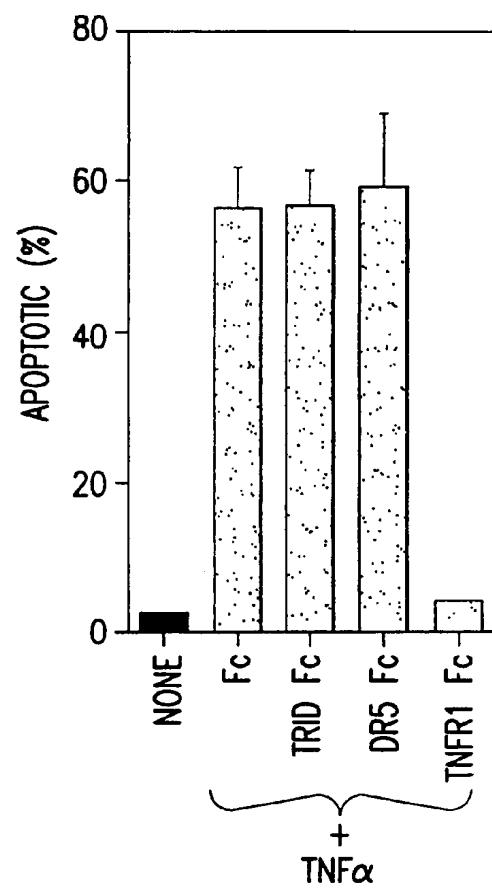


FIG. 6C

DEATH DOMAIN CONTAINING RECEPTOR 5**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of priority under 35 U.S.C. § 119(e) of provisional Application Nos. 60/551,811 and 60/608,429, filed Mar. 11, 2004 and Sep. 10, 2004, respectively. This application is also a Continuation-In-Part and claims benefit of priority under 35 U.S.C. § 120 of non-provisional application Ser. No. 10/648,825, filed Aug. 27, 2003, which claims the benefit of priority under 35 U.S.C. § 119(e) of provisional Application Nos. 60/413,747 and 60/406,307, filed Sep. 27, 2002 and Aug. 28, 2002 respectively. This application is also a Continuation-In-Part and claims benefit of priority under 35 U.S.C. § 120 of non-provisional application Ser. No. 09/565,009, filed on May 4, 2000, which in turn claims the benefit of priority under 35 U.S.C. § 119(e) of provisional Application Nos. 60/148,939, 60/133,238 and 60/132,498, filed Aug. 13, 1999, May 7, 1999 and May 4, 1999 respectively. This Application is also a Continuation-In-Part and claims benefit of priority under 35 U.S.C. § 120 of non-provisional application Ser. No. 09/042,583, filed on Mar. 17, 1998, which in turn claims the benefit of priority under 35 U.S.C. § 119(e) of provisional Application Nos. 60/054,021 and 60/040,846, filed Jul. 29, 1997 and Mar. 17, 1997 respectively. Each of the above-identified priority applications is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a novel member of the tumor necrosis factor family of receptors. More specifically, isolated nucleic acid molecules are provided encoding human Death Domain Containing Receptor 5, or simply "DR5." DR5 polypeptides are also provided, as are vectors, host cells, and recombinant methods for producing the same. The invention relates to the treatment of diseases associated with reduced or increased levels of apoptosis using antibodies specific for DR5, which may be agonists and/or antagonists of DR5 activity. The invention further relates to screening methods for identifying agonists and antagonists of DR5 activity.

RELATED ART

[0003] Numerous biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intra-cellular response.

[0004] For example, tumor necrosis factors (TNF) alpha and beta are cytokines, which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

[0005] Among the ligands, there are included TNF- α , lymphotoxin- α (LT- α , also known as TNF- β), LT- β (found in complex heterotrimer LT- α 2- β), FasL, CD40L, CD27L,

CD30L, 4-1BBL, OX40L and nerve growth factor (NGF). The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., *Biologicals*, 22:291-295 (1994)).

[0006] Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types, which underlie cell ontogeny and functions. (Meager, A., *supra*).

[0007] Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., *Nature* 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R. C. et al., *Science* 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innervation of peripheral structures (Lee, K. F. et al., *Cell* 69:737 (1992)).

[0008] TNF and LT- α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT- α , acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT- α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., *Science* 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

[0009] Moreover, an about 80 amino acid domain near the C-terminus of TNFR-1 (p55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., *Cell* 74:845 (1993)).

[0010] Apoptosis, or programmed cell death, is a physiologic process essential for the normal development and homeostasis of multicellular organisms (H. Steller, *Science* 267:1445-1449 (1995)). Derangements of apoptosis contribute to the pathogenesis of several human diseases including cancer, neurodegenerative disorders, and acquired immune deficiency syndrome (C. B. Thompson, *Science* 267:1456-1462 (1995)). Recently, much attention has focused on the signal transduction and biological function of two cell surface death receptors, Fas/APO-1 and TNFR-1 (J. L. Cleveland et al., *Cell* 81:479-482 (1995); A. Fraser, et al., *Cell* 85:781-784 (1996); S. Nagata et al., *Science* 267:1449-56(1995)). Both are members of the TNF receptor family which also include TNFR-2, low affinity NGFR, CD40, and CD30, among others (C. A. Smith et al., *Science* 248:1019-23 (1990); M. Tewari et al., in *Modular Texts in Molecular and Cell Biology* M. Purton, Heldin, Carl, Ed. (Chapman

and Hall, London, 1995). While family members are defined by the presence of cysteine-rich repeats in their extracellular domains, Fas/APO-1 and TNFR-1 also share a region of intracellular homology, appropriately designated the “death domain”, which is distantly related to the *Drosophila* suicide gene, reaper (P. Golstein, et al., *Cell* 81:185-186 (1995); K. White et al., *Science* 264:677-83 (1994)). This shared death domain suggests that both receptors interact with a related set of signal transducing molecules that, until recently, remained unidentified. Activation of Fas/APO-1 recruits the death domain-containing adapter molecule FADD/MORT1 (A. M. Chinaiyan et al., *Cell* 81: 505-12 (1995); M. P. Boldin et al., *J. Biol. Chem.* 270:7795-8 (1995); F. C. Kischkel et al., *EMBO* 14:5579-5588 (1995)), which in turn binds and presumably activates FLICE/MACH1, a member of the ICE/CED-3 family of pro-apoptotic proteases (M. Muzio et al., *Cell* 85:817-827 (1996); M. P. Boldin et al., *Cell* 85:803-815 (1996)). While the central role of Fas/APO-1 is to trigger cell death, TNFR-1 can signal an array of diverse biological activities-many of which stem from its ability to activate NF- κ B (L. A. Tartaglia et al., *Immunol Today* 13:151-3 (1992)). Accordingly, TNFR-1 recruits the multivalent adapter molecule TRADD, which like FADD also contains a death domain (H. Hsu et al., *Cell* 81:495-504 (1995); H. Hsu, et al., *Cell* 84:299-308 (1996)). Through its associations with a number of signaling molecules including FADD, TRAF2, and RIP, TRADD can signal both apoptosis and NF- κ B activation (H. Hsu et al., *Cell* 84:299-308 (1996); H. Hsu, et al., *Immunity* 4:387-396 (1996)).

[0011] Recently, a new apoptosis-inducing TNF ligand has been discovered. S. R. Wiley et al. (*Immunity* 3:673-682 (1995)) named the molecule—“TNF-related apoptosis-inducing ligand” or simply “TRAIL.” The molecule was also called “Apo-2 ligand” or “Apo-2L.” R. M. Pitt et al., *J. Biol. Chem.* 271:12687-12690 (1996). For convenience, the molecule will be referred to herein as TRAIL.

[0012] Unlike FAS ligand, whose transcripts appear to be largely restricted to stimulated T-cells, significant levels of TRAIL are detected in many human tissues (e.g., spleen, lung, prostate, thymus, ovary, small intestine, colon, peripheral blood lymphocytes, placenta, kidney), and is constitutively transcribed by some cell lines. It has been shown that TRAIL acts independently from the Fas ligand (Wiley et al., supra). It has also been shown that TRAIL activates apoptosis rapidly, within a time frame that is similar to death signaling by Fas/Apo-1L, but much faster than TNF-induced apoptosis. S. A. Marsters et al., *Current Biology* 6:750-752 (1996). The inability of TRAIL to bind TNFR-1, Fas, or the recently identified DR3, suggests that TRAIL may interact with a unique receptor(s).

[0013] The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize additional novel receptors that bind TRAIL.

SUMMARY OF THE INVENTION

[0014] The present invention provides for isolated nucleic acid molecules comprising, or alternatively consisting of,

nucleic acid sequences encoding the amino acid sequence shown in FIG. 1 (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA deposited as ATCC Deposit No. 97920 on Mar. 7, 1997.

[0015] The present invention also provides recombinant vectors, which include the isolated nucleic acid molecules of the invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of DR5 polypeptides or peptides by recombinant techniques.

[0016] The invention further provides an isolated DR5 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

[0017] The present invention also provides diagnostic assays such as quantitative and diagnostic assays for detecting levels of DR5 protein. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of DR5, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors. See, for example, the assays described in Example 20.

[0018] Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes. Cellular responses to TNF-family ligands include not only normal physiological responses, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis—programmed cell death—is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes. Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers, autoimmune disorders, viral infections, inflammation, graft versus host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, toxin-induced liver disease, septic shock, cachexia and anorexia.

[0019] Thus, the invention further provides a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell that expresses the DR5 polypeptide an effective amount of an agonist capable of increasing DR5 mediated signaling. Preferably, DR5 mediated signaling is increased to treat and/or prevent a disease wherein decreased apoptosis is exhibited.

[0020] In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR5 polypeptide an effective amount of an antagonist capable of decreasing DR5 mediated signaling. Preferably, DR5 mediated signaling is decreased to treat and/or prevent a disease wherein increased apoptosis is exhibited.

[0021] The present invention relates to the detection, diagnosis, prognosis and/or treatment of diseases and disorders of cell death, including but not limited to cancers, using compositions comprising polynucleotides encoding DR5, the polypeptides encoded by these polynucleotides and antibodies that immunospecifically bind these polypeptides.

The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders of cell death, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The invention further relates to methods and/or compositions for inhibiting or promoting the production and/or function of the polypeptides of the invention. The invention is based in part on the ability of DR5 to stimulate tumor cell apoptosis and thus prevent tumor progression, as demonstrated in Example 20, below.

[0022] In accordance with one embodiment of the present invention, there is provided an isolated antibody that binds specifically to a DR5 polypeptide, as well as biologically active fragments, analogs and derivatives thereof, together with fragments, analogs and derivatives thereof which may be useful in the diagnosis or treatment of diseases or disorders associated with decreased levels of cell death.

[0023] In one preferred embodiment of the present invention is presented an isolated antibody which is an agonist of DR5 activity and therefore may be useful in the treatment of diseases or disorders associated with decreased levels of cell death including, for example, prostate, pancreatic, hepatic, lung, breast, ovarian, colorectal and hematological cancers.

[0024] In accordance with another embodiment of the present invention, there is provided an isolated antibody that binds specifically to a DR5 polypeptide, as well as biologically active fragments, analogs and derivatives thereof, together with fragments, analogs and derivatives thereof which may be useful in the diagnosis or treatment of diseases or disorders associated with increased levels of cell death.

[0025] In another preferred embodiment of the present invention is presented an isolated antibody which is an antagonist of DR5 activity and therefore may be useful in the treatment of diseases or disorders associated with increased levels of cell death including, for example, myelodysplastic syndrome.

[0026] The present invention also provides pharmaceutical compositions comprising DR5 antibodies, as described above, which may be used for instance, to treat, prevent, prognose and/or diagnose diseases or disorders associated with abnormal levels of cell death and/or conditions associated with such diseases or disorders.

[0027] In preferred embodiments the present invention provides pharmaceutical compositions comprising DR5 agonistic antibodies, which may be used for instance to treat, prevent, prognose and/or diagnose diseases or disorders associated with increased or decreased levels of cell death as well as conditions associated with such diseases or disorders.

[0028] Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below. Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the DR5 polypeptide with a

candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By the invention, a cell expressing the DR5 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

BRIEF DESCRIPTION OF THE FIGURES

[0029] FIG. 1 shows the nucleotide (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of DR5. It is predicted that amino acids from about 1 to about 51 (underlined) constitute the signal peptide (amino acid residues from about -51 to about -1 in SEQ ID NO:2); amino acids from about 52 to about 184 constitute the extracellular domain (amino acid residues from about 1 to about 133 in SEQ ID NO:2); amino acids from about 84 to about 179 constitute the cysteine rich domain (amino acid residues from about 33 to 128 in SEQ ID NO:2); amino acids from about 185 to about 208 (underlined) constitute the transmembrane domain (amino acid residues from about 134 to about 157 in SEQ ID NO:2); and amino acids from about 209 to about 411 constitute the intracellular domain (amino acid residues from about 158 to about 360 in SEQ ID NO:2), of which amino acids from about 324 to about 391 (italicized) constitute the death domain (amino acid residues from about 273 to about 340 in SEQ ID NO:2).

[0030] FIG. 2 shows the regions of similarity between the amino acid sequences of DR5 (HLYBX88), human tumor necrosis factor receptor 1 (h TNFR-1) (SEQ ID NO:3), human Fas protein (SEQ ID NO:4), and the death domain containing receptor 3 (SEQ ID NO:5). The comparison was created with the Megalign program, which is contained in the DNA Star suite of programs, using the Clustal method. Residues that match the consensus are shaded.

[0031] FIG. 3 shows an analysis of the DR5 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, as predicted for the amino acid sequence depicted in FIG. 1 using the default parameters of the recited computer program. In the "Antigenic Index—Jameson-Wolf" graph, amino acid residues about 62 to about 110, about 119 to about 164, about 224 to about 271, and about 275 to about 370 as depicted in FIG. 1 correspond to the shown highly antigenic regions of the DR5 protein. These highly antigenic fragments in FIG. 1 correspond to the following fragments, respectively, in SEQ ID NO:2: amino acid residues from about 11 to about 59, from about 68 to about 113, from about 173 to about 220, and from about 224 to about 319.

[0032] FIG. 4 shows the nucleotide sequences (HAPBU13R and HSBBU76R) of two cDNA molecules, which are related to the nucleotide sequence shown in FIG. 1 (SEQ ID NO:1).

[0033] FIG. 5A is a bar graph showing that overexpression of DR5 induced apoptosis in MCF7 human breast carcinoma cells. FIG. 5B is a bar graph showing that

overexpression of DR5 induced apoptosis in human epithelial carcinoma (HeLa) cells. **FIG. 5C** is a bar graph showing that DR5-induced apoptosis was blocked by caspase inhibitors, CrmA and z-VAD-fmk, but dominant negative FADD was without effect. **FIG. 5D** is an immunoblot showing that, like DR4, DR5 did not interact with FADD and TRADD in vivo. **FIG. 5E** is a bar graph showing that a dominant negative version of a newly identified FLICE-like molecule, FLICE2 (Vincenz, C. et al., *J. Biol. Chem.* 272:6578 (1997)), efficiently blocked DR5-induced apoptosis, while dominant negative FLICE had only partial effect under conditions it blocked. It also shows that TNFR-1 blocked apoptosis effectively.

[0034] **FIG. 6A** is an immunoblot showing that DR5-Fc (as well as DR4 and TRID) specifically bound TRAIL, but not the related cytotoxic ligand TNF α . The bottom panel of **FIG. 6A** shows the input Fc-fusions present in the binding assays. **FIG. 6B** is a bar graph showing that DR5-Fc blocked the ability of TRAIL to induce apoptosis. The data (mean \pm SD) shown in **FIG. 6B** are the percentage of apoptotic nuclei among total nuclei counted (n=4). **FIG. 6C** is a bar graph showing that DR5-Fc had no effect on apoptosis TNF α -induced cell death under conditions where TNFR-1-Fc completely abolished TNF α killing.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0035] The present invention provides isolated nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide encoding a DR5 polypeptide having the amino acid sequence shown in **FIG. 1** (SEQ ID NO:2), or a fragment of this polypeptide. The DR5 polypeptide of the present invention shares sequence homology with other known death domain containing receptors of the TNFR family including human TNFR-1, DR3 and Fas (**FIG. 2**). The nucleotide sequence shown in **FIG. 1** (SEQ ID NO:1) was obtained by sequencing cDNA clones such as HLYBX88, which was deposited on Mar. 7, 1997 at the American Type Culture, 10801 University Boulevard, Manassas, Va., 20110-2209, and given Accession Number 97920. The deposited cDNA is contained in the pSport 1 plasmid (Life Technologies, Gaithersburg, Md.).

Nucleic Acid Molecules

[0036] Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide

sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

[0037] Using the information provided herein, such as the nucleic acid sequence set out in SEQ ID NO:1, a nucleic acid molecule of the present invention encoding a DR5 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule of the invention has been identified in cDNA libraries of the following tissues: primary dendritic cells, endothelial tissue, spleen, chronic lymphocytic leukemia, and human thymus stromal cells.

[0038] The determined nucleotide sequence of the DR5 cDNA of SEQ ID NO:1 contains an open reading frame encoding a protein of about 411 amino acid residues whose initiation codon is at position 130-132 of the nucleotide sequence shown in **FIG. 1** (SEQ ID NO. 1), with a leader sequence of about 51 amino acid residues. Of known members of the TNF receptor family, the DR5 polypeptide of the invention shares the greatest degree of homology with human TNFR-1, FAS and DR3 polypeptides shown in **FIG. 2**, including significant sequence homology over multiple cysteine-rich domains. The homology DR5 shows to other death domain-containing receptors strongly indicates that DR5 is also a death domain containing receptor with the ability to induce apoptosis. DR5 has also now been shown to bind TRAIL.

[0039] As indicated, the present invention also provides the mature form(s) of the DR5 protein of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

[0040] Therefore, the present invention provides a nucleotide sequence encoding the mature DR5 polypeptide having the amino acid sequence encoded by the cDNA contained in the plasmid identified as ATCC Deposit No. 97920, and as shown in **FIG. 1** (SEQ ID NO:2). By the mature DR5 protein having the amino acid sequence encoded by the cDNA contained in the plasmid identified as ATCC Deposit No. 97920, is meant the mature form(s) of the DR5 protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human cDNA contained in the deposited plasmid. As indicated below, the mature DR5 having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 97920, may or may not differ from the predicted "mature" DR5 protein shown in SEQ ID NO:2 (amino acids from about 1 to about 360) depending on the accuracy of the predicted cleavage site based on computer analysis.

[0041] Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch (*Virus Res.* 3:271-286 (1985)) and von Heinje (*Nucleic Acids Res.* 14:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra*. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

[0042] In the present case the predicted amino acid sequence of the complete DR5 polypeptide of the present invention was analyzed by a computer program ("PSORT"). See, K. Nakai and M. Kanehisa, *Genomics* 14:897-911 (1992). PSORT is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids 51 and 52 in FIG. 1 (-1 and 1 in SEQ ID NO:2). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1, -3) rule of von Heinje. von Heinje, *supra*. Thus, the leader sequence for the DR5 protein is predicted to consist of amino acid residues from about 1 to about 51, underlined in FIG. 1 (corresponding to amino acid residues about -51 to about 1 in SEQ ID NO:2), while the predicted mature DR5 protein consists of residues from about 52 to about 411 in FIG. 1 (corresponding to amino acid residues about 1 to about 360 in SEQ ID NO:2).

[0043] As one of ordinary skill would appreciate, due to the possibility of sequencing errors, as well as the variability of cleavage sites for leaders in different known proteins, the predicted DR5 receptor polypeptide encoded by the deposited cDNA comprises about 411 amino acids, but may be anywhere in the range of 401-421 amino acids; and the predicted leader sequence of this protein is about 51 amino acids, but may be anywhere in the range of about 41 to about 61 amino acids. It will further be appreciated that, the domains described herein have been predicted by computer analysis, and accordingly, that depending on the analytical criteria used for identifying various functional domains, the exact "address" of, for example, the extracellular domain, intracellular domain, death domain, cysteine-rich motifs, and transmembrane domain of DR5 may differ slightly. For example, the exact location of the DR5 extracellular domain in FIG. 1 (SEQ ID NO:2) may vary slightly (e.g., the address may "shift" by about 1 to about 20 residues, more likely about 1 to about 5 residues) depending on the criteria used to define the domain. In any event, as discussed further below, the invention further provides polypeptides having various residues deleted from the N-terminus and/or C-terminus of the complete DR5, including polypeptides lacking one or more amino acids from the N-termini of the extracellular domain described herein, which constitute soluble forms of the extracellular domain of the DR5 polypeptides.

[0044] As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known

as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

[0045] By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution.

[0046] However, a nucleic acid molecule contained in a clone that is a member of a mixed clone library (e.g., a genomic or cDNA library) and that has not been isolated from other clones of the library (e.g., in the form of a homogeneous solution containing the clone without other members of the library) or a chromosome isolated or removed from a cell or a cell lysate (e.g., a "chromosome spread", as in a karyotype), is not "isolated" for the purposes of this invention. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

[0047] Isolated nucleic acid molecules of the present invention include DR5 DNA molecules comprising, or alternatively consisting of, an open reading frame (ORF) shown in SEQ ID NO:1; DNA molecules comprising, or alternatively consisting of, the coding sequence for the mature DR5 protein; and DNA molecules which comprise, or alternatively consist of, a sequence substantially different from those described above, but which, due to the degeneracy of the genetic code, still encode the DR5 protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

[0048] In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 which have been determined from the following related cDNAs: HAPBU13R (SEQ ID NO:6) and HSBBU76R (SEQ ID NO:7). The nucleotide sequences of HAPBU13R and HSBBU76R are shown in FIG. 4.

[0049] The nucleotide sequence of an additional related polynucleotide, which has been assigned GenBank Accession number Z66083, is shown in SEQ ID NO:14.

[0050] In another aspect, the invention provides isolated nucleic acid molecules encoding the DR5 polypeptide having an amino acid sequence encoded by the cDNA contained in the plasmid deposited as ATCC Deposit No. 97920 on Mar. 7, 1997. In a further embodiment, nucleic acid molecules are provided, that encode the mature DR5 polypeptide or the full length DR5 polypeptide lacking the N-terminal methionine. The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1 or the nucleotide sequence of the DR5 cDNA contained in the above-described deposited plasmid, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, have uses which include, but are not limited to, as probes for gene mapping by *in situ* hybridization with chromosomes, and for detecting expression of the DR5 gene in human tissue, for instance, by Northern blot analysis.

[0051] The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By fragments of an isolated DNA molecule having the nucleotide sequence shown in SEQ ID NO:1 or having the nucleotide sequence of the deposited cDNA (the cDNA contained in the plasmid deposited as ATCC Deposit No. 97920) are intended DNA fragments at least 20 nt, and more preferably at least 30 nt in length, and even more preferably, at least about 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, or 1200 nucleotides in length, which are useful as DNA probes as discussed above. Of course, DNA fragments corresponding to most, if not all, of the nucleotide sequence shown in SEQ ID NO:1 are also useful as DNA probes. By a fragment at least about 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited DNA or the nucleotide sequence as shown in SEQ ID NO:1. In this context "about" includes the particularly recited size, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

[0052] Representative examples of DR5 polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively consist of, a sequence from about nucleotide 1-130, 130-180, 181-231, 232-282, 283-333, 334-384, 385-435, 436-486, 487-537, 538-588, 589-639, 640-681, 682-732, 733-753, 754-804, 805-855, 856-906, 907-957, 958-1008, 1009-1059, 1060-1098, 1099-1149, 1150-1200, 1201-1251, 1252-1302, 1303-1353, 1354-1362, and 1363 to the end of SEQ ID NO:1, or the complementary DNA strand thereto, or the cDNA contained in the deposited plasmid. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0053] The present invention is further directed to polynucleotides comprising, or alternatively consisting of, isolated nucleic acid molecules, which encode domains of DR5. In one aspect, the invention provides polynucleotides comprising, or alternatively consisting of, nucleic acid molecules, which encode beta-sheet regions of DR5 protein set out in Table I. Representative examples of such polynucleotides include nucleic acid molecules which encode a polypeptide comprising, or alternatively consisting of, one, two, three, four, five, or more amino acid sequences selected from the group consisting of: amino acid residues from about -16 to about -2, amino acid residues from about 2 to about 9, amino acid residues from about 60 to about 67, amino acid residues from about 135 to about 151, amino acid residues from about 193 to about 199, and amino acid residues from about 302 to about 310 in SEQ ID NO:2. In this context "about" includes the particularly recited value and values larger or smaller by several (5, 4, 3, 2, or 1) amino acid residues. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0054] In specific embodiments, the polynucleotide fragments of the invention encode a polypeptide, which demonstrates a DR5 functional activity. By a polypeptide demonstrating a DR5 "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a complete (full-length) or mature DR5 polypeptide, as well as secreted forms of

DR5. Such functional activities include, but are not limited to, biological activity (e.g., ability to induce apoptosis in cells expressing the polypeptide (see e.g., Example 5)), antigenicity (ability to bind (or compete with a DR5 polypeptide for binding) to an anti-DR5 antibody), immunogenicity (ability to generate antibody which binds to a DR5 polypeptide), ability to form multimers, and ability to bind to a receptor or ligand for a DR5 polypeptide (e.g., TRAIL; Wiley et al, *Immunity* 3, 673-682 (1995)).

[0055] The functional activity of DR5 polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

[0056] For example, in one embodiment where one is assaying for the ability to bind or compete with full-length (complete) DR5 polypeptide for binding to anti-DR5 antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[0057] In another embodiment, where a DR5 ligand is identified (e.g. TRAIL), or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, et al., *Microbiol. Rev.* 59:94-123 (1995). In another embodiment, physiological correlates of DR5 binding to its substrates (signal transduction) can be assayed.

[0058] In addition, assays described herein (see Examples 5 and 6), and otherwise known in the art may routinely be applied to measure the ability of DR5 polypeptides and fragments, variants derivatives and analogs thereof to elicit DR5 related biological activity (e.g., ability to induce apoptosis in cells expressing the polypeptide (see e.g., Example 5), and the ability to bind a ligand, e.g., TRAIL (see, e.g., Example 6) *in vitro* or *in vivo*). For example, biological activity can routinely be measured using the cell death assays performed essentially as previously described (Chinnaiyan et al., *Cell* 81:505-512 (1995); Boldin et al., *J. Biol. Chem.* 270:7795-8(1995); Kischkel et al., *EMBO* 14:5579-5588 (1995); Chinnaiyan et al., *J. Biol. Chem.* 271:4961-4965 (1996)) and as set forth in Example 5 below. In one embodiment involving MCF7 cells, plasmids encoding full-length DR5 or a candidate death domain containing receptor are co-transfected with the pLantern reporter construct

encoding green fluorescent protein. Nuclei of cells transfected with DR5 will exhibit apoptotic morphology as assessed by DAPI staining.

[0059] Other methods will be known to the skilled artisan and are within the scope of the invention.

[0060] Preferred nucleic acid fragments of the present invention include, but are not limited to, a nucleic acid molecule encoding a polypeptide comprising, or alternatively consisting of, one, two, three, four, five, or more amino acid sequences selected from the group consisting of: a polypeptide comprising, or alternatively consisting of, the DR5 extracellular domain (amino acid residues from about 52 to about 184 in **FIG. 1** (amino acid residues from about 1 to about 133 in SEQ ID NO:2)); a polypeptide comprising, or alternatively consisting of, the DR5 transmembrane domain (amino acid residues from about 185 to about 208 in **FIG. 1** (amino acid residues from about 134 to about 157 in SEQ ID NO:2)); a polypeptide comprising, or alternatively consisting of, the cysteine rich domain of DR5 (amino acid residues from about 84 to about 179 in **FIG. 1** (from about 33 to about 128 in SEQ ID NO:2)); a polypeptide comprising, or alternatively consisting of, the DR5 intracellular domain (amino acid residues from about 209 to about 411 in **FIG. 1** (amino acid residues from about 158 to about 360 in SEQ ID NO:2)); a polypeptide comprising, or alternatively consisting of, a fragment of the predicted mature DR5 polypeptide, wherein the fragment has a DR5 functional activity (e.g., antigenic activity or biological activity); a polypeptide comprising, or alternatively consisting of, the DR5 receptor extracellular and intracellular domains with all or part of the transmembrane domain deleted; a polypeptide comprising, or alternatively consisting of, the DR5 death domain (amino acid residues from about 324 to about 391 in **FIG. 1** (from about 273 to about 340 in SEQ ID NO:2)); and a polypeptide comprising, or alternatively consisting of, one, two, three, four or more, epitope bearing portions of the DR5 receptor protein. In additional embodiments, the polynucleotide fragments of the invention encode a polypeptide comprising, or alternatively consisting of, any combination of 1, 2, 3, 4, 5, 6, 7, or all 8 of the above members. Since the location of these domains have been predicted by computer graphics, one of ordinary skill would appreciate that the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to 15 residues) depending on the criteria used to define each domain. Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention.

[0061] It is believed one or both of the extracellular cysteine rich motifs of DR5 disclosed in **FIG. 1** is important for interactions between DR5 and its ligands (e.g., TRAIL). Accordingly, specific embodiments of the invention are directed to polynucleotides encoding a polypeptide comprising, or alternatively consisting of, one or both amino acid sequences selected from the group consisting of: amino acid residues 84 to 131, and/or 132 to 179 of the DR5 sequence shown in **FIG. 1** (amino acid residues 33 to 80, and/or 81 to 128 in SEQ ID NO:2). In a specific embodiment the polynucleotides encoding DR5 polypeptides of the invention comprise, or alternatively consist of, both of the extracellular cysteine-rich motifs disclosed in **FIG. 1**.

[0062] In certain embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucle-

otide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding the cysteine-rich domain described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides are also encompassed by the invention. Methods to measure the percent identity of a polynucleotide sequence to a reference polynucleotide sequence are described infra.

[0063] In another embodiment, the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of a polynucleotide, which hybridizes under stringent hybridization conditions to nucleic acids complementary to the cysteine-rich domain encoding polynucleotides described above. The meaning of the phrase "stringent conditions" as used herein is described infra. Polypeptides encoded by such polynucleotides are also contemplated by the invention.

[0064] Preferred nucleic acid fragments of the invention encode a full-length DR5 polypeptide lacking the nucleotides encoding the amino-terminal methionine (nucleotides 130-132 in SEQ ID NO:1) as it is known that the methionine is cleaved naturally and such sequences maybe useful in genetically engineering DR5 expression vectors. Polypeptides encoded by such polynucleotides are also contemplated by the invention.

[0065] In additional embodiments, the polynucleotides of the invention encode functional attributes of DR5. Preferred embodiments of the invention in this regard include fragments that comprise, or alternatively consist of, one, two, three, four, or more of the following functional domains: alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha-amphipathic regions, beta-amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of DR5.

[0066] The data representing the structural or functional attributes of DR5 set forth in **FIG. 3** and/or Table I, as described above, were generated using the various identified modules and algorithms of the DNA^{*}STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Table I can be used to determine regions of DR5, which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or XIV by choosing values that represent regions of the polypeptide, which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

[0067] Certain preferred regions in these regards are set out in **FIG. 3**, but may, as shown in Table I, be represented or identified by using tabular representations of the data presented in **FIG. 3**. The DNA^{*}STAR computer algorithm used to generate **FIG. 3** (set on the original default parameters) was used to present the data in **FIG. 3** in a tabular format (See Table I). The tabular format of the data in **FIG. 3** may be used to easily determine specific boundaries of a preferred region.

[0068] The above-mentioned preferred regions set out in **FIG. 3** and in Table I include, but are not limited to, regions

of the aforementioned types identified by analysis of the amino acid sequence set out in SEQ ID NO:2. As set out in FIG. 3 and in Table I, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions (columns I, III, V, and VII in Table I), Chou-Fasman alpha-regions, beta-regions, and turn-regions (columns II, IV, and VI in Table I), Kyte-Doolittle hydro-

philic regions (column VIII in Table I), Hopp-Woods hydrophobic regions (column IX in Table I), Eisenberg alpha- and beta-amphipathic regions (columns X and XI in Table I), Karplus-Schulz flexible regions (column XII in Table I), Jameson-Wolf regions of high antigenic index (column XII in Table I), and Emini surface-forming regions (column XIV in Table I).

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	A	1.11	-0.70	.	*	.	1.29	2.18
Glu	2	A	1.50	-0.70	.	*	.	1.63	1.69
Gln	3	A	.	.	.	T	.	1.89	-0.73	.	*	.	2.17	2.28	
Arg	4	T	T	.	1.69	-0.76	.	*	.	2.91	3.71
Gly	5	T	T	.	1.87	-0.87	.	*	F	3.40	2.17
Gln	6	T	T	.	1.88	-0.44	.	*	F	2.76	1.93
Asn	7	C	1.29	-0.34	.	*	F	1.87	1.00	
Ala	8	C	0.99	0.16	.	.	F	1.08	1.02	
Pro	9	C	0.53	0.11	.	*	.	0.44	0.79	
Ala	10	A	0.29	0.14	.	*	.	-0.10	0.48	
Ala	11	A	T	.	0.40	0.24	.	.	.	0.10	0.48
Ser	12	A	T	.	0.44	-0.26	.	*	F	0.85	0.61
Gly	13	A	T	.	1.14	-0.69	.	*	F	1.30	1.22
Ala	14	A	T	.	1.32	-1.19	.	*	F	1.30	2.36
Arg	15	A	.	.	.	T	.	.	1.57	-1.19	.	*	F	1.50	2.39
Lys	16	T	.	.	1.94	-1.14	.	.	F	1.50	2.39
Arg	17	T	.	.	1.90	-1.14	.	*	F	1.80	3.66
His	18	C	2.03	-1.21	*	*	F	1.90	1.85	
Gly	19	T	C	2.73	-0.79	*	*	F	2.40	1.43
Pro	20	T	C	2.62	-0.79	*	*	F	2.70	1.43
Gly	21	T	C	1.99	-0.79	*	.	F	3.00	1.82
Pro	22	T	C	1.99	-0.79	.	*	F	2.70	1.86
Arg	23	.	A	C	1.68	-1.21	*	.	F	2.30	2.35
Glu	24	.	A	B	1.43	-1.21	*	.	F	2.10	2.35
Ala	25	.	A	.	.	T	.	.	1.76	-1.14	*	.	F	2.50	1.54
Arg	26	.	A	.	.	T	.	.	1.89	-1.57	*	.	F	2.50	1.54
Gly	27	T	.	.	1.76	-1.14	*	.	F	3.00	1.37
Ala	28	T	.	C	1.43	-0.71	*	*	F	2.70	1.35
Arg	29	T	C	1.54	-0.79	*	*	F	2.66	1.06	
Pro	30	T	C	1.28	-0.79	*	*	F	2.62	2.10	
Gly	31	T	C	0.96	-0.57	*	*	F	2.58	1.54	
Pro	32	T	C	1.34	-0.64	*	*	F	2.54	1.22	
Arg	33	C	1.62	-0.64	*	*	F	2.60	1.58	
Val	34	C	0.70	-0.59	*	*	F	2.34	2.30	
Pro	35	.	.	B	.	.	.	0.06	-0.33	*	*	F	1.58	1.23	
Lys	36	.	.	B	B	.	.	.	-0.41	-0.11	*	.	F	0.97	0.46
Thr	37	.	.	B	B	.	.	.	-1.06	0.57	*	*	F	-0.19	0.52
Leu	38	.	.	B	B	.	.	.	-2.02	0.57	*	*	.	-0.60	0.25
Val	39	.	.	B	B	.	.	.	-1.76	0.79	.	.	.	-0.60	0.09
Leu	40	A	.	B	-2.13	1.29	.	.	.	-0.60	0.06
Val	41	A	.	B	-3.03	1.30	.	.	.	-0.60	0.08
Val	42	A	.	B	-3.53	1.26	.	.	.	-0.60	0.08
Ala	43	A	.	B	-3.53	1.30	.	.	.	-0.60	0.08
Ala	44	A	.	B	-3.49	1.30	.	.	.	-0.60	0.09
Val	45	A	.	B	-3.53	1.34	.	.	.	-0.60	0.10
Leu	46	A	.	B	-2.98	1.34	.	.	.	-0.60	0.07
Leu	47	A	.	B	-2.71	1.23	.	.	.	-0.60	0.09
Leu	48	A	.	B	-2.12	1.23	.	.	.	-0.60	0.13
Val	49	A	.	B	-1.83	0.59	.	.	.	-0.60	0.27
Ser	50	A	.	B	-1.57	0.29	.	*	.	-0.30	0.44
Ala	51	A	A	-1.57	0.10	.	.	.	-0.30	0.54
Glu	52	A	A	-1.64	0.10	.	.	.	-0.30	0.60
Ser	53	A	A	.	B	.	.	.	-1.14	0.14	.	.	.	-0.30	0.31
Ala	54	A	A	.	B	.	.	.	-0.29	0.24	.	.	.	-0.30	0.45
Leu	55	A	A	.	B	.	.	.	0.01	0.14	.	.	.	-0.30	0.45
Ile	56	A	A	.	B	.	.	.	0.60	0.54	.	.	.	-0.60	0.58
Thr	57	A	A	.	B	.	.	.	-0.21	0.16	.	.	F	-0.15	0.96
Gln	58	A	A	.	B	.	.	.	-0.50	0.34	.	.	F	-0.15	0.96
Gln	59	A	A	.	B	.	.	.	-0.12	0.16	.	.	F	0.00	1.38
Asp	60	.	A	.	B	T	.	.	0.69	-0.10	.	.	F	1.00	1.48
Leu	61	.	A	.	.	.	C	1.58	-0.19	.	*	F	0.80	1.48	
Ala	62	.	A	.	.	.	C	2.00	-0.19	.	*	F	0.80	1.48	
Pro	63	.	A	.	.	.	C	1.41	-0.59	.	*	F	1.10	1.73	
Gln	64	.	A	.	.	T	.	.	0.82	-0.09	.	*	F	1.00	2.13

-continued

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Gln	65	A	A	0.61	-0.27	.	*	F	0.60	2.13
Arg	66	A	A	1.42	-0.34	.	*	F	0.60	2.13
Ala	67	A	A	2.01	-0.37	.	*	F	0.94	2.13
Ala	68	A	A	2.27	-0.37	*	*	F	1.28	2.13
Pro	69	A	A	2.38	-0.77	*	*	F	1.92	2.17
Gln	70	.	A	.	.	T	.	.	2.08	-0.77	*	.	F	2.66	4.21
Gln	71	T	T	.	1.67	-0.89	*	*	F	3.40	5.58
Lys	72	T	T	.	2.04	-1.00	.	.	F	3.06	4.84
Arg	73	T	T	.	2.33	-1.00	.	.	F	2.97	4.32
Ser	74	T	C	2.54	-1.01	.	.	F	2.68	3.34
Ser	75	T	C	2.20	-1.41	.	.	F	2.59	2.89
Pro	76	T	T	.	1.39	-0.99	.	.	F	2.70	1.46
Ser	77	T	T	.	0.68	-0.30	.	.	F	2.50	0.90
Glu	78	T	T	.	0.36	-0.11	.	*	F	2.25	0.36
Gly	79	T	.	.	0.44	-0.07	.	.	F	1.80	0.36
Leu	80	T	.	.	0.40	-0.07	.	.	F	1.55	0.42
Cys	81	C	0.58	-0.03	.	.	.	0.95	0.24	
Pro	82	T	C	0.84	0.47	*	.	F	0.15	0.33	
Pro	83	T	T	.	-0.04	0.54	*	.	F	0.35	0.54
Gly	84	T	T	.	0.00	0.54	*	.	.	0.20	0.70
His	85	T	C	0.81	0.36	*	.	.	0.30	0.61	
His	86	C	1.48	-0.07	*	.	.	0.70	0.68		
Ile	87	C	1.34	-0.50	*	*	.	1.19	1.15		
Ser	88	C	1.67	-0.50	*	*	F	1.53	0.84		
Glu	89	T	.	.	2.01	-1.00	*	*	F	2.52	1.21
Asp	90	T	.	.	1.38	-1.50	*	*	F	2.86	2.88
Gly	91	T	T	.	0.52	-1.61	*	*	F	3.40	1.15
Arg	92	T	T	.	1.11	-1.31	*	*	F	2.91	0.47
Asp	93	T	T	.	0.74	-0.93	.	*	F	2.57	0.37
Cys	94	T	T	.	0.79	-0.36	.	*	.	1.78	0.20
Ile	95	T	.	.	0.54	-0.79	.	*	.	1.54	0.21
Ser	96	T	.	.	0.54	-0.03	.	*	.	1.18	0.19
Cys	97	T	T	.	0.43	0.40	.	*	.	0.76	0.36
Lys	98	T	T	.	0.43	0.23	.	.	.	1.34	0.88
Tyr	99	T	T	.	0.86	-0.46	.	*	F	2.52	1.10
Gly	100	T	T	.	1.44	-0.09	.	*	F	2.80	3.22
Gln	101	T	T	.	1.43	-0.27	*	.	F	2.52	2.16
Asp	102	T	T	.	2.07	0.21	*	*	F	1.64	1.99
Tyr	103	T	T	.	1.73	-0.04	*	*	F	1.96	2.73
Ser	104	T	T	.	1.98	0.44	*	.	F	0.78	1.66
Thr	105	T	.	.	2.32	0.44	*	.	F	0.30	1.60
His	106	T	.	.	1.51	0.44	*	.	.	0.15	1.70
Trp	107	T	T	.	0.70	0.37	*	.	.	0.65	1.05
Asn	108	T	T	.	0.24	0.67	.	.	.	0.20	0.60
Asp	109	T	T	.	-0.12	0.97	*	.	.	0.20	0.38
Leu	110	A	.	.	.	T	.	.	-0.62	1.04	*	*	.	-0.20	0.19
Leu	111	.	.	.	B	T	.	.	-0.48	0.81	*	*	.	-0.20	0.10
Phe	112	.	.	.	B	T	.	.	-0.86	0.41	*	*	.	-0.20	0.12
Cys	113	.	.	.	B	T	.	.	-1.17	0.99	*	*	.	-0.20	0.08
Leu	114	.	.	.	B	T	.	.	-1.06	0.79	.	*	.	-0.20	0.13
Arg	115	.	.	.	B	T	.	.	-0.91	0.10	.	*	.	0.10	0.30
Cys	116	.	.	.	B	T	.	.	-0.10	-0.11	.	.	.	0.70	0.30
Thr	117	.	.	.	B	T	.	.	0.30	-0.69	.	*	.	1.00	0.61
Arg	118	.	.	.	B	T	.	.	0.62	-0.99	.	.	F	1.49	0.42
Cys	119	.	.	.	T	T	.	.	1.43	-0.56	*	.	F	2.23	0.77
Asp	120	.	.	.	T	T	.	.	0.47	-1.13	*	.	F	2.57	0.92
Ser	121	.	.	.	T	T	.	.	1.13	-0.97	.	*	F	2.91	0.35
Gly	122	.	.	.	T	T	.	.	0.63	-0.97	.	*	F	3.40	1.13
Glu	123	.	A	.	.	T	.	.	0.22	-0.86	.	*	F	2.51	0.56
Val	124	A	A	0.68	-0.47	.	*	F	1.47	0.56
Glu	125	.	A	.	.	T	.	.	0.01	-0.43	.	*	.	1.38	0.87
Leu	126	.	A	.	.	T	.	.	0.00	-0.29	.	*	.	1.04	0.27
Ser	127	T	C	0.03	0.20	.	*	F	0.45	0.52	
Pro	128	T	T	.	-0.28	0.04	.	*	F	0.93	0.44
Cys	129	T	T	.	0.69	0.53	.	*	F	0.91	0.77
Thr	130	T	T	.	0.69	-0.16	.	*	F	2.24	1.12
Thr	131	T	.	.	1.19	-0.14	.	*	F	2.32	1.16
Thr	132	T	T	.	0.63	-0.09	.	*	F	2.80	3.13
Arg	133	T	T	.	0.18	-0.01	.	.	F	2.52	1.61
Asn	134	T	T	.	0.84	0.07	.	.	F	1.49	0.60
Thr	135	T	T	.	0.49	-0.01	.	.	F	1.81	0.72
Val	136	T	C	0.80	0.07	*	.	.	0.58	0.20	
Cys	137	.	A	.	.	T	.	.	1.11	0.07	*	.	.	0.10	0.21
Gln	138	.	A	B	0.66	-0.33	*	.	.	0.30	0.25

-continued

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Cys	139	.	A	.	.	T	.	.	0.34	-0.39	.	.	0.70	0.34	
Glu	140	A	A	-0.04	-0.54	*	*	F	0.75	0.91
Glu	141	A	A	0.92	-0.33	*	*	F	0.45	0.46
Gly	142	.	A	.	.	T	.	.	1.59	-0.73	.	*	F	1.30	1.67
Thr	143	A	A	1.59	-1.30	.	*	F	0.90	1.67
Phe	144	A	A	2.26	-1.30	.	*	F	0.90	1.67
Arg	145	A	A	1.96	-1.30	.	*	F	0.90	2.81
Glu	146	A	A	1.74	-1.34	.	*	F	0.90	2.61
Glu	147	A	A	2.09	-1.40	.	*	F	0.90	4.66
Asp	148	A	A	1.80	-2.19	.	*	F	0.90	4.12
Ser	149	A	.	.	.	T	.	.	1.83	-1.57	.	*	F	1.30	2.35
Pro	150	A	.	.	.	T	.	.	1.83	-1.00	.	.	F	1.15	0.73
Glu	151	A	.	.	.	T	.	.	1.88	-1.00	*	.	F	1.15	0.85
Met	152	A	.	.	.	T	.	.	1.21	-1.00	*	*	.	1.49	1.28
Cys	153	A	.	.	.	T	.	.	1.32	-0.81	*	*	.	1.68	0.44
Arg	154	A	.	.	.	T	.	.	1.31	-1.24	*	.	.	2.02	0.50
Lys	155	.	.	.	T	T	.	.	1.18	-0.76	*	*	F	2.91	0.73
Cys	156	.	.	.	T	T	.	.	0.51	-0.94	*	.	F	3.40	1.35
Arg	157	.	.	.	T	.	.	.	0.90	-0.94	*	.	F	2.71	0.37
Thr	158	.	.	.	T	.	.	.	1.68	-0.51	*	.	F	2.37	0.28
Gly	159	.	.	.	T	.	.	.	1.22	-0.51	*	.	F	2.43	1.04
Cys	160	.	.	.	T	C	.	.	0.58	-0.66	.	*	F	2.19	0.53
Pro	161	.	.	.	T	T	.	.	0.39	-0.04	.	*	F	2.00	0.36
Arg	162	.	.	.	T	T	.	.	0.32	0.11	.	*	F	1.65	0.27
Gly	163	.	.	.	T	T	.	.	-0.22	-0.31	*	*	.	2.50	1.01
Met	164	.	.	B	B	.	.	.	-0.22	-0.24	*	*	.	1.30	0.48
Val	165	.	.	B	B	.	.	.	0.44	-0.24	*	*	.	1.30	0.24
Lys	166	.	.	B	B	.	.	.	-0.01	-0.24	*	*	.	1.30	0.41
Val	167	.	.	B	.	T	.	.	-0.43	-0.10	*	*	F	1.85	0.22
Gly	168	.	.	.	T	T	.	.	-0.30	-0.23	.	.	F	2.25	0.44
Asp	169	.	.	.	T	T	.	.	0.01	-0.44	.	.	F	2.50	0.34
Cys	170	.	.	.	T	T	.	.	0.57	0.47	.	*	F	1.35	0.48
Thr	171	T	C	.	0.52	0.21	.	*	F	1.20	0.65
Pro	172	.	.	.	T	T	.	.	0.49	-0.21	.	*	F	1.75	0.65
Trp	173	.	.	.	T	T	.	.	0.83	0.47	.	*	F	0.60	0.84
Ser	174	A	.	.	.	T	.	.	0.17	-0.10	.	*	F	1.00	1.01
Asp	175	A	A	-0.02	-0.01	.	.	F	0.45	0.35
Ile	176	A	A	0.26	0.20	*	*	.	-0.30	0.25
Glu	177	A	A	0.51	-0.21	*	.	.	0.30	0.25
Cys	178	A	A	0.80	-0.60	*	.	.	0.60	0.30
Val	179	A	A	0.80	-0.60	*	*	.	0.60	0.74
His	180	A	A	0.46	-0.90	.	*	.	0.60	0.58
Lys	181	A	A	0.46	-0.47	*	.	F	0.60	1.06
Glu	182	A	.	.	.	T	.	.	-0.43	-0.36	*	.	F	1.00	1.00
Ser	183	A	.	.	.	T	.	.	-0.66	-0.31	.	.	F	0.85	0.52
Gly	184	A	.	.	T	T	.	.	-0.14	-0.13	.	.	F	1.25	0.18
Ile	185	A	.	.	T	.	.	.	-0.97	0.30	.	.	.	0.10	0.10
Ile	186	.	B	B	-1.32	0.94	.	*	.	-0.60	0.06
Ile	187	.	B	B	-2.18	1.04	.	.	.	-0.60	0.08
Gly	188	.	B	B	-2.47	1.26	.	*	.	-0.60	0.09
Val	189	.	B	B	-2.71	1.07	.	.	.	-0.60	0.13
Thr	190	A	.	B	-2.68	0.89	.	*	.	-0.60	0.18
Val	191	A	.	B	-2.64	0.84	.	.	.	-0.60	0.14
Ala	192	A	.	B	-2.57	1.06	.	*	.	-0.60	0.14
Ala	193	A	.	B	-3.11	1.10	.	.	.	-0.60	0.08
Val	194	A	.	B	-3.11	1.30	.	.	.	-0.60	0.07
Val	195	A	.	B	-3.39	1.30	.	.	.	-0.60	0.05
Leu	196	A	.	B	-3.39	1.30	.	.	.	-0.60	0.05
Ile	197	A	.	B	-3.50	1.44	.	.	.	-0.60	0.05
Val	198	A	.	B	-3.77	1.59	.	.	.	-0.60	0.06
Ala	199	A	.	B	-3.58	1.59	.	.	.	-0.60	0.06
Val	200	A	.	B	-2.68	1.47	.	.	.	-0.60	0.04
Phe	201	A	.	B	-2.17	0.79	.	.	.	-0.60	0.12
Val	202	A	.	B	-2.09	0.53	.	.	.	-0.60	0.16
Cys	203	A	.	.	T	.	.	.	-2.04	0.71	.	.	.	-0.20	0.17
Lys	204	A	.	.	T	.	.	.	-1.74	0.76	.	.	.	-0.20	0.17
Ser	205	A	.	.	T	.	.	.	-0.84	0.89	.	.	.	-0.20	0.24
Leu	206	A	.	.	T	.	.	.	-0.10	0.24	.	.	.	0.10	0.88
Leu	207	A	A	-0.10	-0.33	.	.	.	0.30	0.88
Trp	208	A	A	-0.24	0.31	.	.	.	-0.30	0.49
Lys	209	A	A	-0.50	0.61	.	.	.	-0.60	0.49
Lys	210	A	A	-0.44	0.36	*	.	.	-0.30	0.91
Val	211	A	A	-0.44	0.43	*	*	.	-0.45	1.36
Leu	212	.	A	B	0.41	0.20	*	*	.	-0.30	0.56

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Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Pro	213	.	A	B	0.36	0.20	*	.	.	-0.30	0.56
Tyr	214	.	.	.	B	T	.	.	-0.58	0.63	*	.	.	-0.20	0.75
Leu	215	.	.	.	B	T	.	.	-1.29	0.67	*	*	.	-0.20	0.64
Lys	216	.	.	.	B	T	.	.	-0.73	0.56	*	.	.	-0.20	0.22
Gly	217	.	.	B	B	.	.	.	-0.27	0.51	*	.	.	-0.60	0.19
Ile	218	.	.	B	B	.	.	.	-0.40	0.19	*	.	.	-0.30	0.23
Cys	219	.	.	B	.	.	T	.	-0.50	-0.07	*	.	.	0.70	0.11
Ser	220	T	T	.	-0.03	0.36	.	*	F	0.65	0.11
Gly	221	T	T	.	-0.08	0.36	.	.	F	0.65	0.16
Gly	222	T	T	.	0.06	-0.33	.	.	F	1.25	0.49
Gly	223	C	0.94	-0.47	.	.	F	0.85	0.57	
Gly	224	C	1.72	-0.86	*	.	F	1.15	0.99	
Asp	225	T	C	1.17	-1.29	.	*	F	1.50	1.97	
Pro	226	T	C	1.51	-1.07	*	.	F	1.84	1.47	
Glu	227	.	.	B	.	T	.	1.97	-1.50	*	.	F	1.98	2.49	
Arg	228	.	.	B	.	T	.	2.01	-1.93	*	.	F	2.32	2.92	
Val	229	T	.	2.06	-1.54	*	.	F	2.86	2.53	
Asp	230	T	T	2.06	-1.59	*	.	F	3.40	1.96	
Arg	231	T	T	2.38	-1.19	*	*	F	3.06	1.73	
Ser	232	T	T	2.17	-1.19	*	.	F	2.72	4.57	
Ser	233	T	T	1.71	-1.40	*	*	F	2.72	4.23	
Gln	234	C	1.98	-0.97	*	*	F	2.32	2.14	
Arg	235	T	C	1.98	-0.47	*	*	F	2.22	1.61
Pro	236	T	C	1.87	-0.86	*	*	F	2.86	2.08	
Gly	237	T	T	2.17	-1.24	.	*	F	3.40	2.01	
Ala	238	T	C	1.61	-1.24	.	*	F	2.86	1.65	
Glu	239	A	0.80	-0.60	.	*	F	1.97	0.79	
Asp	240	A	0.69	-0.34	.	*	F	1.33	0.66	
Asn	241	A	0.90	-0.37	*	.	.	0.99	1.05	
Val	242	A	0.36	-0.87	*	.	.	0.95	1.05	
Leu	243	A	0.09	-0.19	*	.	.	0.50	0.44	
Asn	244	A	.	.	B	.	.	-0.21	0.46	*	.	.	-0.60	0.20	
Glu	245	A	.	.	B	.	.	-1.10	0.44	*	.	.	-0.60	0.37	
Ile	246	A	.	.	B	.	.	-1.91	0.49	*	.	.	-0.60	0.31	
Val	247	A	.	.	B	.	.	-1.06	0.49	*	.	.	-0.60	0.16	
Ser	248	.	.	B	B	.	.	-0.46	0.49	*	.	.	-0.60	0.16	
Ile	249	.	.	B	B	.	.	-0.77	0.91	*	.	.	-0.60	0.35	
Leu	250	.	.	.	B	.	C	-0.77	0.71	.	.	.	-0.40	0.69	
Gln	251	T	C	-0.73	0.47	.	.	F	0.15	0.89	
Pro	252	T	C	-0.09	0.73	.	.	F	0.15	0.94	
Thr	253	T	C	0.21	0.47	.	.	F	0.30	1.76	
Gln	254	T	C	1.10	-0.21	.	.	F	1.20	1.76	
Val	255	.	A	.	.	.	C	1.91	-0.21	.	.	F	0.80	1.97	
Pro	256	.	A	.	.	.	C	1.31	-0.64	.	.	F	1.10	2.37	
Glu	257	A	A	1.52	-0.51	*	.	F	0.90	1.35	
Gln	258	A	A	0.98	-0.91	*	.	F	0.90	3.16	
Glu	259	A	A	0.98	-0.91	*	.	F	0.90	1.51	
Met	260	A	A	1.83	-0.94	*	.	F	0.90	1.51	
Glu	261	A	A	1.83	-0.94	*	.	.	0.75	1.51	
Val	262	A	A	1.24	-0.91	*	.	F	0.90	1.35	
Gln	263	A	A	1.24	-0.41	*	.	F	0.60	1.38	
Glu	264	A	A	1.03	-1.03	*	.	F	0.90	1.38	
Pro	265	A	A	1.32	-0.60	*	.	F	1.18	2.88	
Ala	266	A	A	0.98	-0.76	*	.	F	1.46	2.40	
Glu	267	A	.	.	.	T	.	0.98	-0.73	*	.	F	2.14	1.37	
Pro	268	A	.	.	.	T	.	0.98	-0.09	.	.	F	1.97	0.66	
Thr	269	.	.	.	T	T	.	0.38	-0.11	.	.	F	2.80	1.05	
Gly	270	A	.	.	.	T	.	-0.22	0.00	.	.	F	1.37	0.60	
Val	271	A	0.07	0.69	.	.	.	0.44	0.32	
Asn	272	.	.	B	.	.	.	-0.14	0.64	.	.	.	0.16	0.30	
Met	273	.	.	B	.	.	.	-0.28	0.59	.	.	.	0.18	0.46	
Leu	274	C	0.03	0.59	.	.	.	0.40	0.62		
Ser	275	T	C	0.08	-0.06	.	.	F	1.95	0.66	
Pro	276	T	C	0.93	-0.07	.	.	F	2.25	0.90	
Gly	277	T	C	0.90	-0.69	.	.	F	3.00	1.89	
Glu	278	A	.	.	.	T	.	0.69	-0.87	.	.	F	2.50	1.92	
Ser	279	A	A	0.69	-0.57	.	.	F	1.80	1.02	
Glu	280	A	A	0.99	-0.31	.	.	F	1.05	0.85	
His	281	A	A	0.99	-0.74	.	.	.	1.05	0.85	
Leu	282	A	A	0.74	-0.31	.	.	.	0.30	0.98	
Leu	283	A	A	0.74	-0.20	.	.	.	0.30	0.57	
Glu	284	A	A	0.46	-0.20	.	.	F	0.45	0.73	
Pro	285	A	A	0.46	-0.20	.	.	F	0.45	0.89	
Ala	286	A	A	0.60	-0.89	.	.	F	0.90	1.88	

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Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Glu	287	A	A	1.11	-1.57	.	.	F	0.90	2.13
Ala	288	A	A	1.92	-1.19	.	.	F	0.90	1.84
Glu	289	A	A	2.03	-1.21	*	.	F	0.90	3.16
Arg	290	A	A	2.36	-1.71	*	.	F	0.90	3.57
Ser	291	A	T	.	3.06	-1.71	*	.	F	1.30	6.92
Gln	292	A	T	.	2.24	-2.21	*	.	F	1.30	7.83
Arg	293	A	T	.	2.02	-1.53	.	.	F	1.30	3.30
Arg	294	A	T	.	1.17	-0.84	.	.	F	1.30	2.03
Arg	295	.	.	.	B	T	.	.	0.84	-0.59	.	*	F	1.15	0.87
Leu	296	.	.	B	B	.	.	.	0.56	-0.56	.	*	.	0.60	0.69
Leu	297	.	.	B	B	.	.	.	0.56	-0.06	.	*	.	0.30	0.35
Val	298	.	.	.	B	.	C	0.44	0.34	*	*	.	0.20	0.29	
Pro	299	T	C	-0.01	0.34	*	.	.	0.90	0.61	
Ala	300	T	C	-0.12	0.09	*	*	F	1.35	0.73	
Asn	301	T	C	0.48	-0.60	.	.	F	2.70	1.65	
Glu	302	T	C	0.98	-0.81	.	.	F	3.00	1.65	
Gly	303	C	1.83	-0.76	.	.	F	2.50	2.35		
Asp	304	T	C	1.73	-1.26	.	.	F	2.40	2.54	
Pro	305	T	C	1.51	-1.17	.	*	F	2.10	2.11	
Thr	306	A	.	.	.	T	.	1.62	-0.49	.	*	F	1.30	1.76	
Glu	307	A	.	.	.	T	.	1.62	-0.91	*	*	F	1.30	2.07	
Thr	308	A	.	.	B	.	.	1.30	-0.51	*	*	F	0.90	2.31	
Leu	309	A	.	.	B	.	.	0.60	-0.37	*	*	F	0.45	0.86	
Arg	310	A	.	.	B	.	.	0.81	-0.07	*	*	.	0.30	0.43	
Gln	311	A	.	.	B	.	.	1.12	-0.07	*	*	.	0.30	0.50	
Cys	312	A	.	.	.	T	.	0.42	-0.56	*	*	.	1.15	1.01	
Phe	313	A	.	.	.	T	.	0.14	-0.46	*	*	.	0.70	0.45	
Asp	314	.	.	.	T	T	.	0.96	0.04	*	*	.	0.50	0.26	
Asp	315	A	.	.	.	T	.	0.03	-0.36	*	*	.	0.70	0.81	
Phe	316	A	A	-0.82	-0.24	*	.	.	0.30	0.77	
Ala	317	A	A	-0.37	-0.39	*	.	.	0.30	0.34	
Asp	318	A	A	-0.37	0.04	*	*	.	-0.30	0.32	
Leu	319	A	A	-0.37	0.83	.	.	.	-0.60	0.32	
Val	320	.	A	.	.	.	C	-0.67	0.04	.	.	.	-0.10	0.52	
Pro	321	.	A	.	.	.	C	-0.26	-0.07	.	.	.	0.50	0.42	
Phe	322	.	.	.	T	T	.	0.33	0.84	.	.	.	0.20	0.54	
Asp	323	A	.	.	.	T	.	0.12	0.16	.	.	.	0.25	1.25	
Ser	324	A	.	.	.	T	.	0.12	-0.06	.	.	F	1.00	1.25	
Trp	325	A	.	.	.	T	.	0.38	0.20	*	*	F	0.40	1.19	
Glu	326	A	A	0.70	0.03	*	.	F	-0.15	0.71	
Pro	327	A	A	1.44	0.03	*	.	.	-0.15	1.03	
Leu	328	A	A	0.63	-0.36	*	.	.	0.45	1.96	
Met	329	A	A	0.59	-0.59	*	.	.	0.60	0.93	
Arg	330	A	A	0.07	-0.16	*	.	.	0.30	0.60	
Lys	331	A	A	-0.53	0.10	*	.	.	-0.30	0.60	
Leu	332	A	A	-0.32	0.03	*	.	.	-0.30	0.60	
Gly	333	A	A	0.49	-0.59	*	.	.	0.60	0.51	
Leu	334	A	A	1.09	-0.19	*	.	.	0.30	0.41	
Met	335	A	A	0.09	-0.19	*	*	.	0.30	0.86	
Asp	336	A	A	0.09	-0.19	.	*	F	0.45	0.61	
Asn	337	A	A	0.04	-0.61	*	*	F	0.90	1.48	
Glu	338	A	A	-0.20	-0.66	*	*	F	0.90	1.11	
Ile	339	A	A	0.66	-0.77	*	*	F	0.75	0.67	
Lys	340	A	A	0.67	-0.77	*	*	F	0.75	0.83	
Val	341	A	A	0.67	-0.67	*	.	.	0.60	0.49	
Ala	342	A	A	0.08	-0.67	.	.	.	0.75	1.20	
Lys	343	A	A	-0.51	-0.86	*	.	.	0.60	0.61	
Ala	344	A	A	0.03	-0.36	*	.	.	0.30	0.83	
Glu	345	A	A	-0.04	-0.57	*	.	.	0.60	0.81	
Ala	346	A	A	0.92	-0.57	*	.	.	0.60	0.55	
Ala	347	A	A	1.51	-0.57	*	.	.	0.75	1.07	
Gly	348	A	1.16	-1.07	*	.	.	0.95	1.03	
His	349	A	.	.	.	T	.	0.93	-0.59	.	.	.	1.15	1.47	
Arg	350	A	.	.	.	T	.	0.69	-0.40	.	.	F	1.00	1.20	
Asp	351	A	.	.	.	T	.	0.97	-0.14	.	.	F	1.00	1.90	
Thr	352	A	.	.	.	T	.	0.96	-0.09	.	.	F	1.00	2.02	
Leu	353	A	.	.	B	.	.	0.49	0.03	.	.	.	-0.15	1.02	
Tyr	354	A	.	.	B	.	.	-0.37	0.71	*	.	.	-0.60	0.50	
Thr	355	A	.	.	B	.	.	-0.43	1.40	*	.	.	-0.60	0.24	
Met	356	A	.	.	B	.	.	-0.72	0.91	*	.	.	-0.60	0.59	
Leu	357	A	.	.	B	.	.	-1.27	1.14	*	.	.	-0.60	0.40	
Ile	358	A	.	.	B	.	.	-0.46	1.03	*	*	.	-0.60	0.20	
Lys	359	A	.	.	B	.	.	-0.17	0.94	*	*	.	-0.60	0.33	
Trp	360	A	.	.	B	.	.	-0.17	0.33	*	*	.	0.00	0.81	

-continued

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Val	361	A	.	.	B	.	.	0.09	0.13	*	*	.	0.45	1.66	
Asn	362	T	C	1.01	-0.13	*	.	F	1.95	0.82	
Lys	363	T	C	1.90	-0.13	*	*	F	2.40	1.53	
Thr	364	T	C	1.27	-1.04	*	.	F	3.00	3.44	
Gly	365	T	C	1.26	-1.19	*	.	F	2.70	2.16	
Arg	366	.	A	.	.	T	.	1.26	-1.20	*	.	F	2.20	1.45	
Asp	367	.	A	.	.	.	C	1.22	-0.56	*	.	F	1.55	0.75	
Ala	368	A	A	0.87	-0.54	.	.	F	1.20	1.03	
Ser	369	A	A	0.37	-0.49	.	.	.	0.30	0.76	
Val	370	A	A	-0.10	0.20	.	.	.	-0.30	0.37	
His	371	A	A	-0.21	0.89	.	*	.	-0.60	0.30	
Thr	372	A	A	-0.80	0.39	*	*	.	-0.30	0.38	
Leu	373	A	A	-1.02	0.50	*	*	.	-0.60	0.52	
Leu	374	A	A	-0.72	0.54	*	.	.	-0.60	0.31	
Asp	375	A	A	-0.18	0.04	*	.	.	-0.30	0.38	
Ala	376	A	A	-0.96	0.04	*	.	.	-0.30	0.66	
Leu	377	A	A	-0.99	0.04	*	.	.	-0.30	0.66	
Glu	378	A	A	-0.18	-0.21	*	.	.	0.30	0.39	
Thr	379	A	A	0.74	-0.21	*	*	F	0.45	0.67	
Leu	380	A	A	-0.07	-0.71	*	.	F	0.90	1.59	
Gly	381	A	A	-0.07	-0.71	*	.	F	0.75	0.76	
Glu	382	A	A	0.79	-0.21	*	.	F	0.45	0.53	
Arg	383	A	A	0.79	-0.70	*	.	F	0.90	1.28	
Leu	384	A	A	1.14	-0.99	*	*	F	0.90	2.24	
Ala	385	A	A	1.07	-1.41	*	*	F	0.90	2.59	
Lys	386	A	A	1.41	-0.73	*	.	F	0.75	0.93	
Gln	387	A	A	1.41	-0.73	*	*	F	0.90	1.95	
Lys	388	A	A	1.27	-1.41	*	*	F	0.90	3.22	
Ile	389	A	A	1.27	-1.41	.	*	F	0.90	2.19	
Glu	390	A	A	1.04	-0.73	*	*	F	0.90	1.04	
Asp	391	A	A	0.70	-0.44	.	*	F	0.45	0.43	
His	392	A	A	0.40	-0.06	*	*	.	0.30	0.82	
Leu	393	A	A	0.01	-0.36	*	*	.	0.30	0.64	
Leu	394	A	A	0.94	0.07	*	*	F	-0.15	0.38	
Ser	395	A	.	.	.	T	.	0.24	0.07	*	*	F	0.25	0.55	
Ser	396	A	.	.	.	T	.	-0.36	0.36	*	*	F	0.25	0.58	
Gly	397	.	.	.	T	T	.	-0.57	0.29	.	.	F	0.65	0.70	
Lys	398	A	.	.	.	T	.	-0.57	0.36	.	.	F	0.25	0.82	
Phe	399	A	A	0.24	0.66	.	.	.	-0.60	0.50	
Met	400	.	A	B	.	.	.	0.20	0.27	.	*	.	-0.30	0.88	
Tyr	401	.	A	B	.	.	.	0.50	0.27	.	*	.	-0.30	0.44	
Leu	402	A	A	0.26	0.67	.	*	.	-0.60	0.81	
Glu	403	A	A	0.21	0.39	.	*	.	-0.30	0.82	
Gly	404	A	0.61	-0.23	.	*	F	0.65	0.88	
Asn	405	A	.	.	.	T	.	0.62	-0.60	.	*	F	1.30	1.43	
Ala	406	A	.	.	.	T	.	0.27	-0.79	.	*	F	1.15	0.83	
Asp	407	A	.	.	.	T	.	0.78	-0.17	.	*	F	0.85	0.83	
Ser	408	A	.	.	.	T	.	0.39	-0.21	.	*	F	0.85	0.69	
Ala	409	A	0.34	-0.19	.	*	.	0.50	0.88	
Met	410	A	-0.04	-0.26	.	.	.	0.50	0.67	
Ser	411	A	0.16	0.17	.	.	.	-0.10	0.64	

[0069] Among highly preferred fragments in this regard are those that comprise, or alternatively consist of, regions of DR5 that combine several structural features, such as several of the features set out above. Preferred nucleic acid fragments of the present invention further include nucleic acid molecules encoding a polypeptide comprising, or alternatively consisting of, one, two, three, four, five, or more epitope-bearing portions of the DR5 protein. In particular, such nucleic acid fragments of the present invention include, but are not limited to, nucleic acid molecules encoding a polypeptide comprising, or alternatively consisting of, one, two, three, or more amino acid sequences selected from the group consisting of: amino acid residues from about 62 to about 110 in FIG. 1 (amino acid residues from about 11 to about 59 in SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about

119 to about 164 in FIG. 1 (amino acid residues from about 68 to about 113 in SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 224 to about 271 in FIG. 1 (amino acid residues from about 173 to about 220 in SEQ ID NO:2); and a polypeptide comprising, or alternatively consisting of, amino acid residues from about 275 to about 370 in FIG. 1 (amino acid residues from about 224 to about 319 in SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the DR5 protein. Methods for determining other such epitope-bearing portions of the DR5 protein are described in detail below. In this context “about” includes the particularly recited value and values larger or smaller by several (5, 4, 3, 2, or 1) amino acid residues. Polypeptides encoded by these nucleic acids are also encompassed by the invention.

[0070] Further, the invention includes a polynucleotide comprising, or alternatively consisting of, any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:1 from residue 283 to 1,362, preferably from 283 to 681. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0071] In specific embodiments, the polynucleotides of the invention are less than 100000 kb, 50000 kb, 10000 kb, 1000 kb, 500 kb, 400 kb, 350 kb, 300 kb, 250 kb, 200 kb, 175 kb, 150 kb, 125 kb, 100 kb, 75 kb, 50 kb, 40 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, 7.5 kb, or 5 kb in length.

[0072] In further embodiments, polynucleotides of the invention comprise, or alternatively consisting of, at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, or at least 1000 contiguous nucleotides of DR5 coding sequence, but consist of less than or equal to 1000 kb, 500 kb, 250 kb, 200 kb, 150 kb, 100 kb, 75 kb, 50 kb, 30 kb, 25 kb, 20 kb, 15 kb, 10 kb, or 5 kb of genomic DNA that flanks the 5' or 3' coding nucleotide set forth in FIG. 1 (SEQ ID NO:1). In further embodiments, polynucleotides of the invention comprise, or alternatively consist of, at least 15, at least 30, at least 50, at least 100, or at least 250, at least 500, or at least 1000 contiguous nucleotides of DR5 coding sequence, but do not comprise, or alternatively consist of, all or a portion of any DR5 intron. In another embodiment, the nucleic acid comprising, or alternatively consisting of, DR5 coding sequence does not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the DR5 gene in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

[0073] In another embodiment, the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the sequence complementary to the coding and/or noncoding (i.e., transcribed, untranslated) sequence depicted in SEQ ID NO:1, the cDNA contained in ATCC Deposit No. 97920, and the sequence encoding a DR5 domain, or a polynucleotide fragment as described herein. By “stringent hybridization conditions” is intended overnight incubation at 42°C in a solution comprising, or alternatively consisting of: 50% formamide, 5×SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1×SSC at about 65°C. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0074] By a polynucleotide which hybridizes to a “portion” of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 or 80-150 nt, or the entire length of the reference polynucleotide. By a portion of a polynucleotide of “at least about 20 nt in length,” for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in SEQ ID

NO:1). In this context “about” includes the particularly recited size, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These have uses, which include, but are not limited to, as diagnostic probes and primers as discussed above and in more detail below.

[0075] Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the DR5 cDNA shown in FIG. 1 (SEQ ID NO:1)), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA generated from an oligo-dT primed cDNA library).

[0076] As indicated, nucleic acid molecules of the present invention which encode a DR5 polypeptide may include, but are not limited to, the coding sequence for the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional sequences, such as those encoding a leader or secretory sequence, such as a pre-, pro- or prepro-protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing—including splicing and polyadenylation signals, for example—ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86: 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The “HA” tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson et al., *Cell* 37:767-778(1984). As discussed below, other such fusion proteins include the DR5 receptor fused to Fc at the N- or C-terminus.

[0077] The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs, or derivatives of the DR5 receptor. Variants may occur naturally, such as a natural allelic variant. By an “allelic variant” is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

[0078] Such variants include those produced by nucleotide substitutions, deletions or additions that may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative

amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions, and deletions, which do not alter the properties and activities of the DR5 receptor or portions thereof. Also especially preferred in this regard are conservative substitutions.

[0079] Further embodiments of the invention include isolated nucleic acid molecules that are at least 80% identical, and more preferably at least 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical, to (a) a nucleotide sequence encoding the polypeptide comprising, or alternatively consisting of, the amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the polypeptide comprising, or alternatively consisting of, the amino acid sequence in SEQ ID NO:2, but lacking the amino terminal methionine; (c) a nucleotide sequence encoding the polypeptide comprising, or alternatively consisting of, the amino acid sequence at positions from about 1 to about 360 in SEQ ID NO:2; (d) a nucleotide sequence encoding the polypeptide comprising, or alternatively consisting of, the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 97920; (e) a nucleotide sequence encoding the mature DR5 polypeptide comprising, or alternatively consisting of, the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 97920; (f) a nucleotide sequence that encodes the DR5 extracellular domain comprising, or alternatively consisting of, the amino acid sequence at positions from about 1 to about 133 in SEQ ID NO:2, or the DR5 extracellular domain encoded by the cDNA contained in ATCC Deposit No. 97920; (g) a nucleotide sequence that encodes the DR5 cysteine rich domain comprising, or alternatively consisting of, the amino acid sequence at positions from about 33 to about 128 in SEQ ID NO:2, or the DR5 cysteine rich domain encoded by the cDNA contained in ATCC Deposit No. 97920; (h) a nucleotide sequence that encodes the DR5 transmembrane domain comprising, or alternatively consisting of, the amino acid sequence at positions from about 134 to about 157 of SEQ ID NO:2, or the DR5 transmembrane domain encoded by the cDNA contained in ATCC Deposit No. 97920; (i) a nucleotide sequence that encodes the DR5 intracellular domain comprising, or alternatively consisting of, the amino acid sequence at positions from about 158 to about 360 of SEQ ID NO:2, or the DR5 intracellular domain encoded by the cDNA contained in ATCC Deposit No. 97920; (j) a nucleotide sequence that encodes the DR5 receptor extracellular and intracellular domains with all or part of the transmembrane domain deleted; (k) a nucleotide sequence that encodes the DR5 death domain comprising, or alternatively consisting of, the amino acid sequence at positions from about 273 to about 340 of SEQ ID NO:2, or the DR5 death domain encoded by the cDNA contained in ATCC Deposit No. 97920; (l) a nucleotide sequence that encodes a fragment of the polypeptide of (c) having DR5 functional activity (e.g., antigenic or biological activity); and (m) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), or (l) above. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0080] By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a DR5 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five mismatches per each 100

nucleotides of the reference nucleotide sequence encoding the DR5 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The reference (query) sequence may be the entire DR5 nucleotide sequence shown in FIG. 1 (SEQ ID NO:1) or any polynucleotide fragment (e.g., a polynucleotide encoding the amino acid sequence of a DR5 N and/or C terminal deletion described herein) as described herein.

[0081] As a practical matter, whether any particular nucleic acid molecule is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in SEQ ID NO:1 or to the nucleotide sequence of the deposited cDNA can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

[0082] In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. A determination of whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of this embodiment. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment,

which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score. For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence, which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

[0083] The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in SEQ ID NO:1, the nucleic acid sequence of the deposited cDNAs, or fragments thereof, irrespective of whether they encode a polypeptide having DR5 functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having DR5 functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having DR5 functional activity include, inter alia: (1) isolating the DR5 gene or allelic variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the DR5 gene, as described in Verma et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting DR5 mRNA expression in specific tissues.

[0084] Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in SEQ ID NO:1, the nucleic acid sequence of the deposited cDNAs, or fragments thereof, which do, in fact, encode a polypeptide having DR5 protein functional activity. By "a polypeptide having DR5 functional activity" is intended a polypeptide exhibiting activity similar, but not necessarily identical, to a functional activity of the DR5 protein of the invention (either the full-length (i.e., complete) protein or, preferably, the mature protein), as measured in a particular biological assay. For example, DR5 polypeptide functional activity can be measured by the ability of a polypeptide sequence described herein to form multimers (e.g., homodimers and homotrimers) with complete DR5, and to bind a DR5 ligand (e.g., TRAIL). DR5 polypeptide functional activity can also be measured, for example, by determining the ability of a polypeptide of the invention to induce apoptosis in cells expressing the

polypeptide. These functional assays can be routinely performed using techniques described herein and otherwise known in the art.

[0085] For example, DR5 protein functional activity (e.g., biological activity) can be measured using the cell death assays performed essentially as previously described (A. M. Chinnaian, et al., *Cell* 81:505-12 (1995); M. P. Boldin, et al., *J Biol Chem* 270:7795-8 (1995); F. C. Kischkel, et al., *EMBO* 14:5579-5588 (1995); A. M. Chinnaian, et al., *J Biol Chem* 271:4961-4965 (1996)) and as set forth in Example 5, below. In MCF7 cells, plasmids encoding full-length DR5 or a candidate death domain containing receptor are co-transfected with the pLantern reporter construct encoding green fluorescent protein. Nuclei of cells transfected with DR5 will exhibit apoptotic morphology as assessed by DAPI staining. Similar to TNFR-1 and Fas/APO-1 (M. Muzio, et al., *Cell* 85:817-827 (1996); M. P. Boldin, et al., *Cell* 85:803-815 (1996); M. Tewari, et al., *J Biol Chem* 270:3255-60 (1995)), DR5-induced apoptosis is preferably blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk.

[0086] Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to for example, the nucleic acid sequence of the deposited cDNA, the nucleic acid sequence shown in SEQ ID NO:1, or fragments thereof, will encode a polypeptide "having DR5 protein functional activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having DR5 protein functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

[0087] For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Polynucleotide Assays

[0088] This invention is also related to the use of the DR5 polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a mutated form of DR5 associated with a dysfunction will provide a diagnostic tool that can add or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression, or altered expression of DR5 or a soluble form thereof, such as, for example, tumors or autoimmune disease.

[0089] Individuals carrying mutations in the DR5 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and

autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. (Saiki et al, *Nature* 324:163-166 (1986)). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding DR5 can be used to identify and analyze DR5 expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled DR5 RNA or alternatively, radiolabeled DR5 antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

[0090] Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

[0091] Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high-resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers et al., *Science* 230:1242 (1985)).

[0092] Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton et al., *Proc. Nat'l Acad. Sci. USA* 85: 4397-4401 (1985)).

[0093] Thus, the detection of a specific DNA sequence may be achieved by methods which include, but are not limited to, hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA).

[0094] In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

Vectors and Host Cells

[0095] The present invention also relates to vectors, which include DNA molecules of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

[0096] Host cells can be genetically engineered to incorporate nucleic acid molecules and express polypeptides of the present invention. The polynucleotides may be introduced alone or with other polynucleotides. Such other

polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

[0097] In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well-known techniques for introducing DNA and RNA into cells. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

[0098] Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors are either supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

[0099] A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include chromosomal, episomal and virus-derived vectors e.g., vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

[0100] The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s)), including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name just a few of the well-known promoters. In general, expression constructs will contain sites for transcription, initiation and termination, and, in the transcribed region, a ribosome-binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[0101] In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, such regions will operate by controlling transcription, such as repressor binding sites and enhancers, among others.

[0102] Vectors for propagation and expression generally will include selectable markers. Such markers also may be suitable for amplification or the vectors may contain additional markers for this purpose. In this regard, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells. Such markers include, but are not limited

to, dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing *E. coli* and other bacteria.

[0103] The vector containing the appropriate DNA sequence as described elsewhere herein, as well as an appropriate promoter, and other appropriate control sequences, may be introduced into an appropriate host using a variety of well-known techniques suitable to expression therein of a desired polypeptide. Representative examples of appropriate hosts include bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0104] Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptre99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well-known vectors available to those of skill in the art.

[0105] Selection of appropriate vectors and promoters for expression in a host cell is a well-known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

[0106] The present invention also relates to host cells containing the above-described vector constructs described herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

[0107] Introduction of the construct into the host cell can be effected by calcium phosphate mediated transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods in Molecular Biology* (1986).

[0108] In addition to encompassing host cells containing the vector constructs discussed herein, the invention also

encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., DR5 coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with DR5 polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous DR5 polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous DR5 polynucleotide sequences via homologous recombination (see, e.g., U.S. Pat. No. 5,641,670, issued Jun. 24, 1997; International Publication Number WO 96/29411, published Sep. 26, 1996; International Publication Number WO 94/12650, published Aug. 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0109] The polypeptide may be expressed in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals but also additional heterologous functional regions. Such a fusion protein can be made by ligating polynucleotides of the invention and the desired nucleic acid sequence encoding the desired amino acid sequence to each other, by methods known in the art, in the proper reading frame, and expressing the fusion protein product by methods known in the art. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. For example, in one embodiment, polynucleotides encoding DR5 polypeptides of the invention may be fused to the pelB pectate lyase signal sequence to increase the efficiency to expression and purification of such polypeptides in Gram-negative bacteria. See, U.S. Pat. Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

[0110] Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Additionally, a region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in

a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as the hIL-5-receptor, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., *Journal of Molecular Recognition*, 8:52-58 (1995) and K. Johanson et al., *The Journal of Biological Chemistry*, 270:9459-9471 (1995).

[0111] Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Transgenics and "Knock-Outs"

[0112] The DR5 polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

[0113] Any technique known in the art may be used to introduce the transgene (i.e., nucleic acids of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., *Appl. Microbiol. Biotechnol.* 40:691-698 (1994); Carver et al., *Biotechnology (NY)* 11:1263-1270 (1993); Wright et al., *Biotechnology (NY)* 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA* 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., *Cell* 56:313-321 (1989)); electroporation of cells or embryos (Lo, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., *Science* 259:1745 (1993)); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., *Cell* 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," *Intl. Rev. Cytol.* 115:171-229 (1989), which is incorporated by reference herein in its entirety. See also, U.S. Pat. No. 5,464,764 (Capecchi, et al., Positive-Negative Selection Methods and Vectors); U.S. Pat. No. 5,631,153 (Capecchi, et al., Cells and Non-Human

Organisms Containing Predetermined Genomic Modifications and Positive-Negative Selection Methods and Vectors for Making Same); U.S. Pat. No. 4,736,866 (Leder, et al., Transgenic Non-Human Animals); and U.S. Pat. No. 4,873,191 (Wagner, et al., Genetic Transformation of Zygotes); each of which is hereby incorporated by reference in its entirety. Further, the contents of each of the documents recited in this paragraph are herein incorporated by reference in its entirety.

[0114] Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campbell et al., *Nature* 380:64-66 (1996); Wilmut et al., *Nature* 385:810-813 (1997)), each of which is herein incorporated by reference in its entirety).

[0115] The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals or chimeric animals. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (*Proc. Natl. Acad. Sci. USA* 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (*Science* 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. The content of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

[0116] Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

[0117] Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of

the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

[0118] Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of DR5 polypeptides, studying conditions and/or disorders associated with aberrant DR5 expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

[0119] In further embodiments of the invention, cells that are genetically engineered to express the proteins of the invention, or alternatively, that are genetically engineered not to express the proteins of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells, etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells that express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally. Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Pat. No. 5,399,349; and Mulligan & Wilson, U.S. Pat. No. 5,460,959, each of which is incorporated by reference herein in its entirety).

[0120] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well-known techniques that prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form, which, while allowing for an exchange

of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

DR5 Proteins and Fragments

[0121] The invention further provides for the proteins containing polypeptide sequences encoded by the polynucleotides of the invention.

[0122] The DR5 proteins of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). Accordingly, the present invention relates to monomers and multimers of the DR5 proteins of the invention, their preparation, and compositions (preferably, pharmaceutical compositions) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

[0123] Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only DR5 proteins of the invention (including DR5 fragments, variants, and fusion proteins, as described herein). These homomers may contain DR5 proteins having identical or different polypeptide sequences. In a specific embodiment, a homomer of the invention is a multimer containing only DR5 proteins having an identical polypeptide sequence. In another specific embodiment, a homomer of the invention is a multimer containing DR5 proteins having different polypeptide sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing DR5 proteins having identical or different polypeptide sequences) or a homotrimer (e.g., containing DR5 proteins having identical or different polypeptide sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

[0124] As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing only polypeptide sequences that do not correspond to a polypeptide sequences encoded by the DR5 gene) in addition to the DR5 proteins of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

[0125] Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when proteins of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when proteins of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the DR5 proteins of the invention. Such covalent associations may

involve one or more amino acid residues contained in the polypeptide sequence of the protein (e.g., the polypeptide sequence recited in SEQ ID NO:2 or the polypeptide encoded by the deposited cDNA). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a DR5 fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., U.S. Pat. No. 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a DR5-Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequences from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more DR5 polypeptides of the invention are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include, but are not limited to, those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple DR5 polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0126] Another method for preparing multimer DR5 polypeptides of the invention involves use of DR5 polypeptides fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper domains and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric DR5 proteins are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a soluble DR5 polypeptide fused to a peptide that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric DR5 is recovered from the culture supernatant using techniques known in the art.

[0127] Certain members of the TNF family of proteins are believed to exist in trimeric form (Beutler and Huffel, *Science* 264:667, 1994; Banner et al., *Cell* 73:431 (1993)). Thus, trimeric DR5 may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric DR5.

[0128] In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in Flag®-DR5 or Flag®-DR5 fusion proteins of the invention. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag®-DR5 or Flag®-DR5 fusion proteins of the invention and anti-Flag® antibody.

[0129] The multimers of the invention may be generated using chemical techniques known in the art. For example, proteins desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., U.S. Pat. No. 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more intermolecule cross-links between the cysteine residues located within the polypeptide sequence of the proteins desired to be contained in the multimer (see, e.g., U.S. Pat. No. 5,478,925, which is herein incorporated by reference in its entirety).

[0130] Further, proteins of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide sequence of the protein and techniques known in the art may be applied to generate multimers containing one or more of these modified proteins (see, e.g., U.S. Pat. No. 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the protein components desired to be contained in the multimer of the invention (see, e.g., U.S. Pat. No. 5,478,925, which is herein incorporated by reference in its entirety).

[0131] Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, proteins contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., U.S. Pat. No. 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., U.S. Pat. No. 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., U.S. Pat. No. 5,478,925, which is herein incorporated by reference in its entirety).

[0132] The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an

"isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the DR5 polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988).

[0133] In one embodiment, the invention provides an isolated DR5 polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in SEQ ID NO:2, or a polypeptide or peptide comprising, or alternatively consisting of, a portion (i.e., fragment) of the above polypeptides. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0134] Polypeptide fragments of the present invention include polypeptides comprising, or alternatively consisting of, an amino acid sequence contained in SEQ ID NO:2, encoded by the cDNA contained in the deposited plasmid, or encoded by nucleic acids which hybridize (e.g., under stringent hybridization conditions) to the nucleotide sequence contained in the deposited plasmid, or shown in FIG. 1 (SEQ ID NO:1) or the complementary strand thereto. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise, or alternatively consist of, a member selected from the group consisting of from about amino acid residues -51 to -1, 1 to 27, 28 to 40, 41 to 60, 61 to 83, 84 to 100, 101 to 127, 128 to 133, 134 to 157, 158 to 167, 168 to 180, 181 to 200, 201 to 220, 221 to 240, 241 to 260, 261 to 272, 273 to 310, 311 to 340, and 341 to 360 of SEQ ID NO:2, as well as isolated polynucleotides which encode these polypeptides. Additional representative examples of polypeptide fragments of the invention, include, for example, fragments that comprise, or alternatively consist of, a member selected from the group consisting of from about amino acid residues 1-60, 11-70, 21-80, 31-90, 41-100, 51-110, 61-120, 71-130, 81-140, 91-150, 101-160, 111-170, 121-180, 131-190, 141-200, 151-210, 161-220, 171-230, 181-240, 191-250, 201-260, 211-270, 221-280, 231-290, 241-300, 251-310, 261-320, 271-330, 281-340, 291-350, and 301-360 of SEQ ID NO:2, as well as isolated polynucleotides which encode these polypeptides.

[0135] Moreover, polypeptide fragments can be at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited value, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0136] Preferred polypeptide fragments of the present invention include a polypeptide comprising, or alternatively consisting of, one, two, three, four, five or more amino acid sequences selected from the group consisting of: a polypeptide comprising, or alternatively consisting of, the DR5 receptor extracellular domain (predicted to constitute amino acid residues from about 1 to about 133 in SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, the DR5 cysteine rich domain (predicted to constitute amino acid residues from about 33 to about 128 in SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, the

DR5 receptor transmembrane domain (predicted to constitute amino acid residues from about 134 to about 157 in SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, fragment of the predicted mature DR5 polypeptide, wherein the fragment has a DR5 functional activity (e.g., antigenic activity or biological activity); a polypeptide comprising, or alternatively consisting of, the DR5 receptor intracellular domain (predicted to constitute amino acid residues from about 158 to about 360 in SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, the DR5 receptor extracellular and intracellular domains with all or part of the transmembrane domain deleted; a polypeptide comprising, or alternatively consisting of, the DR5 receptor death domain (predicted to constitute amino acid residues from about 273 to about 340 in SEQ ID NO:2); and a polypeptide comprising, or alternatively consisting of, one, two, three, four or more epitope bearing portions of the DR5 receptor protein. In additional embodiments, the polypeptide fragments of the invention comprise, or alternatively consist of, any combination of 1, 2, 3, 4, 5, 6, 7, or all 8 of the above members. As above, with the leader sequence, the amino acid residues constituting the DR5 receptor extracellular, transmembrane and intracellular domains have been predicted by computer analysis. Thus, as one of ordinary skill would appreciate, the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0137] As discussed above, it is believed that one or both of the extracellular cysteine-rich motifs of DR5 is important for interactions between DR5 and its ligands. Accordingly, in preferred embodiments, polypeptide fragments of the invention comprise, or alternatively consist of, amino acid residues 33 to 80, and/or 81 to 128 of SEQ ID NO:2. In a specific embodiment the polypeptides of the invention comprise, or alternatively consist of, both of the extracellular cysteine-rich motifs disclosed in SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0138] Among the especially preferred fragments of the invention are fragments comprising, or alternatively consisting of, structural or functional attributes of DR5. Such fragments include amino acid residues that comprise, or alternatively consisting of, one, two, three, four or more of the following functional domains: alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., regions of polypeptides consisting of amino acid residues having an antigenic index of or equal to greater than 1.5, as identified using the default parameters of the Jameson-Wolf program) of DR5.

[0139] Certain preferred regions are those disclosed in FIG. 3 and Table I and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in FIG. 1, such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions;

Kyte-Doolittle predicted hydrophilic regions and Hopp-Woods predicted hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0140] In another aspect, the invention provides a peptide or polypeptide comprising, or alternatively consisting of, one, two, three, four, five or more epitope-bearing portions of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0141] As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, J. G. Sutcliffe et al., "Antibodies That React With Predetermined Sites on Proteins," *Science* 219:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

[0142] Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies that bind specifically to a polypeptide of the invention. See, for instance, Wilson et al., *Cell* 37:767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising, or alternatively consisting of, immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0143] Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies that specifically bind the epitope. Further, antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219:660-666 (1983)).

[0144] Non-limiting examples of antigenic polypeptides or peptides that can be used to generate DR5 receptor-specific antibodies include: a polypeptide comprising, or alternatively consisting of, amino acid residues from about 11 to about 59 in SEQ ID NO:2, from about 68 to about 113 in SEQ ID NO:2, from about 173 to about 220 in SEQ ID NO:2, and from about 224 to about 319 in SEQ ID NO:2. In this context "about" includes the particularly recited ranges,

larger or smaller by several (5, 4, 3, 2, or 1) amino acid residues, at either terminus or at both termini. As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the DR5 receptor protein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0145] The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. R. A. Houghten, "General Method for the Rapid Solid-Phase Synthesis of Large Numbers of Peptides: Specificity of Antigen-Antibody Interaction at the Level of Individual Amino Acids," *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Pat. No. 4,631,211 to Houghten et al. (1986).

[0146] Immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985). A preferred immunogenic epitope includes the secreted protein. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as, for example, rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

[0147] Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemocyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as, for example, rabbits, rats, and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 micrograms of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody that can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

[0148] As one of skill in the art will appreciate, DR5 receptor polypeptides of the present invention and the epitope-bearing fragments thereof described herein (e.g., corresponding to a portion of the extracellular domain, such as, for example, amino acid residues 1 to 133 of SEQ ID NO:2) can be combined with heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker et al., *Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric DR5 protein or protein fragment alone (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix-binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers. Polynucleotides encoding these fusion proteins are also encompassed by the invention.

[0149] The techniques of gene shuffling, motif shuffling, exon shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of DR5 thereby effectively generating agonists and antagonists of DR5. See generally, U.S. Pat. Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *BioTechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of DR5 polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired DR5 molecule by homologous, or site-specific, recombination. In another embodiment, DR5 polynucleotides and corresponding polypeptides may be altered through being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination.

[0150] In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of DR5 may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more

heterologous molecules. In preferred embodiments, the heterologous molecules are, for example, TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), Neutrokinin-alpha (International Publication No. WO 98/18921), OPG, nerve growth factor (NGF), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), TR12, TNF-R1, TRAMP/DR3/APO-3/WSL/LARD, TRAIL-R1/DR4/APO-2, TRAIL-R2/DR5, DcR1/TRAIL-R3/TRID/LIT, DcR2/TRAIL-R4, CAD, TRAIL, TRAMP, and v-FLIP. In additional preferred embodiments, the heterologous molecules are, for example, soluble forms of Fas, CD30, CD27, CD40 and 4-IBB.

[0151] In further preferred embodiments, the heterologous molecules are any members of the TNF family.

[0152] To improve or alter the characteristics of DR5 polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

[0153] For instance, for many proteins, including the extracellular domain of a membrane associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. However, even if deletion of one or more amino acids from the N-terminus or C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other DR5 functional activities may still be retained. For example, in many instances, the ability of the shortened protein to induce and/or bind to antibodies which recognize DR5 (preferably antibodies that bind specifically to DR5) will remain irrespective of the size or location of the deletion. In fact, polypeptides composed of as few as six DR5 amino acid residues may often evoke an immune response. Whether a particular polypeptide lacking N-terminal and/or C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

[0154] As mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind DR5 ligand) may still be retained. For example, the ability of shortened

DR5 muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a DR5 mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities.

[0155] It will be recognized in the art that some amino acid sequence of DR5 can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein that determine activity. Such areas will usually comprise residues which make up the ligand binding site or the death domain, or which form tertiary structures which affect these domains.

[0156] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the DR5 amino acid sequence shown in **FIG. 1**, up to the alanine residue at position number 406 and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues n¹-411 of **FIG. 1**, where n¹ is an integer from 2 to 406 corresponding to the position of the amino acid residue in **FIG. 1** (which is identical to the sequence shown as SEQ ID NO:2, with the exception that the amino acid residues in **FIG. 1** are numbered consecutively from 1 through 411 from the N-terminus to the C-terminus, while the amino acid residues in SEQ ID NO:2 are numbered consecutively from -51 through 360 to reflect the position of the predicted signal peptide).

[0157] More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of a member selected from the group consisting of residues: E-2 to S-411; Q-3 to S-411; R-4 to S-411; G-5 to S-411; Q-6 to S-411; N-7 to S-411; A-8 to S-411; P-9 to S-411; A-10 to S-411; A-11 to S-411; S-12 to S-411; G-13 to S-411; A-14 to S-411; R-15 to S-411; K-16 to S-411; R-17 to S-411; H-18 to S-411; G-19 to S-411; P-20 to S-411; G-21 to S-411; P-22 to S-411; R-23 to S-411; E-24 to S-411; A-25 to S-411; R-26 to S-411; G-27 to S-411; A-28 to S-411; R-29 to S-411; P-30 to S-411; G-31 to S-411; P-32 to S-411; R-33 to S-411; V-34 to S-411; P-35 to S-411; K-36 to S-411; T-37 to S-411; L-38 to S-411; V-39 to S-411; L-40 to S-411; V-41 to S-411; V-42 to S-411; A-43 to S-411; A-44 to S-411; V-45 to S-411; L-46 to S-411; L-47 to S-411; L-48 to S-411; V-49 to S-411; S-50 to S-411; A-51 to S-411; E-52 to S-411; S-53 to S-411; A-54 to S-411; L-55 to S-411; I-56 to S-411; T-57 to S-411; Q-58 to S-411; Q-59 to S-411; D-60 to S-411; L-61 to S-411; A-62 to S-411; P-63 to S-411; Q-64 to S-411; Q-65 to S-411; R-66 to S-411; A-67 to S-411; A-68 to S-411; P-69 to S-411; Q-70 to S-411; Q-71 to S-411; K-72 to S-411; R-73 to S-411; S-74 to S-411; S-75 to S-411; P-76 to S-411; S-77 to S-411; E-78 to S-411; G-79 to S-411; L-80 to S-411; C-81 to S-411; P-82 to S-411; P-83 to S-411; G-84 to S-411; H-85 to S-411; H-86 to S-411; I-87 to S-411; S-88 to S-411; E-89 to S-411; D-90 to S-411; G-91 to S-411; R-92 to S-411; D-93 to S-411; C-94 to S-411; I-95 to S-411; S-96 to S-411; C-97 to S-411; K-98 to S-411; Y-99

to S-411; G-100 to S-411; Q-101 to S-411; D-102 to S-411; Y-103 to S-411; S-104 to S-411; T-105 to S-411; H-106 to S-411; W-107 to S-411; N-108 to S-411; D-109 to S-411; L-110 to S-411; L-111 to S-411; F-112 to S-411; C-113 to S-411; L-114 to S-411; R-115 to S-411; C-116 to S-411; T-117 to S-411; R-118 to S-411; C-119 to S-411; D-120 to S-411; S-121 to S-411; G-122 to S-411; E-123 to S-411; V-124 to S-411; E-125 to S-411; L-126 to S-411; S-127 to S-411; P-128 to S-411; C-129 to S-411; T-130 to S-411; T-131 to S-411; T-132 to S-411; R-133 to S-411; N-134 to S-411; T-135 to S-411; V-136 to S-411; C-137 to S-411; Q-138 to S-411; C-139 to S-411; E-140 to S-411; E-141 to S-411; G-142 to S-411; T-143 to S-411; F-144 to S-411; R-145 to S-411; E-146 to S-411; E-147 to S-411; D-148 to S-411; S-149 to S-411; P-150 to S-411; E-151 to S-411; M-152 to S-411; C-153 to S-411; R-154 to S-411; K-155 to S-411; C-156 to S-411; R-157 to S-411; T-158 to S-411; G-159 to S-411; C-160 to S-411; P-161 to S-411; R-162 to S-411; G-163 to S-411; M-164 to S-411; V-165 to S-411; K-166 to S-411; V-167 to S-411; G-168 to S-411; D-169 to S-411; C-170 to S-411; T-171 to S-411; P-172 to S-411; W-173 to S-411; S-174 to S-411; D-175 to S-411; I-176 to S-411; E-177 to S-411; C-178 to S-411; V-179 to S-411; H-180 to S-411; K-181 to S-411; E-182 to S-411; S-183 to S-411; G-184 to S-411; I-185 to S-411; I-186 to S-411; I-187 to S-411; G-188 to S-411; V-189 to S-411; T-190 to S-411; V-191 to S-411; A-192 to S-411; A-193 to S-411; V-194 to S-411; V-195 to S-411; L-196 to S-411; I-197 to S-411; V-198 to S-411; A-199 to S-411; V-200 to S-411; F-201 to S-411; V-202 to S-411; C-203 to S-411; K-204 to S-411; S-205 to S-411; L-206 to S-411; L-207 to S-411; W-208 to S-411; K-209 to S-411; K-210 to S-411; V-211 to S-411; L-212 to S-411; P-213 to S-411; Y-214 to S-411; L-215 to S-411; K-216 to S-411; G-217 to S-411; I-218 to S-411; C-219 to S-411; S-220 to S-411; G-221 to S-411; G-222 to S-411; G-223 to S-411; G-224 to S-411; D-225 to S-411; P-226 to S-411; E-227 to S-411; R-228 to S-411; V-229 to S-411; D-230 to S-411; R-231 to S-411; S-232 to S-411; S-233 to S-411; Q-234 to S-411; R-235 to S-411; P-236 to S-411; G-237 to S-411; A-238 to S-411; E-239 to S-411; D-240 to S-411; N-241 to S-411; V-242 to S-411; L-243 to S-411; N-244 to S-411; E-245 to S-411; I-246 to S-411; V-247 to S-411; S-248 to S-411; I-249 to S-411; L-250 to S-411; Q-251 to S-411; P-252 to S-411; T-253 to S-411; Q-254 to S-411; V-255 to S-411; P-256 to S-411; E-257 to S-411; Q-258 to S-411; E-259 to S-411; M-260 to S-411; E-261 to S-411; V-262 to S-411; Q-263 to S-411; E-264 to S-411; P-265 to S-411; A-266 to S-411; E-267 to S-411; P-268 to S-411; T-269 to S-411; G-270 to S-411; V-271 to S-411; N-272 to S-411; M-273 to S-411; L-274 to S-411; S-275 to S-411; P-276 to S-411; G-277 to S-411; E-278 to S-411; S-279 to S-411; E-280 to S-411; H-281 to S-411; L-282 to S-411; L-283 to S-411; E-284 to S-411; P-285 to S-411; A-286 to S-411; E-287 to S-411; A-288 to S-411; E-289 to S-411; R-290 to S-411; S-291 to S-411; Q-292 to S-411; R-293 to S-411; R-294 to S-411; R-295 to S-411; L-296 to S-411; L-297 to S-411; V-298 to S-411; P-299 to S-411; A-300 to S-411; N-301 to S-411; E-302 to S-411; G-303 to S-411; D-304 to S-411; P-305 to S-411; T-306 to S-411; E-307 to S-411; T-308 to S-411; L-309 to S-411; R-310 to S-411; Q-311 to S-411; C-312 to S-411; F-313 to S-411; D-314 to S-411; D-315 to S-411; F-316 to S-411; A-317 to S-411; D-318 to S-411; L-319 to S-411; V-320 to S-411; P-321 to S-411; F-322 to S-411; D-323 to S-411;

S-324 to S-411; W-325 to S-411; E-326 to S-411; P-327 to S-411; L-328 to S-411; M-329 to S-411; R-330 to S-411; K-331 to S-411; L-332 to S-411; G-333 to S-411; L-334 to S-411; M-335 to S-411; D-336 to S-411; N-337 to S-411; E-338 to S-411; I-339 to S-411; K-340 to S-411; V-341 to S-411; A-342 to S-411; K-343 to S-411; A-344 to S-411; E-345 to S-411; A-346 to S-411; A-347 to S-411; G-348 to S-411; H-349 to S-411; R-350 to S-411; D-351 to S-411; T-352 to S-411; L-353 to S-411; Y-354 to S-411; T-355 to S-411; M-356 to S-411; L-357 to S-411; I-358 to S-411; K-359 to S-411; W-360 to S-411; V-361 to S-411; N-362 to S-411; K-363 to S-411; T-364 to S-411; G-365 to S-411; R-366 to S-411; D-367 to S-411; A-367 to S-411; S-369 to S-411; V-370 to S-411; H-371 to S-411; T-372 to S-411; L-373 to S-411; L-374 to S-411; D-375 to S-411; A-376 to S-411; L-377 to S-411; E-378 to S-411; T-379 to S-411; L-380 to S-411; G-381 to S-411; E-382 to S-411; R-383 to S-411; L-384 to S-411; A-385 to S-411; K-386 to S-411; Q-387 to S-411; K-388 to S-411; I-389 to S-411; E-390 to S-411; D-391 to S-411; H-392 to S-411; L-393 to S-411; L-394 to S-411; S-395 to S-411; S-396 to S-411; G-397 to S-411; K-398 to S-411; F-399 to S-411; M-400 to S-411; Y-401 to S-411; L-402 to S-411; E-403 to S-411; G-404 to S-411; N-405 to S-411; and A-406 to S-411 of the DR5 sequence shown in **FIG. 1** (which is identical to the sequence shown as SEQ ID NO:2, with the exception that the amino acid residues in **FIG. 1** are numbered consecutively from 1 through 411 from the N-terminus to the C-terminus, while the amino acid residues in SEQ ID NO:2 are numbered consecutively from -51 through 360 to reflect the position of the predicted signal peptide).

[0158] The present invention is also directed to nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequences encoding the polypeptides described above. The invention is further directed to nucleic acid molecules comprising, or alternatively consisting of, polynucleotide sequences which encode polypeptides that are at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0159] In another embodiment, N-terminal deletions of the DR5 polypeptide can be described by the general formula n^2 to 184 where n^2 is a number from 1 to 179 corresponding to the amino acid sequence identified in **FIG. 1** (or where n^2 is a number from -51 to 128 corresponding to the amino acid sequence identified in SEQ ID NO:2). In specific embodiments, N-terminal deletions of the DR5 of the invention comprise, or alternatively consist of, a member selected from the group consisting of amino acid residues: E-2 to G-184; Q-3 to G-184; R-4 to G-184; G-5 to G-184; Q-6 to G-184; N-7 to G-184; A-8 to G-184; P-9 to G-184; A-10 to G-184; A-11 to G-184; S-12 to G-184; G-13 to G-184; A-14 to G-184; R-15 to G-184; K-16 to G-184; R-17 to G-184; H-18 to G-184; G-19 to G-184; P-20 to G-184; G-21 to G-184; P-22 to G-184; R-23 to G-184; E-24 to G-184; A-25 to G-184; R-26 to G-184; G-27 to G-184; A-28 to G-184; R-29 to G-184; P-30 to G-184; G-31 to G-184; P-32 to G-184; R-33 to G-184; V-34 to G-184; P-35 to G-184; K-36 to G-184; T-37 to G-184; L-38 to G-184; V-39 to G-184;

L-40 to G-184; V-41 to G-184; V-42 to G-184; A-43 to G-184; A-44 to G-184; V-45 to G-184; L-46 to G-184; L-47 to G-184; L-48 to G-184; V-49 to G-184; S-50 to G-184; A-51 to G-184; E-52 to G-184; S-53 to G-184; A-54 to G-184; L-55 to G-184; I-56 to G-184; T-57 to G-184; Q-58 to G-184; Q-59 to G-184; D-60 to G-184; L-61 to G-184; A-62 to G-184; P-63 to G-184; Q-64 to G-184; Q-65 to G-184; R-66 to G-184; A-67 to G-184; A-68 to G-184; P-69 to G-184; Q-70 to G-184; Q-71 to G-184; K-72 to G-184; R-73 to G-184; S-74 to G-184; S-75 to G-184; P-76 to G-184; S-77 to G-184; E-78 to G-184; G-79 to G-184; L-80 to G-184; C-81 to G-184; P-82 to G-184; P-83 to G-184; G-84 to G-184; H-85 to G-184; H-86 to G-184; I-87 to G-184; S-88 to G-184; E-89 to G-184; D-90 to G-184; G-91 to G-184; R-92 to G-184; D-93 to G-184; C-94 to G-184; I-95 to G-184; S-96 to G-184; C-97 to G-184; K-98 to G-184; Y-99 to G-184; G-100 to G-184; Q-101 to G-184; D-102 to G-184; Y-103 to G-184; S-104 to G-184; T-105 to G-184; H-106 to G-184; W-107 to G-184; N-108 to G-184; D-109 to G-184; L-110 to G-184; L-111 to G-184; F-112 to G-184; C-113 to G-184; L-114 to G-184; R-115 to G-184; C-116 to G-184; T-117 to G-184; R-118 to G-184; C-119 to G-184; D-120 to G-184; S-121 to G-184; G-122 to G-184; E-123 to G-184; V-124 to G-184; E-125 to G-184; L-126 to G-184; S-127 to G-184; P-128 to G-184; C-129 to G-184; T-130 to G-184; T-131 to G-184; T-132 to G-184; R-133 to G-184; N-134 to G-184; T-135 to G-184; V-136 to G-184; C-137 to G-184; Q-138 to G-184; C-139 to G-184; E-140 to G-184; E-141 to G-184; G-142 to G-184; T-143 to G-184; F-144 to G-184; R-145 to G-184; E-146 to G-184; E-147 to G-184; D-148 to G-184; S-149 to G-184; P-150 to G-184; E-151 to G-184; M-152 to G-184; C-153 to G-184; R-154 to G-184; K-155 to G-184; C-156 to G-184; R-157 to G-184; T-158 to G-184; G-159 to G-184; C-160 to G-184; P-161 to G-184; R-162 to G-184; G-163 to G-184; M-164 to G-184; V-165 to G-184; K-166 to G-184; V-167 to G-184; G-168 to G-184; D-169 to G-184; C-170 to G-184; T-171 to G-184; P-172 to G-184; W-173 to G-184; S-174 to G-184; D-175 to G-184; I-176 to G-184; E-177 to G-184; C-178 to G-184; and V-179 to G-184 of the DR5 extracellular domain sequence shown in **FIG. 1** (which is identical to the sequence shown as SEQ ID NO:2, with the exception that the amino acid residues in **FIG. 1** are numbered consecutively from 1 through 411 from the N-terminus to the C-terminus, while the amino acid residues in SEQ ID NO:2 are numbered consecutively from -51 through 360 to reflect the position of the predicted signal peptide).

[0160] The present invention is also directed to nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequences encoding the polypeptides described above. The invention is further directed to nucleic acid molecules comprising, or alternatively consisting of, polynucleotide sequences which encode polypeptides that are at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0161] Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of

the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind DR5 ligand (e.g., TRAIL)) may still be retained. For example, the ability of the shortened DR5 mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a DR5 mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six DR5 amino acid residues may often evoke an immune response.

[0162] Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the DR5 polypeptide shown in **FIG. 1** (SEQ ID NO:2), up to the glutamic acid residue at position number 52, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues 52- m^1 of **FIG. 1** (i.e., SEQ ID NO:2), where m^1 is an integer from 57 to 410 corresponding to the position of the amino acid residue in **FIG. 1** (or where m^1 is an integer from 6 to 360 corresponding to the position of the amino acid residue in SEQ ID NO:2). More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, a member selected from the group consisting of residues: E-52 to M-410; E-52 to A-409; E-52 to S-408; E-52 to D-407; E-52 to A-406; E-52 to N-405; E-52 to G-404; E-52 to E-403; E-52 to L-402; E-52 to Y-401; E-52 to M-400; E-52 to F-399; E-52 to K-398; E-52 to G-397; E-52 to S-396; E-52 to S-395; E-52 to L-394; E-52 to L-393; E-52 to H-392; E-52 to D-391; E-52 to E-390; E-52 to I-389; E-52 to K-388; E-52 to Q-387; E-52 to K-386; E-52 to A-385; E-52 to L-384; E-52 to R-383; E-52 to E-382; E-52 to G-381; E-52 to L-380; E-52 to T-379; E-52 to E-378; E-52 to L-377; E-52 to A-376; E-52 to D-375; E-52 to L-374; E-52 to L-373; E-52 to T-372; E-52 to H-371; E-52 to V-370; E-52 to S-369; E-52 to A-368; E-52 to D-367; E-52 to R-366; E-52 to G-365; E-52 to T-364; E-52 to K-363; E-52 to N-362; E-52 to V-361; E-52 to W-360; E-52 to K-359; E-52 to I-358; E-52 to L-357; E-52 to M-356; E-52 to T-355; E-52 to Y-354; E-52 to L-353; E-52 to T-352; E-52 to D-351; E-52 to R-350; E-52 to H-349; E-52 to G-348; E-52 to A-347; E-52 to A-346; E-52 to E-345; E-52 to A-344; E-52 to K-343; E-52 to A-342; E-52 to V-341; E-52 to K-340; E-52 to I-339; E-52 to E-338; E-52 to N-337; E-52 to D-336; E-52 to M-335; E-52 to L-334; E-52 to G-333; E-52 to L-332; E-52 to K-331; E-52 to R-330; E-52 to M-329; E-52 to L-328; E-52 to P-327; E-52 to E-326; E-52 to W-325; E-52 to S-324; E-52 to D-323; E-52 to F-322; E-52 to P-321; E-52 to V-320; E-52 to L-319; E-52 to D-318; E-52 to A-317; E-52 to F-316; E-52 to D-315; E-52 to D-314; E-52 to F-313; E-52 to C-312; E-52 to Q-311; E-52 to R-310; E-52 to L-309; E-52 to T-308; E-52 to E-307; E-52 to T-306; E-52 to P-305; E-52 to D-304; E-52 to G-303; E-52 to E-302; E-52 to N-301; E-52 to A-300; E-52 to P-299; E-52 to V-298; E-52 to L-297; E-52 to L-296; E-52 to R-295; E-52 to R-294; E-52 to R-293; E-52 to Q-292; E-52 to S-291; E-52 to R-290; E-52 to E-289; E-52

to A-288; E-52 to E-287; E-52 to A-286; E-52 to P-285; E-52 to E-284; E-52 to L-283; E-52 to L-282; E-52 to H-281; E-52 to E-280; E-52 to S-279; E-52 to E-278; E-52 to G-277; E-52 to P-276; E-52 to S-275; E-52 to L-274; E-52 to M-273; E-52 to N-272; E-52 to V-271; E-52 to G-270; E-52 to T-269; E-52 to P-268; E-52 to E-267; E-52 to A-266; E-52 to P-265; E-52 to E-264; E-52 to Q-263; E-52 to V-262; E-52 to E-261; E-52 to M-260; E-52 to E-259; E-52 to Q-258; E-52 to E-257; E-52 to P-256; E-52 to V-255; E-52 to Q-254; E-52 to T-253; E-52 to P-252; E-52 to Q-251; E-52 to L-250; E-52 to I-249; E-52 to S-248; E-52 to V-247; E-52 to I-246; E-52 to E-245; E-52 to N-244; E-52 to L-243; E-52 to V-242; E-52 to N-241; E-52 to D-240; E-52 to E-239; E-52 to A-238; E-52 to G-237; E-52 to P-236; E-52 to R-235; E-52 to Q-234; E-52 to S-233; E-52 to S-232; E-52 to R-231; E-52 to D-230; E-52 to V-229; E-52 to R-228; E-52 to E-227; E-52 to P-226; E-52 to D-225; E-52 to G-224; E-52 to G-223; E-52 to G-222; E-52 to G-221; E-52 to S-220; E-52 to C-219; E-52 to I-218; E-52 to G-217; E-52 to K-216; E-52 to L-215; E-52 to Y-214; E-52 to P-213; E-52 to L-212; E-52 to V-211; E-52 to K-210; E-52 to K-209; E-52 to W-208; E-52 to L-207; E-52 to L-206; E-52 to S-205; E-52 to K-204; E-52 to C-203; E-52 to V-202; E-52 to F-201; E-52 to V-200; E-52 to A-199; E-52 to V-198; E-52 to I-197; E-52 to L-196; E-52 to V-195; E-52 to V-194; E-52 to A-193; E-52 to A-192; E-52 to V-191; E-52 to T-190; E-52 to V-189; E-52 to G-188; E-52 to I-187; E-52 to I-186; E-52 to I-185; E-52 to G-184; E-52 to S-183; E-52 to E-182; E-52 to K-181; E-52 to H-180; E-52 to V-179; E-52 to C-178; E-52 to E-177; E-52 to I-176; E-52 to D-175; E-52 to S-174; E-52 to W-173; E-52 to P-172; E-52 to T-171; E-52 to C-170; E-52 to D-169; E-52 to G-168; E-52 to V-167; E-52 to K-166; E-52 to V-165; E-52 to M-164; E-52 to G-163; E-52 to R-162; E-52 to P-161; E-52 to C-160; E-52 to G-159; E-52 to T-158; E-52 to R-157; E-52 to C-156; E-52 to K-155; E-52 to R-154; E-52 to C-153; E-52 to M-152; E-52 to E-151; E-52 to P-150; E-52 to S-149; E-52 to D-148; E-52 to E-147; E-52 to E-146; E-52 to R-145; E-52 to F-144; E-52 to T-143; E-52 to G-142; E-52 to E-141; E-52 to E-140; E-52 to C-139; E-52 to Q-138; E-52 to C-137; E-52 to V-136; E-52 to T-135; E-52 to N-134; E-52 to R-133; E-52 to T-132; E-52 to T-131; E-52 to T-130; E-52 to C-129; E-52 to P-128; E-52 to S-127; E-52 to L-126; E-52 to E-125; E-52 to V-124; E-52 to E-123; E-52 to G-122; E-52 to S-121; E-52 to D-120; E-52 to C-119; E-52 to R-118; E-52 to T-117; E-52 to C-116; E-52 to R-115; E-52 to L-114; E-52 to C-113; E-52 to F-112; E-52 to L-111; E-52 to L-110; E-52 to D-109; E-52 to N-108; E-52 to W-107; E-52 to H-106; E-52 to T-105; E-52 to S-104; E-52 to Y-103; E-52 to D-102; E-52 to Q-101; E-52 to G-100; E-52 to Y-99; E-52 to K-98; E-52 to C-97; E-52 to S-96; E-52 to I-95; E-52 to C-94; E-52 to D-93; E-52 to R-92; E-52 to G-91; E-52 to D-90; E-52 to E-89; E-52 to S-88; E-52 to I-87; E-52 to H-86; E-52 to H-85; E-52 to G-84; E-52 to P-83; E-52 to P-82; E-52 to C-81; E-52 to L-80; E-52 to G-79; E-52 to E-78; E-52 to S-77; E-52 to P-76; E-52 to S-75; E-52 to S-74; E-52 to R-73; E-52 to K-72; E-52 to Q-71; E-52 to Q-70; E-52 to P-69; E-52 to A-68; E-52 to A-67; E-52 to R-66; E-52 to Q-65; E-52 to Q-64; E-52 to P-63; E-52 to A-62; E-52 to L-61; E-52 to D-60; E-52 to Q-59; E-52 to Q-58; and E-52 to T-57; of the DR5 sequence shown in **FIG. 1** (which R-66; E-52 to Q-65; E-52 to Q-64; E-52 to P-63; E-52 to A-62; E-52 to L-61; residues in **FIG. 1** are numbered consecutively from 1

through 411 from the N-terminus to the C-terminus, while the amino acid residues in SEQ ID NO:2 are numbered consecutively from -51 through 360 to reflect the position of the predicted signal peptide).

[0163] The present invention is also directed to nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequences encoding the polypeptides described above. The invention is further directed to nucleic acid molecules comprising, or alternatively consisting of, polynucleotide sequences which encode polypeptides that are at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0164] In another embodiment, C-terminal deletions of the DR5 polypeptide can be described by the general formula 52-m² where m² is a number from 57 to 183 corresponding to the amino acid sequence identified in FIG. 1 (SEQ ID NO:2). In specific embodiments, C-terminal deletions of the DR5 of the invention comprise, or alternatively, consist of, a member selected from the group consisting of residues: E-52 to S-183; E-52 to E-182; E-52 to K-181; E-52 to H-180; E-52 to V-179; E-52 to C-178; E-52 to E-177; E-52 to I-176; E-52 to D-175; E-52 to S-174; E-52 to W-173; E-52 to P-172; E-52 to T-171; E-52 to C-170; E-52 to D-169; E-52 to G-168; E-52 to V-167; E-52 to K-166; E-52 to V-165; E-52 to M-164; E-52 to G-163; E-52 to R-162; E-52 to P-161; E-52 to C-160; E-52 to G-159; E-52 to T-158; E-52 to R-157; E-52 to C-156; E-52 to K-155; E-52 to R-154; E-52 to C-153; E-52 to M-152; E-52 to E-151; E-52 to P-150; E-52 to S-149; E-52 to D-148; E-52 to E-147; E-52 to E-146; E-52 to R-145; E-52 to F-144; E-52 to T-143; E-52 to G-142; E-52 to E-141; E-52 to E-140; E-52 to C-139; E-52 to Q-138; E-52 to C-137; E-52 to V-136; E-52 to T-135; E-52 to N-134; E-52 to R-133; E-52 to T-132; E-52 to T-131; E-52 to T-130; E-52 to C-129; E-52 to P-128; E-52 to S-127; E-52 to L-126; E-52 to E-125; E-52 to V-124; E-52 to E-123; E-52 to G-122; E-52 to S-121; E-52 to D-120; E-52 to C-119; E-52 to R-118; E-52 to T-117; E-52 to C-116; E-52 to R-115; E-52 to L-114; E-52 to C-113; E-52 to F-112; E-52 to L-111; E-52 to L-110; E-52 to D-109; E-52 to N-108; E-52 to W-107; E-52 to H-106; E-52 to T-105; E-52 to S-104; E-52 to Y-103; E-52 to D-102; E-52 to Q-101; E-52 to G-100; E-52 to Y-99; E-52 to K-98; E-52 to C-97; E-52 to S-96; E-52 to I-95; E-52 to C-94; E-52 to D-93; E-52 to R-92; E-52 to G-91; E-52 to D-90; E-52 to E-89; E-52 to S-88; E-52 to I-87; E-52 to H-86; E-52 to H-85; E-52 to G-84; E-52 to P-83; E-52 to P-82; E-52 to C-81; E-52 to L-80; E-52 to G-79; E-52 to E-78; E-52 to S-77; E-52 to P-76; E-52 to S-75; E-52 to S-74; E-52 to R-73; E-52 to K-72; E-52 to Q-71; E-52 to Q-70; E-52 to P-69; E-52 to A-68; E-52 to A-67; E-52 to R-66; E-52 to Q-65; E-52 to Q-64; E-52 to P-63; E-52 to A-62; E-52 to L-61; E-52 to D-60; E-52 to Q-59; E-52 to Q-58; and E-52 to T-57 of the DR5 extracellular domain sequence shown in FIG. 1 (SEQ ID NO:2).

[0165] The present invention is also directed to nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide sequence at least 80%, 85%, 90%, 92%,

95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequences encoding the polypeptides described above. The invention is further directed to nucleic acid molecules comprising, or alternatively consisting of, polynucleotide sequences which encode polypeptides that are at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptides described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

[0166] The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of a DR5 polypeptide, which may be described generally as having residues n¹-m¹ and/or n²-m² of FIG. 1 (i.e., SEQ ID NO:2), where n¹, n², m¹, and m² are integers as described above.

[0167] Also included are a nucleotide sequence encoding a polypeptide consisting of a portion of the complete DR5 amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 97920, where this portion excludes from 1 to about 78 amino acids from the amino terminus of the complete amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 97920, or from 1 to about 233 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 97920. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

[0168] Preferred amongst the N- and C-terminal deletion mutants are those comprising, or alternatively consisting of, only a portion of the extracellular domain; i.e., within residues 52-184, since any portion therein is expected to be soluble.

[0169] It will be recognized in the art that some amino acid sequence of DR5 can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein that determine activity. Such areas will usually comprise residues which make up the ligand binding site or the death domain, or which form tertiary structures which affect these domains.

[0170] Thus, the invention further includes variations of the DR5 protein that show substantial DR5 protein activity or which include regions of DR5, such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J. U. et al., *Science* 247:1306-1310 (1990).

[0171] Thus, the fragment, derivative, or analog of the polypeptide of SEQ ID NO:2, or that encoded by the deposited cDNA, may be (i) one in which at least one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue(s), and more preferably at least one but less than ten conserved amino acid residues) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent

group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein. Polynucleotides encoding these fragments, derivatives or analogs are also encompassed by the invention.

[0172] Of particular interest are substitutions of charged amino acids with another charged amino acids and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the DR5 protein. Additionally, one or more of the amino acid residues of the polypeptides of the invention (e.g., arginine and lysine residues) may be deleted or substituted with another residue to eliminate undesired processing by proteases such as, for example, furins or kexins. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard et al., *Clin Exp Immunol.* 2:331-340 (1967); Robbins et al., *Diabetes* 36:838-845 (1987); Cleland et al. *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

[0173] The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade et al., *Nature* 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF-alpha to only one of the two known types of TNF receptors. Thus, the DR5 receptor of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

[0174] As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table II).

TABLE II

Conservative Amino Acid Substitution	
Aromatic	Phenylalanine Tryptophan
Hydrophobic	Tyrosine Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

[0175] In specific embodiments, the number of substitutions, additions or deletions in the amino acid sequence of

FIG. 1 and/or any of the polypeptide fragments described herein (e.g., the extracellular domain or intracellular domain) is 75, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 30-20, 20-15, 20-10, 15-10, 10-1, 5-10, 1-5, 1-3 or 1-2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0176] Amino acids in the DR5 protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or in vitro, or in vitro proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., *J. Mol. Biol.* 224:899-904 (1992) and de Vos et al. *Science* 255:306-312 (1992)).

[0177] Additionally, protein engineering may be employed to improve or alter the characteristics of DR5 polypeptides. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

[0178] Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see e.g., Carter et al., *Nucl. Acids Res.* 13:4331 (1986); and Zoller et al., *Nucl. Acids Res.* 10:6487 (1982)), cassette mutagenesis (see e.g., Wells et al., *Gene* 34:315 (1985)), and restriction selection mutagenesis (see e.g., Wells et al., *Philos. Trans. R. Soc. London SerA* 317:415 (1986)).

[0179] Thus, the invention also encompasses DR5 derivatives and analogs that have one or more amino acid residues deleted, added, or substituted to generate DR5 polypeptides that are better suited for expression, scale up, etc., in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions on any one or more of the glycosylation recognition sequences in the DR5 polypeptides of the invention, and/or an amino acid deletion at the second position of any one or more such recognition sequences will prevent glycosylation of the DR5 at the modified tripeptide sequence (see, e.g., Miyajima et al., *EMBO J* 5(6):1193-1197).

[0180] The polypeptides of the present invention also include a polypeptide comprising, or alternatively consisting of, one, two, three, four, five or more amino acid sequences selected from the group consisting of: the polypeptide encoded by the deposited cDNA (the deposit having ATCC

Accession Number 97920) including the leader; the mature polypeptide encoded by the deposited the cDNA minus the leader (i. e., the mature protein); a polypeptide comprising, or alternatively consisting of, amino acids from about -51 to about 360 in SEQ ID NO:2; a polypeptide comprising, or alternatively consisting of, amino acids from about -50 to about 360 in SEQ ID NO:2; a polypeptide comprising, or alternatively consisting of, amino acids from about 1 to about 360 in SEQ ID NO:2; a polypeptide comprising, or alternatively consisting of, the DR5 extracellular domain; a polypeptide comprising, or alternatively consisting of, the DR5 cysteine rich domain; a polypeptide comprising, or alternatively consisting of, the DR5 transmembrane domain; a polypeptide comprising, or alternatively consisting of, the DR5 intracellular domain; a polypeptide comprising, or alternatively consisting of, the extracellular and intracellular domains with all or part of the transmembrane domain deleted; and a polypeptide comprising, or alternatively consisting of, the DR5 death domain; as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, or 99% identical to the polypeptides described above, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0181] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a DR5 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the DR5 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0182] As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in FIG. 1 (SEQ ID NO:2), the amino acid sequence encoded by the deposited cDNA, or fragments thereof, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

[0183] In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100-residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90-residue subject sequence is compared with a 100-residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence, which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

[0184] The polypeptide of the present invention have uses that include, but are not limited to, use as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns and as a source for generating antibodies

that bind the polypeptides of the invention, using methods well known to those of skill in the art.

[0185] The present application is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the DR5 polypeptide sequence set forth herein as $n^1\text{-}m^1$, and/or $n^2\text{-}m^2$. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific DR5 N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0186] In certain preferred embodiments, DR5 proteins of the invention comprise fusion proteins as described above wherein the DR5 polypeptides are those described as $n^1\text{-}m^1$, and $n^2\text{-}m^2$, herein. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[0187] The present inventors have discovered that the DR5 polypeptide is a 411-residue protein exhibiting three main structural domains. First, the ligand-binding domain (extracellular domain) was identified within residues from about 52 to about 184 in **FIG. 1** (amino acid residues from about 1 to about 133 in SEQ ID NO:2). Second, the transmembrane domain was identified within residues from about 185 to about 208 in **FIG. 1** (amino acid residues from about 134 to about 157 in SEQ ID NO:2). Third, the intracellular domain was identified within residues from about 209 to about 411 in **FIG. 1** (amino acid residues from about 158 to about 360 in SEQ ID NO:2). Importantly, the intracellular domain includes a death domain at residues from about 324 to about 391 (amino acid residues from about 273 to about 340 in SEQ ID NO:2). Further preferred fragments of the polypeptide shown in **FIG. 1** include the mature protein from residues about 52 to about 411 (amino acid residues from about 1 to about 360 in SEQ ID NO:2), and soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain.

[0188] The invention further provides DR5 polypeptides encoded by the deposited cDNA including the leader and DR5 polypeptide fragments selected from the mature protein, the extracellular domain, the transmembrane domain, the intracellular domain, the death domain, and all combinations thereof.

[0189] In addition, proteins of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller, M., et al., Nature 310:105-111 (1984)). For example, a peptide corresponding to a fragment of the DR5 polypeptides of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the DR5 polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino

butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0190] Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see, e.g., Carter et al., *Nucl. Acids Res.* 13:4331 (1986); and Zoller et al., *Nucl. Acids Res.* 10:6487 (1982)), cassette mutagenesis (see, e.g., Wells et al., *Gene* 34:315 (1985)), restriction selection mutagenesis (see, e.g., Wells et al., *Philos. Trans. R. Soc. London SerA* 317:415 (1986)).

[0191] The invention additionally, encompasses DR5 polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin; etc.

[0192] Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[0193] Also provided by the invention are chemically modified derivatives of DR5, which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Pat. No. 4,179,337). The chemical moieties for derivation may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0194] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on

biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0195] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Pat. No. 5,643,575; Morpurgo et al., *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev et al., *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti et al., *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0196] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulphydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0197] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a protein via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0198] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety

from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation, which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0199] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis et al., *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Pat. No. 4,002,531; U.S. Pat. No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0200] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monomethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{CISO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to arnine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

[0201] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Pat. No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0202] The number of polyethylene glycol moieties attached to each protein of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

[0203] As mentioned, DR5 polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques, which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given DR5 polypeptide. Also, a given DR5 polypeptide may contain many types of modifications. DR5 polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic DR5 polypeptides may result from natural posttranslational processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS—STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., *Meth Enzymol* 182:626-646 (1990); Rattan et al., *Ann NY Acad Sci* 663:48-62 (1992)).

[0204] The DR5 polypeptides can be recovered and purified from chemical synthesis and recombinant cell cultures by standard methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography (“HPLC”) is employed for purification. Well-known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

[0205] DR5 polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of DR5. Among these are applications in the treatment and/or prevention of tumors, parasitic infections, bacterial infections, viral infections, restenosis, and graft vs. host disease; to induce resistance to parasites, bacteria and viruses; to induce proliferation of T-cells, endothelial cells and certain hematopoietic cells; to regulate anti-viral responses; and to treat and/or prevent certain autoimmune diseases after stimulation of DR5 by an agonist. Additional applications relate to diagnosis, treatment, and/or prevention of disorders of cells, tissues and organisms. These aspects of the invention are discussed further below.

[0206] The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the

polypeptide having an amino acid sequence of SEQ ID NO:2, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in the cDNA deposited as ATCC Deposit No. 97920 or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:1 or contained in the cDNA deposited as ATCC Deposit No. 97920 under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:1), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

[0207] In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide described herein. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. The term “epitopes,” as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An “immunogenic epitope” is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an “antigenic epitope.” The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen et al., *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983).

[0208] Fragments that function as epitopes may be produced by any conventional means. (See, e.g., Houghten, *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985), further described in U.S. Pat. No. 4,631,211).

[0209] As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R. A., “Antibodies That React With Predetermined Sites on Proteins,” *Science* 219:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

[0210] Non-limiting examples of antigenic polypeptides or peptides that can be used to generate DR5-specific antibodies include: a polypeptide comprising, or alternatively consisting of, amino acid residues from about 62 to about 110 in FIG. 1 (about 62 to about 110 in SEQ ID NO:2); a polypeptide comprising, or alternatively consisting of, amino acid residues from about 119 to about 164 in FIG. 1 (about 119 to about 164 in SEQ ID NO:2); a polypeptide

comprising, or alternatively consisting of, amino acid residues from about 224 to about 271 in **FIG. 1** (about 224 to about 271 in SEQ ID NO:2); and a polypeptide comprising, or alternatively consisting of, amino acid residues from about 275 to about 370 in **FIG. 1** (about 275 to about 370 in SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the DR5 protein.

[0211] The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R. A., "General Method for the Rapid Solid-Phase Synthesis of Large Numbers of Peptides: Specificity of Antigen-Antibody Interaction at the Level of Individual Amino Acids," *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPs)" process is further described in U.S. Pat. No. 4,631,211 to Houghten et al. (1986). As one of skill in the art will appreciate, DR5 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker et al., *Nature* 331:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric DR5 protein or protein fragment alone (Fountoulakis et al., *J Biochem.* 270:3958-3964 (1995)).

Antibodies

[0212] The present invention further relates to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, preferably an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i. e., molecules that contain an antigen-binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0213] Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also

included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati et al.

[0214] The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt et al., *J. Immunol.* 147:60-69 (1991); U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelnik et al., *J. Immunol.* 148:1547-1553 (1992).

[0215] Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention that they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies that specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

[0216] Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies that bind polypeptides encoded by polynucleotides, which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10⁻²M, 10⁻²M, 5×10⁻³M, 10⁻³M, 5×10⁻⁴M, 10⁻⁴M, 5×10⁻⁵M,

10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

[0217] Antibodies that bind DR5 receptor polypeptides may bind them as isolated polypeptides or in their naturally occurring state. By “isolated polypeptide” is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an “isolated polypeptide” are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the DR5 polypeptide is substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). Thus, antibodies of the present invention may bind recombinantly produced DR5 receptor polypeptides.

[0218] In a specific embodiment, antibodies of the present invention bind a full-length DR5 receptor expressed on the surface of a cell comprising a polynucleotide encoding amino acids 1 to 411 of SEQ ID NO:2 operably associated with a regulatory sequence that controls gene expression. In another specific embodiment, antibodies of the present invention bind a full-length DR5 receptor expressed on the surface of a cell comprising a polynucleotide encoding the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 97920, operably associated with a regulatory sequence that controls gene expression.

[0219] In preferred embodiments, antibodies of the present invention bind the mature DR5 receptor expressed on the surface of a cell comprising a polynucleotide encoding amino acids about 52 to about 411 of SEQ ID NO:2 operably associated with a regulatory sequence that controls gene expression. In other preferred embodiments, antibodies of the present invention bind the mature DR5 receptor expressed on the surface of a cell comprising a polynucleotide encoding the amino acid sequence of the mature polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920, operably associated with a regulatory sequence that controls gene expression.

[0220] In preferred embodiments, antibodies of the present invention bind the extracellular domain of a DR5 receptor expressed on the surface of a cell comprising a polynucleotide encoding amino acids about 52 to about 184 of SEQ ID NO:2 operably associated with a regulatory sequence that controls gene expression. In other preferred embodiments, antibodies of the present invention bind the extracellular domain of a DR5 receptor expressed on the surface of a cell comprising a polynucleotide encoding the amino acid sequence of the extracellular domain of a polypeptide encoded by the cDNA contained in ATCC Deposit No. 97920, operably associated with a regulatory sequence that controls gene expression.

[0221] The present invention also provides antibodies that bind DR5 polypeptides that act as either DR5 agonists or DR5 antagonists. In specific embodiments, the antibodies of the invention stimulate apoptosis of DR5 expressing cells. In other specific embodiments, the antibodies of the invention inhibit TRAIL binding to DR5. In other specific embodiments, the antibodies of the invention upregulate DR5 expression.

[0222] The present invention also provides antibodies that inhibit apoptosis of DR5 expressing cells. In other specific embodiments, the antibodies of the invention downregulate DR5 expression.

[0223] In further embodiments, the antibodies of the invention have a dissociation constant (KD) of 10^{-7} M or less. In preferred embodiments, the antibodies of the invention have a dissociation constant (KD) of 10^{-9} M or less.

[0224] The present invention further provides antibodies that stimulate apoptosis of DR5 expressing cells better than an equal concentration of TRAIL polypeptide stimulates apoptosis of DR5 expressing cells.

[0225] The present invention further provides antibodies that stimulate apoptosis of DR5 expressing cells equally well in the presence or absence of antibody cross-linking reagents; and/or stimulate apoptosis with equal or greater potency as an equal concentration of TRAIL in the absence of a cross-linking antibody or other cross-linking agent.

[0226] In further embodiments, antibodies of the invention have an off rate (k_{off}) of 10^{-3} /sec or less. In preferred embodiments, antibodies of the invention have an off rate (k_{off}) of 10^{-4} /sec or less. In other preferred embodiments, antibodies of the invention have an off rate (k_{off}) of 10^{-5} /sec or less.

[0227] The present invention further encompasses methods and compositions for killing of cells expressing DR5 on their surface, comprising, or alternatively consisting of, contacting anti-DR5 antibodies of the invention with such cells expressing DR5 on their surface.

[0228] In specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing DR5 on their surface, comprising, or alternatively consisting of, contacting anti-DR5 antibodies of the invention with such cells expressing DR5 on their surface.

[0229] In further specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising amino acids about 52 to about 184 of SEQ ID NO:2 on their surface, comprising, or alternatively consisting of, contacting anti-DR5 antibodies of the invention with such cells expressing said polypeptide on their surface.

[0230] In further specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920 on their surface, comprising, or alternatively consisting of, contacting anti-DR5 antibodies of the invention with such cells expressing said polypeptide on their surface.

[0231] The present invention further encompasses methods and compositions for killing of cells expressing DR5 on their surface, comprising, or alternatively consisting of, administering to an animal, anti-DR5 antibodies of the invention in an amount effective to kill such DR5 expressing cells.

[0232] In specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing DR5 on their surface, comprising,

or alternatively consisting of, administering to an animal, anti-DR5 antibodies of the invention in an amount effective to induce apoptosis in such DR5 expressing cells.

[0233] In further specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising amino acids about 52 to about 184 of SEQ ID NO:2 on their surface, comprising, or alternatively consisting of, administering to an animal, anti-DR5 antibodies of the invention in an amount effective to induce apoptosis in such cells expressing said polypeptide on their surface.

[0234] In further specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920 on their surface, comprising, or alternatively consisting of, administering to an animal, anti-DR5 antibodies of the invention in an amount effective to induce apoptosis such cells expressing said polypeptide on their surface.

[0235] The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0236] Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies that disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies that do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand or receptor activity by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

[0237] The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies that bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies that bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies that activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation. The antibodies may be specified as agonists, antagonists or inverse agonists for biolog-

cal activities comprising the specific biological activities of the peptides of the invention disclosed herein. Thus, the invention further relates to antibodies that act as agonists or antagonists of the polypeptides of the present invention. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Pat. No. 5,811,097; Deng et al., *Blood* 92(6):1981-1988 (1998); Chen et al., *Cancer Res.* 58(16):3668-3678 (1998); Harrop et al., *J. Immunol.* 161(4):1786-1794 (1998); Zhu et al., *Cancer Res.* 58(15):3209-3214 (1998); Yoon et al., *J. Immunol.* 160(7):3170-3179(1998); Prat et al., *J. Cell. Sci.* 111(Pt2):237-247 (1998); Pitard et al., *J. Immunol. Methods* 205(2):177-190 (1997); Liautard et al., *Cytokine* 9(4):233-241 (1997); Carlson et al., *J. Biol. Chem.* 272(17):11295-11301 (1997); Taryman et al., *Neuron* 14(4):755-762 (1995); Muller et al., *Structure* 6(9):1153-1167 (1998); Bartunek et al., *Cytokine* 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

[0238] Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Example 20, below, as well as Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

[0239] Furthermore, antibodies of the present invention may be used to cause death of cells which express polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

[0240] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 396,387.

[0241] The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous

chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0242] The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen of interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Such adjuvants are also well known in the art.

[0243] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Thus, the term "monoclonal antibody" is not limited to antibodies produced through hybridoma technology. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma and recombinant and phage display technology.

[0244] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well-known in the art and are discussed in detail in Example 11. Briefly, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0245] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybri-

doma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

[0246] Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

[0247] For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles, which carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phages used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186(1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187:9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0248] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0249] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston

et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See, e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska. et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

[0250] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0251] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modi-

fied embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B-cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 96/34096; WO 96/33735; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.) and GenPharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0252] Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

[0253] Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444 (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

[0254] Antibodies of the invention may also include multimeric forms of antibodies. For example, antibodies of the invention may take the form of antibody dimers, trimers, or higher-order multimers of monomeric immunoglobulin molecules. Dimers of whole immunoglobulin molecules or of F(ab')_n fragments are tetravalent, whereas dimers of Fab fragments or scFv molecules are bivalent. Individual monomers within an antibody multimer may be identical or different, i.e., they may be heteromeric or homomeric antibody multimers. For example, individual antibodies within a multimer may have the same or different binding specificities.

[0255] Multimerization of antibodies may be accomplished through natural aggregation of antibodies or through chemical or recombinant linking techniques known in the art. For example, some percentage of purified antibody preparations (e.g., purified IgG1 molecules) spontaneously form protein aggregates containing antibody homodimers, and other higher-order antibody multimers. Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art. For example, heterobifunctional crosslinking agents including, but not limited to, SMCC [succinimidyl 4-(maleimidomethyl)cyclohexane-1-carboxylate] and SATA [N-succinimidyl S-acetylthioacetate] (available, for example, from Pierce Biotechnology, Inc. (Rockford, Ill.)) can be used to form antibody multimers. An exemplary protocol for the formation of antibody homodimers is given in Ghetie et al., *Proceedings of the National Academy of Sciences USA* (1997) 94:7509-7514, which is hereby incorporated by reference in its entirety. Antibody homodimers can be converted to Fab'2 homodimers through digestion with pepsin. Alternatively, antibodies can be made to multimerize through recombinant DNA techniques. IgM and IgA naturally form antibody multimers through the interaction with the J chain polypeptide. Non-IgA or non-IgM molecules, such as IgG molecules, can be engineered to contain the J chain interaction domain of IgA or IgM, thereby conferring the ability to form higher order multimers on the non-IgA or non-IgM molecules. (see, for example, Chintalacharuvu et al., (2001) *Clinical Immunology* 101:21-31. and Frigerio et al., (2000) *Plant Physiology* 123:1483-94., both of which are hereby incorporated by reference in their entireties.) ScFv dimers can also be formed through recombinant techniques known in the art; an example of the construction of scFv dimers is given in Goel et al., (2000) *Cancer Research* 60:6964-6971 which is hereby incorporated by reference in its entirety. Antibody multimers may be purified using any suitable method known in the art, including, but not limited to, size exclusion chromatography.

[0256] A. Polynucleotides Encoding Antibodies.

[0257] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:2.

[0258] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0259] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a

particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably polyA+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0260] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0261] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.* 278:457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0262] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human

antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

[0263] Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-554) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., 1988, *Science* 242:1038-1041).

[0264] B. Methods of Producing Antibodies

[0265] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[0266] Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, e.g., a heavy or light chain of an antibody of the invention, requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods that are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0267] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0268] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 1986, *Gene* 45:101; Cockett et al., 1990, *Bio/Technology* 8:2).

[0269] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors that direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0270] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned

individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0271] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, *Methods in Enzymol.* 153:51-544).

[0272] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[0273] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express the antibody molecule may be engineered. Rather than using expression vectors, which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci, which in turn can be cloned and expanded into cell lines. This method may advantageously be used to

engineer cell lines that express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0274] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1972, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes can be employed in tk-, hprt- or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5): 155-215); and hygro, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1, which are incorporated by reference herein in their entirities.

[0275] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

[0276] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers, which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0277] Once an antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an immunolog-

bulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

[0278] C. Antibody Conjugates

[0279] The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention to generate fusion proteins. Also encompassed are antibodies of the invention recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention. Furthermore, the antibodies may be specific for polypeptides (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Alternatively, antibodies of the present invention may be used to target conjugated polypeptides and/or compounds to particular cell types, either in vitro or in vivo, by fusing or conjugating the antibodies of the present invention to the polypeptides and/or compounds to be targeted.

[0280] Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. Also, antibodies of the present invention fused or conjugated to polypeptides and/or compounds may be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Pat. No. 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452 (1991), which are incorporated by reference in their entireties.

[0281] The present invention further includes compositions comprising the polypeptides, including antibodies, of the present invention fused or conjugated to antibody domains other than the variable regions. Furthermore, the present invention includes compositions comprising the antibodies of the present invention fused or conjugated to heterologous antibody domains other than variable regions. For example, the polypeptides including antibodies of the present invention may be fused or conjugated to a heterologous antibody Fc region, or portion thereof.

[0282] The antibody portion fused to a polypeptide and/or antibody of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides including antibodies, may also be fused or conjugated to the above antibody portions to form

multimers. For example, Fc portions fused to the polypeptides including antibodies of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides including antibodies of the present invention to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides including antibodies of the present invention to antibody portions are known in the art. See, e.g., U.S. Pat. Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991); Zheng et al., *J. Immunol.* 154:5590-5600 (1995); and Vil et al., *Proc. Natl. Acad. Sci. USA* 89:11337-11341(1992) (said references incorporated by reference in their entireties).

[0283] As discussed, supra, the polypeptides including antibodies of the present invention may be fused or conjugated to the above antibody portions to increase the in vivo half-life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides including antibodies of the present invention may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Trauneker et al., *Nature* 331:84-86 (1988).

[0284] The polypeptides including antibodies of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing, agonizing and/or antagonizing other molecules, than the monomeric secreted antibody, protein, antibody fragment or protein fragment alone. (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)). In many cases, the heterologous Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5 receptor, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).

[0285] The present invention further includes compositions comprising the antibodies of the present invention fused or conjugated to human serum albumin to increase the in vivo half-life of the antibodies or for use in immunoassays using methods known in the art. Further, the antibodies of the present invention may be fused or conjugated to human serum albumin to facilitate purification. In many cases, the human serum albumin part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. See e.g., U.S. Pat. No. 5,876,969, EP Patent 0413622, and U.S. Pat. No. 5,766,883, herein incorporated by reference in their entirety.

[0286] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such

as a peptide to facilitate their purification. In preferred embodiments, the marker amino acid sequence is a hexahistidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

[0287] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment and/or prevention regimens. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. See, for example, U.S. Pat. No. 4,741,900 for metal ions that can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbellifluorone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{111}In or ^{99}Tc .

[0288] Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytoidal agent, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0289] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[0290] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0291] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

[0292] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980, which is incorporated herein by reference in its entirety.

[0293] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

[0294] Additionally, antibodies of the invention may be modified by post-translational modifications encompassed including, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends, attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression.

[0295] Also provided by the invention are chemically modified antibody derivatives, which may provide additional advantages such as increased solubility, stability and circulating time of the antibody, or decreased immunoge-

nicity (see, U.S. Pat. No. 4,179,337). The chemical moieties for derivation may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The antibodies may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0296] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0297] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Pat. No. 5,643,575; Morpurgo et al., *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev et al., *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti et al., *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0298] The polyethylene glycol molecules (or other chemical moieties) should be attached to the antibody with consideration of effects on binding specificity and agonistic and/or antagonistic properties of the antibody.

[0299] As described supra, there are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride) and polyethylene glycol may be attached to antibodies via linkage to any of a number of amino acid residues. Furthermore, one may specifically desire antibodies chemically modified at the N-terminus.

[0300] Polyethylene glycol may be attached to the antibody either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis et al., *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Pat. No. 4,002,531; U.S. Pat. No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0301] As described supra, polyethylene glycol can also be attached to antibodies using a number of different intervening linkers. See e.g., U.S. Pat. No. 5,612,460, the entire disclosure of which is incorporated herein by reference.

[0302] The number of polyethylene glycol moieties attached to each antibody of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated antibodies of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per antibody molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

[0303] As described supra, antibodies may be modified by natural processes, such as posttranslational processing, or by chemical modification techniques, which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given antibody. Also, a given antibody may contain many types of modifications.

[0304] Modifications may include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS—STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., *Meth Enzymol* 182:626-646 (1990); Rattan et al., *Ann NY Acad Sci* 663:48-62 (1992)).

[0305] D. Assays for Antibody Binding

[0306] The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0307] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at

pH 7.2, 1% Trayslol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C., adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C., washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0308] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%-20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ³²P or ¹²⁵I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[0309] ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0310] The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be

determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ³H or ¹²⁵I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest is conjugated to a labeled compound (e.g., ³H or ¹²⁵I) in the presence of increasing amounts of an unlabeled second antibody.

[0311] E. Antibody Based Therapies

[0312] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating and/or preventing one or more of the disorders or conditions described herein. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof as described herein).

[0313] While not intending to be bound to theory, DR5 receptors are believed to induce programmed cell death by a process which involves the association/cross-linking of death domains between different receptor molecules. Further, DR5 ligands (e.g., TRAIL) that induce DR5 mediated programmed cell death are believed to function by causing the association/cross-linking of DR5 death domains. Thus, agents (e.g., antibodies) that prevent association/cross-linking of DR5 death domains will prevent DR5 mediated programmed cell death, and agents (e.g., antibodies) that facilitate the association/cross-linking of DR5 death domains will induce DR5 mediated programmed cell death.

[0314] As noted above, DR5 receptors have been shown to bind TRAIL. DR5 receptors are also known to be present in a number of tissues and on the surfaces of a number of cell types. These tissues and cell types include primary dendritic cells, endothelial tissue, spleen, lymphocytes of patients with chronic lymphocytic leukemia, and human thymus stromal cells. Further, as explained in more detail below, TRAIL has been shown to induce apoptosis and to inhibit the growth of tumor cells *in vivo*. Additionally, TRAIL activities are believed to be modulated, at least in part, through interaction with DR4 and DR5 receptors.

[0315] TRAIL is a member of the TNF family of cytokines, which has been shown to induce apoptotic cell death in a number of tumor cell lines, and appears to mediate its apoptosis inducing effects through interaction with DR4 and DR5 receptors. These death domain-containing receptors are believed to form membrane-bound self-activating signaling complexes, which initiate apoptosis through cleavage of caspases.

[0316] In addition to DR4 and DR5 receptors, TRAIL also binds to several receptors proposed to be "decoy" receptors, DcR2 (a receptor with a truncated death domain), DcR1 (a GPI-anchored receptor), and OPG (a secreted protein which binds to another member of the TNF family, RANKL).

[0317] Further, recent studies have shown that the rank-order of affinities of TRAIL for the recombinant soluble forms of its receptors is strongly temperature dependent. In particular, at 37 C, DR5 has the highest affinity for TRAIL and OPG having the lowest affinity.

[0318] The DR4 and DR5 receptor genes, as well as genes encoding two decoy receptors, have been shown to be located on human chromosome 8p21-22. Further, this region of the human genome is frequently disrupted in head and neck cancers.

[0319] It has recently been found that the FaDu nasopharyngeal cancer cell line contains an abnormal chromosome 8p21-22 region. (Ozoren et al., *Int. J. Oncol.* 16:917-925 (2000).) In particular, a homozygous deletion involving DR4, but not DR5, has been found in these cells. (Ozoren et al., *Int. J. Oncol.* 16:917-925 (2000).) The homozygous loss within the DR4 receptor gene in these FaDu cells encompasses the DR4 receptor death domain. This disruption of the DR4 receptor death domain is associated with resistance to TRAIL-mediated cytotoxicity. Further, re-introduction of a wild-type DR4 receptor gene has been shown to both lead to apoptosis and restoration of TRAIL sensitivity of FaDu cells. (Ozoren et al., *Int. J. Oncol.* 16:917-925 (2000).) These data indicate that the DR4 receptor gene may be inactivated in human cancers and DR4 receptor gene disruption may contribute to resistance to TRAIL therapy. It is expected that similar results would be found in cells having analogous deletions in the DR5 gene.

[0320] It has also been shown that overexpression of the cytoplasmic domain of the DR4 receptor in human breast, lung, and colon cancer cell lines leads to p53-independent apoptotic cell death which involves the cleavage of caspases. (Xu et al., *Biochem. Biophys. Res. Commun.* 269:179-190 (2000).) Further, DR4 cytoplasmic domain overexpression has also been shown to result in cleavage of both poly(ADP-ribose) polymerase (PARP) and a DNA fragmentation factor (ie., ICAD-DFF45). (Xu et al., *Biochem. Biophys. Res. Commun.* 269:179-190 (2000).) In addition, despite similar levels of DR4 cytoplasmic domain protein as compared to cancer cells tested, normal lung fibroblasts have been shown to be resistant to DR4 cytoplasmic domain overexpression and show no evidence of caspase-cleavage. (Xu et al., *Biochem. Biophys. Res. Commun.* 269:179-190 (2000).) Again, similar results are expected with cells that overexpress the cytoplasmic domain of DR5. Thus, the cytoplasmic domains of the DR4 and DR5 receptors are useful as agents for inducing apoptosis, for example, in cancer cells.

[0321] Further, overexpression of the cyclin-dependent kinase inhibitor p21 (WAF1/CIP1), as well as the N-terminal 91 amino acids of this protein, has cell cycle-inhibitory activity and inhibits DR4 cytoplasmic domain-dependent caspase cleavage. Thus, DR4 receptors are also involved in the regulation of cell cycle progression. As above, similar results are expected with the DR5 receptor. Thus, the DR4 and DR5 receptors, as well as agonists and antagonists of these receptors, are useful for regulating cell cycle progression.

[0322] Antibodies that bind to DR5 receptors are useful for treating and/or preventing diseases and conditions associated with increased or decreased DR5-induced apoptotic cell death. Further, these antibodies vary in the effect they

have on DR5 receptors. These effects differ based on the specific portions of the DR5 receptor to which the antibodies bind, the three-dimensional conformation of the antibody molecules themselves, and/or the manner in which they interact with the DR5 receptor. Thus, antibodies that bind to the extracellular domain of a DR5 receptor can either stimulate or inhibit DR5 activities (e.g., the induction of apoptosis). Antibodies that stimulate DR5 receptor activities (e.g., by facilitating the association between DR5 receptor death domains) are DR5 agonists, and antibodies that inhibit DR5 receptor activities (e.g., by blocking the binding of TRAIL and/or preventing the association between DR5 receptor death domains) are DR5 antagonists.

[0323] Antibodies of the invention which function as agonists and antagonists of DR5 receptors include antigen-binding antibody fragments such as Fab and F(ab')₂ fragments, Fd, single-chain Fvs (scFv), di sulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain, as well as polyclonal, monoclonal and humanized antibodies. Divalent antibodies are preferred as agonists. Each of these antigen-binding antibody fragments and antibodies are described in more detail elsewhere herein.

[0324] In view of the above, antibodies of the invention, as well as other agonists, are useful for stimulating DR5 death domain activity to promote apoptosis in cells which express DR5 receptors (e.g., cancer cells). Antibodies of this type are useful for prevention and/or treating diseases and conditions associated with increased cell survival and/or insensitivity to apoptosis-inducing agents (e.g., TRAIL), such as solid tissue cancers (e.g., skin cancer, head and neck tumors, breast tumors, endothelioma, lung cancer, osteoblastoma, osteoclastoma, and Kaposi's sarcoma) and leukemias.

[0325] Antagonists of the invention (e.g., anti-DR5 antibodies) function by preventing DR5 mediated apoptosis and are useful for preventing and/or treating diseases associated with increased apoptotic cell death. Examples of such diseases include diabetes mellitus, AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, toxin-induced liver disease, septic shock, cachexia and anorexia.

[0326] As noted above, DR5 receptors are present on the surfaces of T-cells. Thus, agonists of the invention (e.g., anti-DR5 receptor antibodies) are also useful for inhibiting T-cell mediated immune responses, as well as preventing and/or treating diseases and conditions associated with increased T-cell proliferation. Diseases and conditions associated with T-cell mediated immune responses and increased T-cell proliferation include graft-v-host responses and diseases, osteoarthritis, psoriasis, septicemia, inflammatory bowel disease, inflammation in general, autoimmune diseases, and T-cell leukemias.

[0327] When an agonist of the invention is administered to an individual for the treatment and/or prevention of a disease or condition associated with increased T-cell populations or increased cell proliferation (e.g., cancer), the antagonist may be co-administered with another agent, which induces apoptosis (e.g., TRAIL) or otherwise inhibits cell proliferation (e.g., an anti-cancer drug). Combination therapies of this nature, as well as other combination therapies, are discussed below in more detail.

[0328] Further, antagonists of the invention (e.g., anti-DR5 receptor antibodies) are also useful for enhancing

T-cell mediated immune responses, as well as preventing and/or treating diseases and conditions associated with decreased T-cell proliferation. Antibodies of the invention, which block the binding of DR5 receptor ligands to DR5 receptors or interfere with DR5 receptor conformational changes associated with membrane signal transduction can inhibit DR5-mediated T-cell apoptosis. The inhibition of DR5 mediated apoptosis can, for examples, either result in an increase in the expansion rate of in vivo T-cell populations or prevent a decrease in the size of such populations. Thus, antagonists of the invention can be used to prevent and/or treat diseases or conditions associated with decreased or decreases in T-cell populations. Examples of such diseases and conditions included acquired immune deficiency syndrome (AIDS) and related afflictions (e.g., AIDS related complexes), T-cell immunodeficiencies, radiation sickness, and T-cell depletion due to radiation and/or chemotherapy.

[0329] When an antagonist of the invention is administered to an individual for the treatment and/or prevention of a disease or condition associated with decreased T-cell populations, the antagonist may be co-administered with an agent, which activates and/or induces lymphocyte proliferation (e.g., a cytokine). Combination therapies of this nature, as well as other combination therapies, are discussed below in more detail.

[0330] Similarly, agonists and antagonists of the invention (e.g., anti-DR5 receptor antibodies) are also useful when administered alone or in combination with another therapeutic agent for either inhibiting or enhancing B-cell mediated immune responses, as well as preventing and/or treating diseases and conditions associated with increased or decreased B-cell proliferation.

[0331] Anti-DR5 antibodies are thus useful for treating and/or preventing malignancies, abnormalities, diseases and/or conditions involving tissues and cell types which express DR5 receptors (e.g., endothelial cells). Further, malignancies, abnormalities, diseases and/or conditions which can be treated and/or prevented by the induction of programmed cell death in cells which express DR5 receptors can be treated and/or prevented using DR5 receptor agonists of the invention. Similarly, malignancies, abnormalities, diseases and/or conditions which can be treated and/or prevented by inhibiting programmed cell death in cells which express DR5 receptors can be treated and/or prevented using DR5 receptor antagonists of the invention.

[0332] Further, antibodies of the invention, as well as other agonists, are useful for stimulating DR5 death domain activity in endothelial cells, resulting in anti-angiogenic activity. Antibodies of this type are useful for prevention and/or treating diseases and conditions associated with hypervascularization and neovascularization, such as rheumatoid arthritis and solid tissue cancers (e.g., skin cancer, head and neck tumors, breast tumors, endothelioma, osteoblastoma, osteoclastoma, and Kaposi's sarcoma), as well as diseases and conditions associated with chronic inflammation.

[0333] Diseases and conditions associated with chronic inflammation, such as ulcerative colitis and Crohn's disease, often show histological changes associated with the ingrowth of new blood vessels into the inflamed tissues. Agonists of the invention, which stimulate the activity of DR5 death domains, will induce apoptosis in endothelial

cells that express these receptors. As a result, agonists of the invention can inhibit the formation of blood and lymph vessels and, thus, can be used to prevent and/or treat diseases and conditions associated with hypervascularization and neovascularization.

[0334] Other diseases and conditions associated with angiogenesis which can be prevented and/or treated using agonists of the invention include hypertrophic and keloid scarring, proliferative diabetic retinopathy, arteriovenous malformations, atherosclerotic plaques, hemophilic joints, nonunion fractures, Osler-Weber syndrome, psoriasis, pyogenic granuloma, scleroderma, trachoma, menorrhagia, and vascular adhesions.

[0335] Further, agents that inhibit DR5 death domain activity (e.g., DR5 antagonists) are also useful for preventing and/or treating a number of diseases and conditions associated with decreased vascularization. As indicated above, examples of antagonists of DR5 receptor activity include anti-DR5 receptor antibodies. These antibodies can function, for examples, by either binding to DR5 receptors and blocking the binding of ligands which stimulate DR5 death domain activity (e.g., TRAIL) or inhibiting DR5 receptor conformational changes associated with membrane signal transduction.

[0336] An example of a condition associated with decreased vascularization that can be treated using antagonists of the invention is delayed wound healing. The elderly, in particular, often heal at a slower rate than younger individuals. Antagonists of the invention can thus prevent and/or inhibit apoptosis from occurring in endothelial cells at wound sites and thereby promote wound healing in healing impaired individuals, as well as in individuals who heal at "normal" rates. Thus, antagonists of the invention can be used to promote and/or accelerate wound healing. Antagonists of the invention are also useful for treating and/or preventing other diseases and conditions including restenosis, myocardial infarction, peripheral arterial disease, critical limb ischemia, angina, atherosclerosis, ischemia, edema, liver cirrhosis, osteoarthritis, and pulmonary fibrosis.

[0337] A number of additional malignancies, abnormalities, diseases and/or conditions which can be treated using the agonists and antagonists of the invention are set out elsewhere herein, for example, in the section below entitled "Therapeutics".

[0338] The antibodies of the present invention may be used therapeutically in a number of ways. For example, antibodies that bind polynucleotides or polypeptides of the present invention can be administered to an individual (e.g., a human) either locally or systemically. Further, these antibodies can be administered alone, in combination with another therapeutic agent, or associated with or bound to a toxin.

[0339] In a specific embodiment, antibodies or antibody compositions of the invention are administered in combination with DAB₃₈₉EGF, a diphtheria toxin fused to Epidermal Growth Factor. DAB₃₈₉EGF is described in Shaw et al., (1991) *The Journal of Biological Chemistry*, 266:21118-24, which is hereby incorporated by reference in its entirety. In a specific embodiment, antibodies or antibody compositions of the invention are administered in combination with

DAB₃₈₉EGF for the treatment of cancer, such as brain cancers and epithelial cancers. In a specific embodiment, antibodies or antibody compositions of the invention are administered in combination with DAB₃₈₉EGF for the treatment of astrocytomas. In a specific embodiment, antibodies or antibody compositions of the invention are administered in combination with DAB₃₈₉EGF for the treatment of glioblastoma multiforme (GBM).

[0340] The present invention provides antibodies, which may be administered in combination with one or more therapeutic agents and/or procedures in the treatment, prevention, amelioration and/or cure of cancers. In preferred embodiments, agonistic antibodies of the invention may be administered in combination with one or more therapeutic agents and/or procedures in the treatment, prevention, amelioration and/or cure of cancers.

[0341] Therapeutic agents, useful in the treatment, prevention, amelioration and/or cure of cancers, with which antibodies of the present invention may be administered, include, for example, biological agents (e.g., inhibitors of signaling pathways, inhibitors of gene transcription, inhibitors of multi-drug resistance (MDR) mechanisms, inhibitors of angiogenesis, inhibitors of matrix metalloproteinases, hormones and hormone antagonists, and compounds of unknown mechanism), chemotherapeutic agents (e.g., alkylating agents, antimetabolites, farnesyl transferase inhibitors, mitotic spindle inhibitors (plant-derived alkaloids), nucleotide analogs, platinum analogs, and topoisomerase inhibitors), corticosteroids, gene therapies, immunotherapeutic agents (e.g., monoclonal antibodies, cytokines and vaccines), phototherapy, radiosensitizing agents, treatment support agents (e.g., anti-emetic agents, analgesic agents and hematopoietic agents), and other miscellaneous drug types. Therapeutic procedures, useful in the treatment, prevention, amelioration and/or cure of cancers, with which agonistic antibodies of the present invention may be administered, include, for example, but are not limited to, surgical procedures and radiation therapies.

[0342] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, prevention, amelioration and/or cure of cancers.

[0343] In specific embodiments, antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment, prevention, amelioration and/or cure of cancers including, but not limited to, 81C6 (Anti-tenascin monoclonal antibody), 2-chlorodeoxyadenosine, A007 (4'-dihydroxybenzophenone-2, 4-dinitrophenylhydrazone), Abarelix® (Abarelix-Depot-M®, PPI-149, R-3827); Abiraterone acetate® (CB-7598, CB-7630), ABT-627 (ET-1 inhibitor), ABX-EGF (anti-EGFr MAb), Acetylinaline (CI-994, GOE-5549, GOR-5549, PD-130636), AG-2034 (AG-2024, AG-2032, GARFT [glycinamide ribonucleoside transformylase] inhibitor), Alanosine, Aldesleukin (IL-2, Proleukin®), Alemtuzumab® (Campath®), Alitretinoin (Panretin®, LGN-1057), Allopurinol (Aloprim®, Zyloprim®), Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Amifostine (Ethyol®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminoglutethimide (Cytadren®), Aminolevulinic acid (Levulan®, Kerastick®), Aminopterin, Amsacrine, Anastrozole (Arimidex®), Angiostatin, Annamycin (AR-

522, annamycin LF, Aronex®), Anti-idiotype therapy (BsAb), Anti-CD19/CD3 MAb (anti-CD19/CD3 scFv, anti-NHL MAb), APC-8015 (Provence®, Dendritic cell therapy), Aplidine (Aplidin®, Aplidina®), Arabinosylguanine (Ara-G, GW506U78, Nelzarabine®, Compound 506U78), Arsenic trioxide (Trisenox®, ATO, Atrivex®), Avorelin® (Meterelin®, MF-6001, EP-23904), B43-Genistein (anti-CD19 Ab/genistein conjugate), B43-PAP (anti-CD19 Ab/pokeweed antiviral protein conjugate), B7 antibody conjugates, BAY 43-9006 (Raf kinase inhibitor), BBR 3464, Betathine (Beta-LT), Bevacizumab® (Anti-VEGF monoclonal antibody, rhuMAb-VEGF), Bexarotene (Targretin®, LGD 1069), BIBH-1 (Anti-FAP MAb), BIBX-1382, Biclatamide (Casodex®), Biricodar dictrate (Incel®, Incel MDR Inhibitor), Bleomycin (Blenoxane®), BLP-25 (MUC-1 peptide), BLyS antagonists, BMS-214662 (BMS-192331, BMS-193269, BMS-206635), BNP-1350 (BNPI-1100, Karenitecins), Boronated Protoporphyrin Compound (PDIT, Photodynamic Immunotherapy), Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), Budesonide (Rhinocort®), Busulfan (Busulfex®, Myleran®), C225 (IMC-225, EGFR inhibitor, Anti-EGFr MAb, Cetuximab®), C242-DM1 (huC242-DM1), Cabergoline (Dostinex®), Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Carbendazin® (FB-642), Carboplatin (Paraplatin®, CBDCA), Carboxyamidotriazole (NSC 609974, CAI, L-651582), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), CC49-zeta gene therapy, CEA-cide® (Labetuzumab®, Anti-CEA monoclonal antibody, hMN-14), Cea Vac® (MAb 3H1), Celecoxib (Celebrex®), CEP-701 (KT-5555), Cereport® (Lobradimil®, RMP-7), Chlorambucil (Leukeran®), CHML (Cytotropic Heterogeneous Molecular Lipids), Cholecalciferol, CI-1033 (Pan-erbB RTK inhibitor), Cilengitide (EMD-121974, integrin alphavbeta3 antagonist), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (Intra-Dose®, FocaCist®), Cisplatin-liposomal (SPI-077), 9-cis retinoic acid (9-cRA), Cladribine (2-CdA, Leustatin®), Clofarabine (chloro-fluoro-araA), Clonidine hydrochloride (Duraclon®), CMB-401 (Anti-PEM MAb/calicheamycin), CMT-3 (COL-3, Metastat), Cordycepin, Cotara® (chTNT-1/B, [¹³¹I]-chTNT-1/B), CN-706, CP-358774 (Tarceva®, OSI-774, EGFR inhibitor), CP-609754, CP IL-4-toxin (IL-4 fusion toxin), CS-682, CT-2584 (Apra®, CT-2583, CT-2586, CT-3536), CTP-37 (Avicine®, hCG blocking vaccine), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, Depo-Cyt®), D-limonene, DAB389-EGF (EGF fusion toxin), Dacarbazine (DTIC), Daclizumab® (Zenapax®), Dactinomycin (Cosmegen®), Daunomycin (Daunorubicin®, Cerubidine®), Daunorubicin (DaunoXome®, Daunorubicin®, Cerubidine®), DeaVac® (CEA anti-idiotype vaccine), Decitabine (5-aza-2'-deoxycytidine), Declopamride (Oxi-104), Denileukin diftitox (Ontak®), Depsipeptide (FR901228, FK228), Dexamethasone (Decadron®), Dexrazoxane (Zinecard®), Diethylstilbestrol (DES), Dihydro-5-azacytidine, Docetaxel (Taxotere®, Taxane®), Dolasetron mesylate (Anzemet®), Dolastatin-10 (DOLA-10, NSC-376128), Doxorubicin (Adriamycin®, Doxil®, Rubex®), DPPE, DX-8951f (DX-8951), Edatrexate, EGF-P64k Vaccine, Elliott's B Solution®, EMD-121974, Endostatin, Eniluracil (776c85), EO9 (EO1, EO4, EO68, EO70, EO72), Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Epratuzumab® (Lymphocide®, humanized anti-CD22, HAT), Erythropoietin (EPO®, Epo-

gen®, Procrit®), Estramustine (Emcyt®), Etanidazole (Radanyl®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Exemestane (Aromasin®, Nikidess®), Exetecan mesylate (DX-8951, DX-8951f), Exisulind (SAAND, Aptosyn®, cGMP-PDE2 and 5 inhibitor), F19 (Anti-FAP monoclonal antibody, iodinated anti-FAP MAb), Fadrozole (Afema®, Fadrozole hydrochloride, Arensin®), Fenretinide® (4HPR), Fentanyl citrate (Actiq®), Filgrastim (Neupogen®, G-CSF), FK-317 (FR-157471, FR-70496), Flavopiridol (HMR-1275), Fly3/flk2 ligand (Mobista®), Fluasterone, Fludarabine (Fludara®, FAMP), Fludeoxyglucose (F-18®), Fluorouracil (5-FU, Adrucil®, Fluropex®, Efudex®), Flutamide (Eulexin®), FMdC (KW-2331, MDL-101731), Formestane (Lentaron®), Fotemustine (Muphoran®, Mustophoran®), FUDR (Flouxuridine®), Fulvestrant (Faslodex®), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Gadolinium texaphyrin (Motexafin gadolinium, Gd-Tex®, Xcytrin®), Galarubicin hydrochloride (DA-125), GBC-590, Gastrimune® (Anti-gastrin-17 immunogen, anti-g17), Gemcitabine (Gemto®, Gemzar®), Gentuzumabozogamicin (Mylotarg®), GL331, Globo H hexasaccharide (Globo H-KLH®), Glufosfamide® (β -D-glucosyl-isofosfamide mustard, D19575, INN), Goserelin acetate (Zoladex®), Granisetron (Kytril®), GVAX (GM-C SF gene therapy), Her-2/Neu vaccine, Hercepting (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), HSPPC-96 (HSP cancer vaccine, gp96 heat shock protein-peptide complex), Hu1D10 (anti-HLA-DR MAb, SMART 1D10), HumalYMP (anti-CD20 MAb), Hydrocortisone, Hydroxyurea (Hydrea®), Hypericin® (VIMRxyn®), I-131 Lipiodol®, ibritumomab® tiuxetan (Zevalin®), Idarubicin (Idamycin®, DMDR, IDA), Ifosfamide (IFEX®), Imatinib mesylate (STI-571, Imatinib®, Glivec®, Gleevec®, Ab1 tyrosine kinase inhibitor), INGN-101 (p53 gene therapy/retrovirus), INGN-201 (p53 gene therapy/adenovirus), Interferon alpha (Alfaferone®, Alpha-IFO), Interferon alpha 2a (Intron A®), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), Interleukin-2 (ProleukinR®), Intoplicine (RP 60475), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), ISIS-2053 (PKC-alpha antisense), ISIS-2503 (Ras antisense), ISIS-3521 (PKC-alpha antisense), ISIS-5132 (K-ras/raf antisense), Isoretinooin (13-CRA, 13-cis retinoic acid, Accutane®), Ketoconazole (Nizoral®), KRN-8602 (MX, MY-5, NSC-619003, MX-2), L-778123 (Ras inhibitors), L-asparaginase (Elspar®, Crastinin®, Asparaginase medac®, Kidrolase®), Leflunomide (SU-101, SU-0200), Letrozole (Femara®), Leucovorin (Leucovorin®, Wellcovorin®), Leuprolide acetate (Viadur®, Lupron®, Leuprorel®, Eligard®), Levectin® (cytorectin+ IL-2 gene, IL-2 gene therapy), Levamisole (Ergamisol®), Liaoazole (Liazal, Liazol, R-75251, R-85246, Ro-85264), Lmb-2 immunotoxin (anti-CD25 recombinant immuno toxin, anti-Tac(Fv)-PE38), Lometrexol (T-64, T-904064), Lomustine (CCNU®, CeeNU®), LY-335979, Lym-1 (131-I LYM-1), Lymphoma vaccine (Genitope), Mannan-MUC1 vaccine, Marimastat® (BB-2516, TA-2516, MMP inhibitor), MDX-447 (MDX-220, BAB-447, EMD-82633, H-447, anti-EGFr/FcGammaR1r), Mechlorethamine (Nitrogen Mustard, HN₂, Mustargen®), Megestrol acetate (Megace®, Pallace®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Mercaptopurine (6-mercaptopurine, 6-MP), Mesna (Mesnex®), Methotrexate® (MTX, Mexate®, Folex®), Methoxsalen (Uvadex®), 2-Methoxyestradiol (2-ME, 2-ME2), Methylprednisolone (Solumedrol®), Methyltestosterone (Android-10®, Testred®, Virilon®), MGV, Mitomycin C (Mitomycin®, Mutamycin®, Mito Extra®), Mitoxantrone (Novantrone®, DHAD), Mitumomab® (BEC-2, EMD-60205), Mivobulin isethionate (CI-980), MN-14 (Anti-CEA immunoradiotherapy, ¹³¹I-MN-14, ¹⁸⁸Re-MN-14), Motexafin Lutetium (Lutrin®, Oprin®, Lu-Tex®, lutetium texaphyrin, Lucyn®, Antrin®, MPV-2213 ad (Finroazole®), MS-209, Muc-1 vaccine, NaPro Paclitaxel, Nclarabine (Compound 506, U78), Neovastat® (AE-941, MMP inhibitor), Neogene compounds (Oncomyc-NG, Ressten-NG, myc antisense), Nilutamide (Nilandron®), NovoMAb-G2 scFv (NovoMAb-G2 IgM), O6-benzylguanine (BG, Procept®), Octreotide acetate (Sandostatin LAR® Depot), Odanasetron (Zofran®), Onconase (Ranpirnase®), OncoVAX-CL, OncoVAX-CL Jenner (GA-733-2 vaccine), OncoVAX-P (OncoVAX-PrPSA), Onyx-015 (p53 gene therapy), Oprelvekin (Neumage®), Orzel (Tegafur+ Uracil+Leucovorin), Oxaliplatin (Eloxatine®, Eloxatin®), Pacis® (BCG, live), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Pamidronate (Aredia®), PC SPES, Pegademase (Adagen®, Pegademase bovine), Pegaspargase® (Oncospas®), Peldesine (BCX-34, PNP inhibitor), Pemetrexed disodium (Alimta®, MTA, multitargeted anti-folate, LY 231514), Pentostatin (Nipent®, 2-deoxycoformycin), Perfosfamide (4-hydroperoxycyclophosphamide, 4-HC), Perillyl alcohol (perilla alcohol, perillic alcohol, perillol, NSC-641066), Phenylbutyrate, Pirarubicin (THP), Pivaloyloxymethyl butyrate (AN-9, Pivanex®), Porfimer sodium (Photofrin®), Prednisone, Prinomastat® (AG-3340, MMP inhibitor), Procarbazine (Matulane®), PROSTVAC, Providence Portland Medical Center Breast Cancer Vaccine, PS-341 (LDP-341, 26S proteosome inhibitor), PSMA MAb (Prostate Specific Membrane Antigen monoclonal antibody), Pyrazoloacridine (NSC-366140, PD-115934), Quinine, R115777 (Zarnestra®), Raloxifene hydrochloride (Evista®, Keoxifene hydrochloride), Raltitrexed (Tomudex®, ZD-1694), Rebeccamycin, Retinoic acid, R-flurbiprofen (Flurizan®, E-7869, MPC-7869), RFS-2000 (9-nitrocampothecan, 9-NC, rubitecan®), Rituximab® (Rituxan®, anti-CD20 MAb), RSR-13 (GSJ-61), Satraplatin (BMS-182751, JM-216), SCH 6636, SCH-66336, Sizofilan® (SPG, Sizofiran®, Schizophyllan®, Sonifilan®), SKI-2053R (NSC-D644591), Sobuzoxane (MST-16, Perazolin®), Squalamine (MSI-1256F), SR-49059 (vasopressin receptor inhibitor, V1a), Streptozocin (Zanosar®), SU5416 (Semaxanib®, VEGF inhibitor), SU6668 (PDGF-TK inhibitor), T-67 (T-138067, T-607), Talc (Sclerosol®), Tamoxifen (Nolvadex®), Taurolidine (Taurolin®), Temozolamide (Temodar®, NSC 362856), Teniposide (VM-26, Vumon®), TER-286, Testosterone (Andro®, Androderm®, Testoderm TTS®, Testoderm®, Depo-Testosterone®, Androgel®, depoAndro®), Tf-CRM107 (Transferrin-CRM-107), Thalidomide, Theratope, Thioguanine (6-thioguanine, 6-TG), Thiotapec (triethylenethiophosphaoramide, Thioplex®), Thymosin alpha 1 (Zadaxin®, Thymalfasin®), Tiazofurin (Tiazole®), Tirapazamine (SR-259075, SR-4233, Tiazofurin®, Win-59075), TNP-470 (AGM-1470, Fumagillin), Tocladesine (8-Cl-cAMP), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Toremifene (Estrimex®, Fareston®), Tositumomab® (Bexxar®), Tretinoin (Retin-A®, Atragen®, ATRA, Vesanoide®), TriAb® (anti-idiotype antibody immune stimulator), Trilostane (Modrefen®), Triptorelin pamoate (Trelstar Depot®,

Decapeptyl®), Trimetrexate (Neutrexin®), Troxacitabine (BCH-204, BCH-4556, Troxatyl®), TS-1, UCN-01 (7-hydroxystaurosporine), Valrubicin (Valstar®), Valspodar (PSC 833), Vapreotide® (BMY-41606), Vaxid (B-cell lymphoma DNA vaccine), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), Vindesine (Eldisine®, Fildesin®), Vinorelbine (Navelbine®), Vitaxin® (LM-609, integrin alphavbeta3 antagonistic MAbs), WF10 (macrophage regulator), WHI-P131, WT1 Vaccine, XR-5000 (DACA), XR-9576 (XR-9351, P-glycoprotein/MDR inhibitor), ZD-9331, ZD-1839 (IRESSA®), and Zoledronate (Zometa®).

[0344] In one embodiment, antibodies of the present invention may be administered in combination with a taxane. In another embodiment, antibodies of the present invention may be administered in combination with a taxane for the treatment of cancers that are resistant to individual chemotherapies. In a specific embodiment, antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®). In a specific embodiment, antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) for the treatment of cancers that are resistant to individual chemotherapies.

[0345] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a taxane. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a taxane for the treatment of cancers that are resistant to individual chemotherapies. In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®). In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) for the treatment of cancers that are resistant to individual chemotherapies

[0346] In one embodiment, antibodies of the present invention may be administered in combination with a platinum-based chemotherapeutic. In another embodiment, antibodies of the present invention may be administered in combination with a platinum-based chemotherapeutic for the treatment of cancers that are resistant to individual chemotherapies. In another specific embodiment, antibodies of the invention may be administered in combination with Carboplatin (Paraplatin®, CBDCA). In another specific embodiment, antibodies of the present invention may be administered in combination with Carboplatin (Paraplatin®, CBDCA) for the treatment of cancers that are resistant to individual chemotherapies.

[0347] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a platinum-based chemotherapeutic. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a platinum-based chemotherapeutic for the treatment of cancers that are resistant to individual chemotherapies. In another specific embodiment, agonistic antibodies of the invention may be administered in combination with Carboplatin (Paraplatin®, CBDCA). In another specific embodiment, agonistic antibodies of the present invention may be administered in combination with Carboplatin (Paraplatin®, CBDCA) for the treatment of cancers that are resistant to individual chemotherapies.

[0348] In one embodiment, antibodies of the present invention may be administered in combination with a topo-

somerase inhibitor. In another embodiment, antibodies of the present invention may be administered in combination with a topoisomerase inhibitor for the treatment of cancers that are resistant to individual chemotherapies. In a specific embodiment, antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1). In a specific embodiment, antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) for the treatment of cancers that are resistant to individual chemotherapies.

[0349] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a topoisomerase inhibitor. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a topoisomerase inhibitor for the treatment of cancers that are resistant to individual chemotherapies. In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1). In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) for the treatment of cancers that are resistant to individual chemotherapies.

[0350] In one embodiment, antibodies of the present invention may be administered in combination with a fluoropyrimidine. In another embodiment, antibodies of the present invention may be administered in combination with a fluoropyrimidine for the treatment of cancers that are resistant to individual chemotherapies. In another specific embodiment, antibodies of the invention may be administered in combination with Fluorouracil (5-FU, Adrucil®). In another specific embodiment, antibodies of the present invention may be administered in combination with Fluorouracil (5-FU, Adrucil®) for the treatment of cancers that are resistant to individual chemotherapies.

[0351] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a fluoropyrimidine. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a fluoropyrimidine for the treatment of cancers that are resistant to individual chemotherapies. In another specific embodiment, agonistic antibodies of the invention may be administered in combination with Fluorouracil (5-FU, Adrucil®). In another specific embodiment, agonistic antibodies of the present invention may be administered in combination with Fluorouracil (5-FU, Adrucil®) for the treatment of cancers that are resistant to individual chemotherapies.

[0352] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, prevention, amelioration and/or cure of cancers.

[0353] In further specific embodiments, antibodies of the present invention may be administered in combination with one or more combinations of therapeutic agents useful in the treatment, prevention, amelioration and/or cure of cancers including, but not limited to, 9-aminocamptotheacin+G-CSF, Adriamycin®+Blenoxane+Vinblastine+Dacarbazine (ABVD), BCNU (Carmustine)+Etoposide+Ara-C (Cytarabine)+Melphalen (BEAM), Bevacizumab®+Leucovorin,

Bleomycin+Etoposide+Platinol® (Cisplatin) (BEP), Bleomycin+Etoposide+Adriamycin+Cyclophosphamide+Vincristine+Procarbazine+Prednisone (BEACOPP), Bryostatin+Vincristine, Busulfan+Melphalan, Carboplatin+Cereport®, Carboplatin+Cyclophosphamide, Carboplatin+Paclitaxel, Carboplatin+Etoposide+Bleomycin (CEB), Carboplatin+Etoposide+Thiotepa, Cisplatin+Cyclophosphamide, Cisplatin+Docetaxel, Cisplatin+Doxorubicin, Cisplatin+Etoposide, Cisplatin+Gemcitabine, Cisplatin+Interferon alpha, Cisplatin+Irinotecan, Cisplatin+Paclitaxel, Cisplatin+Teniposide, Cisplatin+Vinblastine, Cisplatin+Vindesine, Cisplatin+Vinorelbine, Cisplatin+Cytarabine+Ifosfamide, Cisplatin+Ifosfamide+Vinblastine, Cisplatin+Vinblastine+Mitomycin C, Cisplatin+Vincristine+Fluorouracil, Cisplatin+Vincristine+Lomustine, Cisplatin+Vinorelbine+Gemcitabine, Cisplatin+Carmustine+Dacarbazine+Tamoxifen, Cisplatin+Cyclophosphamide+Etoposide+Vincristine, Cisplatin (Platinol®)+Oncovint+Doxorubicin (Adriamycin®)+Etoposide (CODE), Cisplatin+Cytarabine+Ifosfamide+Etoposide+Methotrexate, Cyclophosphamide+Adriamycin® (Doxorubicin), Cyclophosphamide+Melphalan, Cyclophosphamide+SCH 6636, Cyclophosphamide+Adriamycin®+Cisplatin (Platinol®) (CAP), Cyclophosphamide+Adriamycin®+Vincristine (CAV), Cyclophosphamide+Doxorubicin+Teniposide+Prednisone, Cyclophosphamide+Doxorubicin+Teniposide+Prednisone+Interferon alpha, Cyclophosphamide+Epirubicin+Cisplatin (Platinol®) (CEP), Cyclophosphamide+Epirubicin+Fluorouracil, Cyclophosphamide+Methotrexate+Fluorouracil (CMF), Cyclophosphamide+Methotrexate+Vincristine (CMV), Cyclophosphamide+Adriamycin®+Methotrexate+Fluorouracil (CAMF), Cyclophosphamide+Adriamycin®+Methotrexate+Procarbazine (CAMP), Cyclophosphamide+Adriamycin®+Vincristine+Etoposide (CAV-E), Cyclophosphamide+Adriamycin®+Vincristine+Prednisone (CHOP), Cyclophosphamide+Novantrone® (Mitoxantrone)+Vincristine (Oncovorin)+Prednisone (CNOP), Cyclophosphamide+Adriamycin®+Vincristine+Prednisone+Rituximab (CHOP+Rituximab), Cyclophosphamide+Adriamycin®+Vincristine+Teniposide (CAV-T), Cyclophosphamide+Adriamycin®+Vincristine alternating with Platinol®+Etoposide (CAV/PE), Cyclophosphamide+BCNU (Carmustine)+VP-16 (Etoposide) (CBV), Cyclophosphamide+Vincristine+Prednisone (CVP), Cyclophosphamide+Oncovin®+Methotrexate+Fluorouracil (COMF), Cytarabine+Methotrexate, Cytarabine+Bleomycin+Vincristine+Methotrexate (CytaBOM), Dactinomycin+Vincristine, Dexamethasone+Cytarabine+Cisplatin (DHAP), Dexamethasone+Ifosfamide+Cisplatin+Etoposide (DICE), Docetaxel+Gemcitabine, Docetaxel+Vinorelbine, Doxorubicin+Vinblastine+Mechlorethamine+Vincristine+Bleomycin+Etoposide+Prednisone (Stanford V), Epirubicin+Gemcitabine, Estramustine+Docetaxel, Estramustine+Navelbine, Estramustine+Paclitaxel, Estramustine+Vinblastine, Etoposide (Vepesid®)+Ifosfamide+Cisplatin (Platinol®) (VIP), Etoposide+Vinblastine+Adriamycin (EVA), Etoposide (Vepesid®)+Ifosfamide+Cisplatin+Epirubicin (VIC-E), Etoposide+Methylprednisolone+Cytarabine+Cisplatin (ESHAP), Etoposide+Prednisone+Ifosfamide+Cisplatin (EPIC), Fludarabine+Mitoxantrone+Dexamethasone (FMD), Fludarabine+Dexamethasone+Cytarabine (ara-C)+Cisplatin (Platinol®) (FluDAP), Fluorouracil+Bevacizumab®, Fluorouracil+CeaVac®, Fluorouracil+Leucovorin, Fluorouracil+Levamisole, Flu-

rouracil+Oxaliplatin, Fluorouracil+Raltitrexed, Fluorouracil+SCH 6636, Fluorouracil+Trimetrexate, Fluorouracil+Leucovorin+Bevacizumab®, Fluorouracil+Leucovorin+Oxaliplatin, Fluorouracil+Leucovorin+Trimetrexate, Fluorouracil+Oncovin®+Mitomycin C (FOMi), Hydrazine+Adriamycin®+Methotrexate (HAM), Ifosfamide+Docetaxel, Ifosfamide+Etoposide, Ifosfamide+Gemcitabine, Ifosfamide+Paclitaxel, Ifosfamide+Vinorelbine, Ifosfamide+Carboplatin+Etoposide (ICE), Ifosfamide+Cisplatin+Doxorubicin, Irinotecan+C225 (Cetuximab®), Irinotecan+Docetaxel, Irinotecan+Etoposide, Iinotecan+Fluorouracil, Irinotecan+Gemcitabine, Mechlorethamine+Oncovin® (Vincristine)+Procarbazine (MOP), Mechlorethamine+Oncovin® (Vincristine)+Procarbazine+Prednisone (MOPP), Mesna+Ifosfamide+Idarubicin+Etoposide (MIZE), Methotrexate+Interferon alpha, Methotrexate+Vinblastine, Methotrexate+Cisplatin, Methotrexate with leucovorin rescue+Bleomycin+Adriamycin+Cyclophosphamide+Oncovorin+Dexamethasone (m-BACOD), Mitomycin C+Ifosfamide+Cisplatin (Platinol®) (MIP), Mitomycin C+Vinblastine+Paraplatin® (MVP), Mitoxantrone+Hydrocortisone, Mitoxantrone+Prednisone, Oncovin®+SCH 6636, Oxaliplatin+Leucovorin, Paclitaxel+Doxorubicin, Paclitaxel+SCH 6636, Paraplatin®+Docetaxel, Paraplatin®+Etoposide, Paraplatin®+Gemcitabine, Paraplatin®+Interferon alpha, Paraplatin®+Irinotecan, Paraplatin®+Paclitaxel, Paraplatin®+Vinblastine, Carboplatin (Paraplatin®)+Vincristine, Paraplatin®+Vindesine, Paraplatin®+Vinorelbine, Pemetrexed disodium+Gemcitabine, Platinol® (Cisplatin)+Vinblastine+Bleomycin (PVb), Prednisone+Methotrexate+Adriamycin+Cyclophosphamide+Etoposide (ProMACE), Procarbazine+Lomustine, Procarbazine+Lomustine+Vincristine+Thioguanine, Procarbazine+Oncovin®+CCNU®+Cyclophosphamide (POCC), Quinine+Doxorubicin, Quinine+Mitoxantrone+Cytarabine, Thiotepa+Etoposide, Thiotepa+Busulfan+Cyclophosphamide, Thiotepa+Busulfan+Melphalan, Thiotepa+Etoposide+Carmustine, Thiotepa+Etoposide+Carboplatin, Topotecan+Paclitaxel, Trimetrexate+Leucovorin, Vinblastine+Doxorubicin+Thiotepa, Vinblastine+Bleomycin+Etoposide+Carboplatin, Vincristine+Lomustine+Prednisone, Vincristine (Oncovin®)+Adriamycin®+Dexamethasone (VAD), Vincristine (Oncovin®)+Adriamycin®+Procarbazine (VAP), Vincristine+Dactinomycin+Cyclophosphamide, and Vinorelbine+Gemcitabine.

[0354] In one embodiment, antibodies of the present invention may be administered in combination with a taxane and a platinum-based chemotherapeutic. In another embodiment, antibodies of the present invention may be administered in combination with a taxane and a platinum-based chemotherapeutic for the treatment of cancers that are resistant to individual chemotherapies. In a specific embodiment, antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) and Carboplatin (Paraplatin®, CBDCA). In another specific embodiment, antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) and Carboplatin (Paraplatin®, CBDCA) for the treatment of cancers that are resistant to individual chemotherapies.

[0355] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a taxane and a platinum-based chemotherapeutic. In another

embodiment, agonistic antibodies of the present invention may be administered in combination with a taxane and a platinum-based chemotherapeutic for the treatment of cancers that are resistant to individual chemotherapies. In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) and Carboplatin (Paraplatin®, CBDCA). In another specific embodiment, agonistic antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) and Carboplatin (Paraplatin®, CBDCA) for the treatment of cancers that are resistant to individual chemotherapies.

[0356] In one embodiment, antibodies of the present invention may be administered in combination with a topoisomerase inhibitor and a fluoropyrimidine. In another embodiment, antibodies of the present invention may be administered in combination with a topoisomerase inhibitor and a fluoropyrimidine for the treatment of cancers that are resistant to individual chemotherapies. In a specific embodiment, antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) and Fluorouracil (5-FU, Adrucil®). In another specific embodiment, antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) and Fluorouracil (5-FU, Adrucil®) for the treatment of cancers that are resistant to individual chemotherapies.

[0357] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a topoisomerase inhibitor and a fluoropyrimidine. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a topoisomerase inhibitor and a fluoropyrimidine for the treatment of cancers that are resistant to individual chemotherapies. In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) and Fluorouracil (5-FU, Adrucil®). In another specific embodiment, agonistic antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) and Fluorouracil (5-FU, Adrucil®) for the treatment of cancers that are resistant to individual chemotherapies.

[0358] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described combinations of therapeutic agents in the treatment, prevention, amelioration and/or cure of cancers.

[0359] Antibodies of the present invention may be administered in combination with one or more therapeutic agents described above to treat, prevent, ameliorate and/or cure cancers of any tissue known to express DR5 receptor. In preferred embodiments, agonistic antibodies of the present invention are administered in combination with one or more therapeutic agents described above to treat, prevent, ameliorate and/or cure cancers of any tissue known to express DR5 receptor.

[0360] Tissues known to express DR5 receptor include, but are not limited to, heart, placenta, lung, liver, skeletal muscle, pancreas, spleen, thymus, prostate, testis, uterus, ovary, small intestine, colon, brain kidney, bone marrow, skin, pituitary, cartilage and blood.

[0361] In specific embodiments antibodies of the present invention may be administered in combination with one or more therapeutic agents, as described above, in the treatment, prevention, amelioration and/or cure of solid tissue cancers (e.g., skin cancer, prostate cancer, pancreatic cancer, hepatic cancer, lung cancer, ovarian cancer, colorectal cancer, head and neck tumors, breast tumors, endothelioma, osteoblastoma, osteoclastoma, Ewing's sarcoma, and Kaposi's sarcoma), as well as hematological cancers (e.g., leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, multiple myeloma).

[0362] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more therapeutic agents, as described above, in the treatment, prevention, amelioration and/or cure of solid tissue cancers (e.g., skin cancer, prostate cancer, pancreatic cancer, hepatic cancer, lung cancer, ovarian cancer, colorectal cancer, head and neck tumors, breast tumors, endothelioma, osteoblastoma, osteoclastoma, Ewing's sarcoma, and Kaposi's sarcoma), as well as hematological cancers (e.g., leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, multiple myeloma).

[0363] In specific embodiments antibodies of the present invention are used to treat, ameliorate and/or prevent skin cancers including basal cell carcinoma, squamous cell carcinoma and malignant melanoma. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and /or therapeutic agents to treat, ameliorate and/or prevent skin cancers.

[0364] In preferred embodiments agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent skin cancers including basal cell carcinoma, squamous cell carcinoma and malignant melanoma. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and /or therapeutic agents to treat, ameliorate and/or prevent skin cancers.

[0365] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of skin cancers including, but not limited to, Bleomycin (Blenoxane®), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cisplatin (Platinol®, CDDP), Dacarbazine (DTIC), Interferon alpha 2b (Intron A®), Interleukin-2 (ProleukinR®), Tamoxifen (Nolvadex®), Temozolamide (Temodar®, NSC 362856), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), and Vindesine (Eldisine®, Fildesin®). Combinations of therapeutic agents useful in the treatment of skin cancers include, but are not limited to, Cisplatin+Carmustine+Dacarbazine+Tamoxifen.

[0366] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of skin cancers.

[0367] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent head and neck cancers including brain cancers. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent head and neck cancers including brain cancers.

Brain cancers which may be treated using antibodies of the present invention include, but are not limited to, gliomas such as astrocytomas and oligodendromas, non-glial tumors such as neuronal, meningeal, ependymal and choroid plexus cell tumors, and metastatic brain tumors such as those originating as breast, lung, prostate and skin cancers.

[0368] In further preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent head and neck cancers including brain cancers. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent head and neck cancers including brain cancers. Brain cancers which may be treated using agonistic antibodies of the present invention include, but are not limited to, gliomas such as astrocytomas and oligodendromas, non-glial tumors such as neuronal, meningeal, ependymal and choroid plexus cell tumors, and metastatic brain tumors such as those originating as breast, lung, prostate and skin cancers.

[0369] In one preferred embodiment, agonistic antibodies of the invention are used to treat brain tumors. In a further preferred embodiment, agonistic antibodies of the invention are used to treat glioblastoma multiforme.

[0370] Antibodies of the present invention may be administered in combination with one or more radiological procedures useful in the treatment of brain cancers including, but not limited to, external beam radiation therapy, stereotactic radiation therapy, conformal radiation therapy, intensity-modulated radiation therapy (IMRT), and radiosurgery.

[0371] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more radiological procedures useful in the treatment of brain cancers including, but not limited to, external beam radiation therapy, stereotactic radiation therapy, conformal radiation therapy, intensity-modulated radiation therapy (IMRT), and radiosurgery.

[0372] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of brain cancers including, but not limited to, Bleomycin (Blenoxane®), Busulfan (Busulfex®, Myleran®), Carboplatin (Paraplatin®, CBDCA), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (IntraDose®, FocaCist®), Cyclophosphamide (Cytoxan®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®), Dacarbazine (DTIC®), Dactinomycin (Cosmegen®), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Docetaxel (Taxotere®, Taxane®), Dexamethasone (Decadron®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fluorouracil (5-FU, Adrucil®), Hydroxyurea (Hydrea®), Ifosfamide (IFEX®), Lomustine (CCNU®, CeeNU®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Mercaptopurine (6-mercaptopurine, 6-MP), Methchlorethamine (Nitrogen Mustard, HN2, Mustargen®), Methotrexate® (MTX, Mexate®, Folex®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexit®), Procarbazine (Matulane®), Temozolamide (Temodar®, NSC 362856), Teniposide (VM-26, Vumon®), Thioguanine (6-thioguanine, 6-TG), Thiotepa (triethylenethiophosphoramide), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), and Vincristine (Oncovin®, Onco TCS®, VCR, Leurochristine®).

[0373] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of brain cancers.

[0374] Further examples of therapeutic agents useful in the treatment of brain cancers which may be administered in combination with antibodies of the present invention include, but are not limited to, 81C6 (Anti-tenascin monoclonal antibody), BIBX-1382, Cereport® (Lobradimil®, RMP-7), Cilengitide® (EMD-121974, integrin alphavbeta3 antagonist), CMT-3 (Metastat®), Cotara® (chTNT-1/B, [¹³¹I]-chTNT-1/B), CP IL-4-toxin (IL-4 fusion toxin), Fenretinide® (4HPPR), Fotemustine (Muphoran®, Mustophoran®), Gemcitabine (Gemto®, Gemzar®), Hypericin® (VRXyn®), Imatinib mesylate (STI-571, Imatinib®, Glivec®, Gleevec®, Abl tyrosine kinase inhibitor), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Leflunomide (SU-101, SU-0200), Mivobulin isethionate (CI-980), O6-benzylguanine (BG, Procept®), Prinomastat® (AG-3340, MMP inhibitor), R115777 (Zarnestra®), SU6668 (PDGF-TK inhibitor), T-67 (T-138067, T-607), Tamoxifen (Nolvadex®), Tf-CRM107 (Transferrin-CRM-107), Thalidomide, Tiazofurin (Tiazole®), Vapretotide® (BMY-41606), Vinorelbine (Navelbine®), and XR-5000 (DACA).

[0375] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of brain cancers.

[0376] Preferred combinations of therapeutic agents useful in the treatment of brain cancers which may be administered in combination with antibodies of the present invention include, but are not limited to, Busulfan+Melphalan, Carboplatin+Cereport®, Carboplatin+Etoposide, Carboplatin+Etoposide+Thiotepa, Cisplatin+Etoposide, Cisplatin+Cytarabine+Ifosfamide, Cisplatin+Vincristine+Lomustine, Cisplatin+Cyclophosphamide+Etoposide+Vincristine, Cisplatin+Cytarabine+Ifosfamide+Etoposide+Methotrexate, Cyclophosphamide+Melphalan, Cytarabine+Methotrexate, Dactinomycin+Vincristine, Mechlorethamine+Oncovin® (Vincristine)+Procarbazine (MOP), Mechlorethamine+Oncovin® (Vincristine)+Procarbazine+Prednisone (MOPP), Carboplatin (Paraplatin)+Etoposide, Carboplatin (Paraplatin®)+Vincristine, Procarbazine+Lomustine, Procarbazine+Lomustine+Vincristine, Procarbazine+Lomustine+Vincristine+Thioguanine, Thiotepa+Etoposide, Thiotepa+Etoposide+Carmustine, Thiotepa+Etoposide+Carboplatin, Vinblastine+Bleomycin+Etoposide+Carboplatin, and Vincristine+Lomustine+Prednisone.

[0377] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described combinations of therapeutic agents in the treatment, amelioration and/or prevention of brain cancers.

[0378] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent breast cancer. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and /or therapeutic agents to treat, ameliorate and/or prevent breast cancer. Breast cancers which may be treated using antibodies of the present invention include, but are not limited to, ductal carcinoma, stage

I, stage II, stage III and stage IV breast cancers as well as invasive breast cancer and metastatic breast cancer.

[0379] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent breast cancer. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and /or therapeutic agents to treat, ameliorate and/or prevent breast cancer. Breast cancers which may be treated using agonistic antibodies of the present invention include, but are not limited to, ductal carcinoma, stage I, stage II, stage III and stage IV breast cancers as well as invasive breast cancer and metastatic breast cancer.

[0380] In one preferred embodiment, agonistic antibodies of the invention are used to treat metastatic breast cancer.

[0381] Antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of breast cancer.

[0382] In preferred embodiments, agonistic antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of breast cancer.

[0383] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of breast cancer including, but not limited to, Amifostine (Ethylol®), Aminoglutethimide (Cyadren®), Anastrozole (Arimidex®), Bleomycin (Blenoxane®), Capecitabine (Xeloda®), Doxifluridine®, oral 5-FU), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (IntraDose®, FocaCist®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Docetaxel (Taxotere®, Taxane®), Doxorubicin (Adriamycin®), Doxil®, Rubex®), Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Exemestane (Aromasin®, Nikidess®), Fadrozole (Afema®, Fadrozole hydrochloride, Arensin®), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®)), Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), Ifosfamide (IFEX®), Letrozole (Femara®), Leucovorin (Leucovorin®, Wellcovorin®), Mechlorethamine (Nitrogen Mustard, HN2, Mustargen®), Megestrol acetate (Megace®, Pallace®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Methotrexate® (MTX, Mexate®, Folex®), Methyltestosterone (Android-10®, Testred®, Vironol®), Mitomycin C (Mitomycin®, Mutamycin®, Mito Extra®), Orzel® (Tegafur+Uracil+Leucovorin), Paclitaxel (Paxene®, Taxol®), Sobuzoxane (MST-16, Perazolin®), Tamoxifen (Nolvadex®), Testosterone (Andro®, Androderm®, Testoderm TTS®, Testoderm®, Depo-Testosterone®, Androgel®, depoAndro®), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), and Vinorelbine (Navelbine®).

[0384] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of breast cancers.

[0385] Further examples of therapeutic agents useful in the treatment of breast cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Aldesleukin (IL-2, Proleukin®), Altretamine (Hexalen®, hexamethylmelamine, Hex-

astat®), Angiostatin, Annamycin (AR-522, annamycin LF, Aronex®), Bircodar dicitrate (Incel®, Incel MDR Inhibitor), Boronated Protoporphyrin Compound (PDIT, Photodynamic Immunotherapy), Bryostatin-1 (Bryostatin, BMY-45618, NSC-339555), Busulfan (Busulfex®, Myleran®)), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), D-limonene, Dacarbazine (DTIC), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Dolastatin-10 (DOLA-10, NSC-376128), DPPE, DX-8951f (DX-8951), EMD-121974, Endostatin, EO9 (EO1, EO4, EO68, EO70, EO72), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fluasterone, Fludarabine (Fludara®, FAMP), Flutamide (Eulexin®), Formestane (Lentaron®), Fulvestrant (Faslodex®), Galarubicin hydrochloride (DA-125), Gemcitabine (Gemto®, Gemzar®), Her-2/Neu vaccine, Hydroxyurea (Hydrea®), Idarubicin (Idamycin®, DMDR, IDA), Interferon alpha 2a (Intron A®), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), Irinotecan (Camptosar®, CPT-11, Topotecan®, CaptoCPT-1), Ketoconazole (Nizoral®), KRN-8602 (MX, MY-5, NSC-619003, MX-2), L-asparaginase (Elspar®), Leuprolide acetate (Viadur®, Lupron®), Lomustine (CCNU®, CeeNU®), LY-335979, Mannan-MUC1 vaccine, 2-Methoxyestradiol (2-ME, 2-ME2), Mitoxantrone (Novantrone®, DHAD), Motexafin Lutetium (Lutrin®, Optrin®, Lu-Tex®), lutetium texaphyrin, Lucyn®, Antrin®), MPV-2213ad (Finrozole®), MS-209, Muc-1 vaccine, NaPro Paclitaxel, Perillyl alcohol (perilla alcohol, perillie alcohol, perillol, NSC-641066), Pirarubicin (THP), Procabazine (Matulane®), Providence Portland Medical Center Breast Cancer Vaccine, Pyrazoloacridine (NSC-366140, PD-115934), Raloxifene hydrochloride (Evista®, Keoxifene hydrochloride), Raltitrexed (Tomudex®, ZD-1694), Rebecamycin, Streptozocin (Zanosar®), Temozolamide (Temodar®, NSC 362856), Theratope, Thiotepa (triethylenethiophosphoaramide, Thioplex®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Toremifene (Estrimex®, Fareston®), Trilostane (Modrefen®), and XR-9576 (XR-9351, P-glycoprotein/MDR inhibitor).

[0386] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of breast cancers.

[0387] Preferred combinations of therapeutic agents useful in the treatment of breast cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Cyclophosphamide+ Adriamycin® (Doxorubicin), Cyclophosphamide+ Epirubicin+Fluorouracil, Cyclophosphamide+ Methotrexate+Fluorouracil (CMF), Paclitaxel+ Doxorubicin, and Vinblastine+Doxorubicin+Thiotepa.

[0388] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of breast cancers.

[0389] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent lung cancer. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent lung cancer. Lung cancer which

may be treated using antibodies of the present invention includes, but is not limited to, non-small cell lung cancer (NSCLC) including early stage NSCLC (i.e., Stage IA/IB and Stage IIA/IIB), Stage IIIA NSCLC, Stage II(unresectable)/IIIB NSCLC and Stage IV NSCLC, small cell lung cancer (SCLC) including limited stage SCLC and extensive stage SCLC as well as Malignant Pleural Mesothelioma.

[0390] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent lung cancer. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent lung cancer. Lung cancer which may be treated using agonistic antibodies of the present invention includes, but is not limited to, non-small cell lung cancer (NSCLC) including early stage NSCLC (i.e., Stage IA/IB and Stage IIA/IIB), Stage IIIA NSCLC, Stage II(unresectable)/IIIB NSCLC and Stage IV NSCLC, small cell lung cancer (SCLC) including limited stage SCLC and extensive stage SCLC as well as Malignant Pleural Mesothelioma.

[0391] In one preferred embodiment, agonistic antibodies of the invention are used to treat non-small cell lung cancers.

[0392] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of lung cancer including, but not limited to, BAY 43-9006 (Raf kinase inhibitor), Carboplatin (Paraplatin®, CBDCA), Chlorambucil (Leukeran®), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (Intra-Dose®, FocaCist®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Docetaxel (Taxotere®, Taxane®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Edatrexate, Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepeside), Gemcitabine (Gemto®, Gemzar®), Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), Ifosfamide (IFEX®), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Lomustine (CCNU®, CeeNU®), Mechlorethamine (Nitrogen Mustard, HN2, Mustargen), Melphalan (L-PAM, Alkeran®D, Phenylalanine mustard), Methotrexate® (MTX, Mexate®, Folex®), Mitomycin C (Mitomycin®, Mutamycin®, Mito Extra®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Porfimer sodium (Photofrin®), Procarbazine (Matulane®), SKI-2053R (NSC-D644591), Teniposide (VM-26, Vumon®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), Vindesine (Eldisine®, Fildesin®), and Vinorelbine (Navelbine®).

[0393] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of lung cancers.

[0394] Further examples of therapeutic agents useful in the treatment of lung cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, ABX-EGF (anti-EGFr MAb), Acetyldinaline (CI-994), AG-2034 (AG-2024, AG-2032, GARFT [glycinamide ribonucleoside transformylase] inhibitor), Alanosine, Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Angiostatin, Aplidine (Aplidin®, Aplidina®), BBR 3464, Bexarotene (Targretin®, LGD1069), BIBH-1 (Anti-FAP MAb), BIBX-1382, BLP-25 (MUC-1 peptide), Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), Budesonide (Rhinocort®), C225 (IMC-225, EGFR inhibitor, Anti-EGFr MAb, Cetuximab®), Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Carboxyamidotriazole (NSC 609974, CAI, L-651582), CEA-cide® (Labetuzumab®, Anti-CEA monoclonal antibody, hMN-14), Cereport® (Lobradimil®, RMP-7), Cl-1033 (Pan-erbB RTK inhibitor), Cilengitide® (EMD-121974, integrin alphavbeta3 antagonist), 9-cis retinoic acid (9-cRA), Cisplatin-liposomal (SPI-077), CMB-401 (Anti-PEM MAb/calicheamycin), CMT-3 (Metastat®), CP-358774 (Tarceva(g, OSI-774, EGFR inhibitor), CT-2584 (Apra®), DAB389-EGF (EGF fusion toxin), DeaVac® (CEA anti-idiotype vaccine), Decitabine (5-aza-2'-deoxyytidine), Diethylnor-spermine (DENSPM), Dihydro-5-azacytidine, EGF-P64k Vaccine, Endostatin, Etanidazole (Radinil®), Exetecan mesylate (DX-8951, DX-8951f), Exisulind (SAAND, Aptosyn®, cGMP-PDE2 and 5 inhibitor), FK-317 (FR-157471, FR-70496), Flavopiridol (HMR-1275), Fotemustine (Muphoran®, Mustophoran®), G3139 (Genasense®, GenetaAnticode®, Bcl-2 antisense), Gadolinium texaphyrin (Motexafin gadolinium, Gd-Tex®, Xcytrin®), GBC-590, GL331, Galarubicin hydrochloride (DA-125), Glufosfamide® (β -D-glucosyl-isofosfamide mustard, D19575, INN), GVAX (GM-CSF gene therapy), INGN-101 (p53 gene therapy/retrovirus), INGN-201 (p53 gene therapy/ad-enovirus), Irofulven (MGI-114), ISIS-2053, ISIS-3521 (PKC-alpha antisense), ISIS-5132 (K-ras/raf antisense), Isotretinoin (13-CRA, 13-cis retinoic acid, Accutane®), Lometrexol (T-64, T-904064), Marimastat® (BB-2516, TA-2516, MMP inhibitor), MDX-447 (BAB-447, EMD-82633, H-447, anti-EGFr/FcGammaR1r), MGV, Mitumomab® (BEC-2, EMD-60205), Mivobulin isethionate (CI-980), Neovastat® (AE-941, MMP inhibitor), Onconase (Ranpirnase®), Onyx-015 (p53 gene therapy), Pemetrexed disodium (Alimta®, MTA, multitargeted antifolate, LY 231514), Pivaloyloxymethyl butyrate (AN-9, Pivanex®), Prinomastat® (AG-3340, MMP inhibitor), PS-341 (LDP-341, 26S proteosome inhibitor), Pyrazoloacridine (NSC-366140, PD-115934), R115777 (Zarnestra®), Raltitrexed (Tomudex®, ZD-1694), R-flurbiprofen (Flurizan®, E-7869, MPC-7869), RFS-2000 (9-nitrocamptothecan, 9-NC, rubitecang), RSR-13 (GSJ-61), Satraplatin (BMS-182751, JM-216), SCH-66336, Sizofilan® (SPG, Sizofiran®, Schizophyllan®, Sonifilan®), Squalamine (MSI-1256F), SR-49059 (vasopressin receptor inhibitor, V1a), SU5416 (Semaxanib®, VEGF inhibitor), Taurolidine (Taurolin®), Temozolamide (Temodar®, NSC 362856), Thalidomide, Thymosin alpha I (Zadaxin®, Thymalfasin®), Tirapazamine (SR-259075, SR-4233, Tirazone®, Win-59075), TNP-470 (AGM-1470), TriAbg (anti-idiotype antibody immune stimulator), Tretinoin (Retin-A®, Atragen®V, ATRA, Vesanoïd®), Troxacitabine (BCH-204, BCH-4556, Troxatyl®), Vitaxin®) (LM-609, integrin alphavbeta3 antagonistic MAb), XR-9576 (P-glycoprotein/MDR inhibitor), and ZD-1839 (IRESSA®).

[0395] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of lung cancers.

[0396] In one embodiment, antibodies of the present invention may be administered in combination with a tax-

ane. In another embodiment, antibodies of the present invention may be administered in combination with a taxane for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies. In a specific embodiment, antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®). In a specific embodiment, antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies.

[0397] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a taxane. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a taxane for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies. In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®). In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies.

[0398] In one embodiment, antibodies of the present invention may be administered in combination with a platinum-based chemotherapeutic. In another embodiment, antibodies of the present invention may be administered in combination with a platinum-based chemotherapeutic for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies. In another specific embodiment, antibodies of the invention may be administered in combination with Carboplatin (Paraplatin®, CBDCA). In another specific embodiment, antibodies of the present invention may be administered in combination with Carboplatin (Paraplatin®, CBDCA) for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies.

[0399] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a platinum-based chemotherapeutic. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a platinum-based chemotherapeutic for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies. In another specific embodiment, agonistic antibodies of the invention may be administered in combination with Carboplatin (Paraplatin®, CBDCA). In another specific embodiment, agonistic antibodies of the present invention may be administered in combination with Carboplatin (Paraplatin®, CBDCA) for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies.

[0400] Preferred combinations of therapeutic agents useful in the treatment of lung cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Cisplatin+Docetaxel, Cisplatin+Etoposide, Cisplatin+Gemcitabine, Cisplatin+Interferon alpha, Cisplatin+Irinotecan, Cisplatin+Paclitaxel, Cisplatin+Teniposide, Cisplatin+Vinblastine, Cisplatin+Vindesine, Cisplatin+Vinorelbine, Cisplatin+Vinblastine+Mitomycin C, Cisplatin+Vinorelbine+Gemcitabine,

Cisplatin (Platinol®)+Oncovin®+Doxorubicin (Adriamycin®)+Etoposide (CODE), Cyclophosphamide+Adriamycin®+Cisplatin (Platinol®) (CAP), Cyclophosphamide+Adriamycin®+Vincristine (CAV), Cyclophosphamide+Epirubicin+Cisplatin (Platinol®) (CEP), Cyclophosphamide+Methotrexate+Vincristine (CMV), Cyclophosphamide+Adriamycin®, Methotrexate+Fluorouracil (CAMF), Cyclophosphamide+Adriamycin®, Methotrexate+Procarbazine (CAMP), Cyclophosphamide+Adriamycin®, Vincristine+Etoposide (CAV-E), Cyclophosphamide+Adriamycin®, Vincristine+Teniposide (CAV-T), Cyclophosphamide+Oncovin®, Methotrexate+Fluorouracil (COMF), Cyclophosphamide+Adriamycin®+Vincristine, alternating with Cisplatin+Etoposide (CAV/PE), Docetaxel+Gemcitabine, Docetaxel+Vinorelbine, Etoposide (Vepesid®)+Ifosfamide+Cisplatin (Platinol®) (VIP), Etoposide (Vepesid®)+Ifosfamide, Cisplatin+Epirubicin (VIC-E), Fluorouracil+Oncovin®+Mitomycin C (FOMi), Hydrazine+Adriamycin®+Methotrexate (HAM), Ifosfamide+Docetaxel, Ifosfamide+Etoposide, Ifosfamide+Gemcitabine, Ifosfamide+Paclitaxel, Ifosfamide+Vinorelbine, Ifosfamide+Carboplatin+Etoposide (ICE), Iinotecan+Docetaxel, Irinotecan+Etoposide, Iinotecan+Gemcitabine, Methotrexate+Cisplatin, Methotrexate+Interferon alpha, Methotrexate+Vinblastine, Mitomycin C+Ifosfamide+Cisplatin (Platinol®) (MIP), Mitomycin C+Vinblastine+Paraplatin® (MVP), Paraplatin+Docetaxel, Paraplatin®+Etoposide, Paraplatin®+Gemcitabine, Paraplatin®+Interferon alpha, Paraplatin®+Irinotecan, Paraplatin®+Paclitaxel, Paraplatin®+Vinblastine, Paraplatin®+Vindesine, Paraplatin®+Vinorelbine, Procarbazine+Oncovin®+CCNU® (Lomustine)+Cyclophosphamide (POCC), Vincristine (Oncovin®)+Adriamycin®+Procarbazine (VAP), and Vinorelbine+Gemcitabine.

[0401] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of lung cancers.

[0402] In one embodiment, antibodies of the present invention may be administered in combination with a taxane and a platinum-based chemotherapeutic. In another embodiment, antibodies of the present invention may be administered in combination with a taxane and a platinum-based chemotherapeutic for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies. In a specific embodiment, antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) and Carboplatin (Paraplatin®, CBDCA). In another specific embodiment, antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) and Carboplatin (Paraplatin®, CBDCA) for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies.

[0403] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a taxane and a platinum-based chemotherapeutic. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a taxane and a platinum-based chemotherapeutic for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies. In a specific embodiment,

agonistic antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) and Carboplatin (Paraplatin®, CBDCA). In another specific embodiment, agonistic antibodies of the present invention may be administered in combination with Docetaxel (Taxotere®) and Carboplatin (Paraplatin®, CBDCA) for the treatment of lung cancers, such as non-small cell lung cancer, that are resistant to individual chemotherapies.

[0404] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent colorectal cancer. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent colorectal cancer. Colorectal cancers which may be treated using antibodies of the present invention include, but are not limited to, colon cancer (e.g., early stage colon cancer (stage I and II), lymph node positive colon cancer (stage III), metastatic colon cancer (stage IV)) and rectal cancer.

[0405] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent colorectal cancer. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent colorectal cancer. Colorectal cancers which may be treated using agonistic antibodies of the present invention include, but are not limited to, colon cancer (e.g., early stage colon cancer (stage I and II), lymph node positive colon cancer (stage III), metastatic colon cancer (stage IV)) and rectal cancer.

[0406] In one preferred embodiment, agonistic antibodies of the invention are used to treat colon cancer.

[0407] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of colorectal cancer including, but not limited to, Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Leucovorin (Leucovorin®, Wellcovorin®), and Levamisole (Ergamisol®). In one embodiment, antibodies of the present invention may be administered in combination with a topoisomerase inhibitor. In another embodiment, antibodies of the present invention may be administered in combination with a topoisomerase inhibitor for the treatment of colon cancer that is resistant to individual chemotherapies. In a specific embodiment, antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1). In a specific embodiment, antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) for the treatment of colon cancer that is resistant to individual chemotherapies.

[0408] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a topoisomerase inhibitor. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a topoisomerase inhibitor for the treatment of colon cancer that is resistant to individual chemotherapies. In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-

1). In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) for the treatment of colon cancer that is resistant to individual chemotherapies.

[0409] In one embodiment, antibodies of the present invention may be administered in combination with a fluoropyrimidine. In another embodiment, antibodies of the present invention may be administered in combination with a fluoropyrimidine for the treatment of colon cancer that is resistant to individual chemotherapies. In another specific embodiment, antibodies of the invention may be administered in combination with Fluorouracil (5-FU, Adrucil®). In another specific embodiment, antibodies of the present invention may be administered in combination with Fluorouracil (5-FU, Adrucil®) for the treatment of colon cancer that is resistant to individual chemotherapies.

[0410] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a fluoropyrimidine. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a fluoropyrimidine for the treatment of colon cancer that is resistant to individual chemotherapies. In another specific embodiment, agonistic antibodies of the invention may be administered in combination with Fluorouracil (5-FU, Adrucil®). In another specific embodiment, agonistic antibodies of the present invention may be administered in combination with Fluorouracil (5-FU, Adrucil®) for the treatment of colon cancer that is resistant to individual chemotherapies.

[0411] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of colorectal cancers.

[0412] Preferred combinations of therapeutic agents useful in the treatment of colorectal cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Fluorouracil+Leucovorin, and Fluorouracil+Levamisole.

[0413] In one embodiment, antibodies of the present invention may be administered in combination with a topoisomerase inhibitor and a fluoropyrimidine. In another embodiment, antibodies of the present invention may be administered in combination with atropoisomerase inhibitor and a fluoropyrimidine for the treatment of colon cancer, that are resistant to individual chemotherapies. In a specific embodiment, antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) and Fluorouracil (5-FU, Adrucil®). In another specific embodiment, antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) and Fluorouracil (5-FU, Adrucil®) for the treatment of colon cancer that is resistant to individual chemotherapies.

[0414] In one embodiment, agonistic antibodies of the present invention may be administered in combination with a topoisomerase inhibitor and a fluoropyrimidine. In another embodiment, agonistic antibodies of the present invention may be administered in combination with a topoisomerase inhibitor and a fluoropyrimidine for the treatment of colon

cancer, that are resistant to individual chemotherapies. In a specific embodiment, agonistic antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) and Fluorouracil (5-FU, Adrucil®). In another specific embodiment, agonistic antibodies of the present invention may be administered in combination with Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1) and Fluorouracil (5-FU, Adrucil®) for the treatment of colon cancer, that are resistant to individual chemotherapies.

[0415] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of colorectal cancers.

[0416] Further examples of therapeutic agents useful in the treatment of colorectal cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Aminocamptothevin (9-AC, 9-Aminocamptothevin, NSC 603071), Aplidine (Aplidin®, Aplidina®), Bevacizumab® (Anti-VEGF monoclonal antibody, rhuMAb-VEGF), C225 (IMC-225, EGFR inhibitor, Anti-EGFr MAb, Cetuximab®), C242-DM1 (huC242-DM1), CC49-zeta gene therapy, CEA-cide® (Labetuzumab®g, Anti-CEA monoclonal antibody, hMN-14), CeaVac® (MAb 3H 1), CP-609754, CTP-37 (Avicine®, hCG blocking vaccine), Declopamide (Oxi-104), Eniluracil (776c85), F19 (Anti-FAP monoclonal antibody, iodinated anti-FAP MAB), FMdC (KW-2331, MDL-101731), FUDR (Floxuridine®), Gemcitabine (Gemto®, Gemzar®), Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), Intoplicine (RP 60475), L-778123 (Ras inhibitors), Leuvelectin® (cytotoxin+IL-2 gene, IL-2 gene therapy), MN-14 (Anti-CEA immunoradiotherapy, ¹³¹I-MN-14, ¹⁸⁸Re-MN-14), OncoVAX-CL, OncoVAX-CL-Jenner (GA-733-2 vaccine). Orzel® (Tegafur+Uracil+Leucovorin), Oxaliplatin (Eloxatine®, Eloxatin®), Paclitaxel-DHA (Taxoprexin®), Pemetrexed disodium (Alimta®, MTA, multitargeted antifolate, LY 231514), R115777 (Zarnestra®), Raltitrexed (Tomudex®, ZD-1694), SCH 66336, SU5416 (Semaxanib®, VEGF inhibitor), Tocladesine (8-Cl-cAMP), Trimetrexate (Neutrexin®), TS-1, and ZD-9331.

[0417] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of colorectal cancers.

[0418] Further exemplary combinations of therapeutic agents useful in the treatment of colorectal cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Aminocamptothevin+G-CSF, Bevacizumab®+Fluorouracil, Bevacizumab®+Leucovorin, Bevacizumab®+Fluorouracil+Leucovorin, Cyclophosphamide+SCH 6636, Fluorouracil+CeaVac®, Fluorouracil+Oxaliplatin, Fluorouracil+Raltitrexed, Fluorouracil+SCH 6636, Fluorouracil+Trimetrexate, Fluorouracil+Leucovorin+Oxaliplatin, Fluorouracil+Leucovorin+Trimetrexate, Irinotecan+C225 (Cetuximab®), Oncovin®+SCH 6636, Oxaliplatin+Leucovorin, Paclitaxel+SCH 6636, Pemetrexed disodium+Gemcitabine, and Trimetrexate+Leucovorin.

[0419] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or

more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of colorectal cancers.

[0420] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent prostate cancer. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent prostate cancer. Prostate cancer which may be treated using antibodies of the present invention includes, but is not limited to, benign prostatic hyperplasia, malignant prostate cancer (e.g., stage I, stage II, stage III or stage IV) and metastatic prostate cancer.

[0421] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent prostate cancer. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent prostate cancer. Prostate cancer which may be treated using agonistic antibodies of the present invention includes, but is not limited to, benign prostatic hyperplasia, malignant prostate cancer (e.g., stage I, stage II, stage III or stage IV) and metastatic prostate cancer.

[0422] In one preferred embodiment, agonistic antibodies of the invention are used to treat malignant prostate cancer. In a further preferred embodiment, agonistic antibodies of the invention are used to treat metastatic prostate cancer.

[0423] Antibodies of the present invention may be administered in combination with one or more surgical, radiological and/or hormonal procedures useful in the treatment of prostate cancer including, but not limited to, prostatectomy (e.g., radical retropubic prostatectomy), external beam radiation therapy, brachytherapy, orchectomy and hormone treatment (e.g., LHRH agonists, androgen receptor inhibitors).

[0424] In preferred embodiments, agonistic antibodies of the present invention may be administered in combination with one or more surgical, radiological and/or hormonal procedures useful in the treatment of prostate cancer including, but not limited to, prostatectomy (e.g., radical retropubic prostatectomy), external beam radiation therapy, brachytherapy, orchectomy and hormone treatment (e.g., LHRH agonists, androgen receptor inhibitors).

[0425] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of prostate cancer including, but not limited to, Aminoglutethimide (Cytadren®), Biclutamide (Casodex®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Diethylstilbestrol (DES), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Flutamide (Eulexin®), Hydrocortisone, Ketoconazole (Nizoral®), Leuprolide acetate (Via-dur®, Lupron®, Leuprorel®, Eligard®), Mitoxantrone (Novantrone®, DHAD), Nilutamide (Nilandron®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), PC SPES, Prednisone, Triptorelin pamoate (Trelstar Depot®, Decapeptyl®), and Vinblastine (Velban®, VLB).

[0426] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of prostate cancers.

[0427] Further examples of therapeutic agents useful in the treatment of prostate cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Abarelix® (Abarelix-Depot-Me, PPI-149, R-3827); Abiraterone acetate® (CB-7598, CB-7630), ABT-627 (ET-1 inhibitor), APC-8015 (Provence®, Dendritic cell therapy), Avorelin® (Meterelin®, MF-6001, EP-23904), CEP-701 (KT-5555), CN-706, CT-2584 (Apra®, CT-2583, CT-2586, CT-3536), GBC-590, Globo H hexasaccharide (Globo H-KLH®), Interferon alpha 2a (Intron A®), Liarozole (Liazal, Liazol, R-75251, R-85246, Ro-85264), MDX-447 (MDX-220, BAB-447, EMD-82633, H-447, anti-EGFr/FcGammaR1r), OncoVAX-P (OncoVAX-PrPSA), PROSTVAC, PS-341 (LDP-341, 26S proteosome inhibitor), PSMA MAb (Prostate Specific Membrane Antigen monoclonal antibody), and R-flurbiprofen (Flurizan®, E-7869, MPC-7869).

[0428] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of prostate cancers.

[0429] Preferred combinations of therapeutic agents useful in the treatment of prostate cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Docetaxel+Estramustine, Mitoxantrone+Hydrocortisone, Mitoxantrone+Prednisone, Navelbine+Estramustine, Paclitaxel+Estramustine, and Vinblastine+Estramustine.

[0430] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of prostate cancers.

[0431] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent pancreatic cancer. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent pancreatic cancer. Pancreatic cancers which may be treated using antibodies of the present invention include, but are not limited to, adenocarcinoma, endocrine (islet cell) tumors, tumors confined to the pancreas, locally advanced pancreatic cancer and metastatic pancreatic cancer.

[0432] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent pancreatic cancer. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent pancreatic cancer. Pancreatic cancers which may be treated using agonistic antibodies of the present invention include, but are not limited to, adenocarcinoma, endocrine (islet cell) tumors, tumors confined to the pancreas, locally advanced pancreatic cancer and metastatic pancreatic cancer.

[0433] In one preferred embodiment, agonistic antibodies of the invention are used to treat locally advanced pancreatic cancer. In a further preferred embodiment, agonistic antibodies of the invention are used to treat metastatic pancreatic cancer.

[0434] Antibodies of the present invention may be administered in combination with one or more surgical and/or

radiological procedures useful in the treatment of pancreatic cancer including, but not limited to, pancreaticoduodenectomy (Whipple resection).

[0435] In preferred embodiments, agonistic antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of pancreatic cancer including, but not limited to, pancreaticoduodenectomy (Whipple resection).

[0436] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of pancreatic cancer including, but not limited to, Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Cisplatin (Platinol®, CDDP), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®), Gemcitabine (Gemto®, Gemzarg), and Irinotecan (Camptosar®, CPT-11, Topotecacin®, CaptoCPT-1).

[0437] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of pancreatic cancers.

[0438] Preferred combinations of therapeutic agents useful in the treatment of pancreatic cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Cisplatin+Gemcitabine, CP-358774+Gemcitabine, Docetaxel+Gemcitabine, Irinotecan+Fluorouracil, Irinotecan+Gemcitabine, and Paclitaxel+Gemcitabine.

[0439] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of pancreatic cancers.

[0440] Further examples of therapeutic agents useful in the treatment of pancreatic cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, ABX-EGF (anti-EGFr MAb), Acetyldinaline (CI-994, GOE-5549, GOR-5549, PD-130636), BMS-214662 (BMS-192331, BMS-193269, BMS-206635), BNP-1350 (BNPI-1100, Karenitecins), C225 (IMC-225, EGFR inhibitor, Anti-EGFr MAb, Cetuximab®), C242-DM1 (huC242-DMI, SB-408075), Carbendazin® (FB-642), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), CMT-3 (COL-3, Metastat®), CP-358774 (Tarceva®, OSI-774, EGFR inhibitor), Docetaxel (Taxotere®, Taxane®), Exetecan mesylate (DX-8951, DX-8951f), Flavopiridol (HMR-1275), Gastrimmune® (Anti-gastrin-17 immunogen, anti-g17), GBC-590, Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), HSPPC-96 (HSP cancer vaccine, gp96 heat shock protein-peptide complex), Irofulven (MGI-114), ISIS-2503 (Ras antisense), Onyx-015 (p53 gene therapy), Paclitaxel (Paxene®, Taxol®), Pemetrexed disodium (Alimta®, MTA, multitargeted antifolate, LY 231514), Perillyl alcohol (perilla alcohol, perillie alcohol, perillol, NSC-641066), RFS-2000 (9-nitrocamptothecan, 9-NC, rubitecan®), and Rituximab® (Rituxan®, anti-CD20 MAb).

[0441] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of pancreatic cancers.

[0442] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent hepatic cancer. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent hepatic cancer. Hepatic cancers which may be treated using antibodies of the present invention include, but are not limited to, hepatocellular carcinoma, malignant hepatoma, cholangiocarcinoma, mixed hepatocellular cholangiocarcinoma or hepatoblastoma.

[0443] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent hepatic cancer. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent hepatic cancer. Hepatic cancers which may be treated using agonistic antibodies of the present invention include, but are not limited to, hepatocellular carcinoma, malignant hepatoma, cholangiocarcinoma, mixed hepatocellular cholangiocarcinoma or hepatoblastoma.

[0444] In one preferred embodiment, agonistic antibodies of the invention are used to treat hepatoblastoma. In one further preferred embodiment, agonistic antibodies of the invention are used to treat hepatocellular carcinoma.

[0445] Antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of hepatic cancers including, but not limited to, partial hepatectomy, liver transplant, radiofrequency ablation, laser therapy, microwave therapy, cryosurgery, percutaneous ethanol injection, hepatic arterial infusion, hepatic artery ligation, chemoembolization and external beam radiation therapy.

[0446] In preferred embodiments, agonistic antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of hepatic cancers including, but not limited to, partial hepatectomy, liver transplant, radiofrequency ablation, laser therapy, microwave therapy, cryosurgery, percutaneous ethanol injection, hepatic arterial infusion, hepatic artery ligation, chemoembolization and external beam radiation therapy.

[0447] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of hepatic cancer including, but not limited to, Aldesleukin (IL-2, Proleukin®), Cisplatin (Platinol®, CDDP), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®), I-131 Lipiodol®, Ifosfamide (IFEX®), Megestrol acetate (Megace®, Pallace®), Pravastatin sodium (Pravachol®), and Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®).

[0448] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of hepatic cancers.

[0449] Preferred combinations of therapeutic agents useful in the treatment of hepatic cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Cisplatin+Doxorubicin, Cisplatin+Etoposide, Cisplatin+Vincristine+Fluorouracil, and Ifosfamide+Cisplatin+Doxorubicin.

[0450] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of hepatic cancers.

[0451] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent ovarian cancer. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent ovarian cancer. Ovarian cancers which may be treated using antibodies of the present invention include, but are not limited to, epithelial carcinoma, germ cell tumors and stromal tumors.

[0452] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent ovarian cancer. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent ovarian cancer. Ovarian cancers which may be treated using agonistic antibodies of the present invention include, but are not limited to, epithelial carcinoma, germ cell tumors and stromal tumors.

[0453] In one preferred embodiment, agonistic antibodies of the invention are used to treat germ cell tumors. In one further preferred embodiment, agonistic antibodies of the invention are used to treat epithelial carcinoma.

[0454] Antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of ovarian cancer including, but not limited to, hysterectomy, oophorectomy, hysterectomy with bilateral salpingo-oophorectomy, omentectomy, tumor debulking, external beam radiation therapy and intraperitoneal radiation therapy.

[0455] In preferred embodiments, agonistic antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of ovarian cancer including, but not limited to, hysterectomy, oophorectomy, hysterectomy with bilateral salpingo-oophorectomy, omentectomy, tumor debulking, external beam radiation therapy and intraperitoneal radiation therapy.

[0456] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of ovarian cancer including, but not limited to, Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Bleomycin (Blenoxane®), Carboplatin (Paraplatin®, CBDCA), Cisplatin (Platinol®, CDDP), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Dactinomycin (Cosmegen®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®), Gemcitabine (Gemto®, Gemzar®), Ifosfamide (IFEX®), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Leucovorin (Leucovorin®, Wellcovorin®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Paclitaxel (Paxene®, Taxol®), Tamoxifen

(Nolvadex®), Vinblastine (Velban®, VLB) and Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®).

[0457] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of ovarian cancers.

[0458] Preferred combinations of therapeutic agents useful in the treatment of ovarian cancer which may be administered in combination with antibodies of the present invention include, but are not limited to, Bleomycin+Etoposide+Platinol® (Cisplatin) (BEP), Carboplatin+Cyclophosphamide, Carboplatin+Paclitaxel, Carboplatin+Etoposide+Bleomycin (CEB), Cisplatin+Cyclophosphamide, Cisplatin+Etoposide, Cisplatin+Paclitaxel, Cisplatin+Ifosfamide+Vinblastine, Fluorouracil+Leucovorin, Platinol® (Cisplatin)+Vinblastine+Bleomycin (PVB), and Vincristine+Dactinomycin+Cyclophosphamide.

[0459] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of ovarian cancers.

[0460] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent Ewing's sarcoma. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent Ewing's sarcoma. Ewing's sarcoma family tumors which may be treated using antibodies of the present invention include, but are not limited to, Ewing's tumor of bone (ETB), extraosseus Ewing's (EOE), primitive neuroectodermal tumors (PNET or peripheral neuroepithelioma) and Askin's tumor.

[0461] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent Ewing's sarcoma. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent Ewing's sarcoma. Ewing's sarcoma family tumors which may be treated using agonistic antibodies of the present invention include, but are not limited to, Ewing's tumor of bone (ETB), extraosseus Ewing's (EOE), primitive neuroectodermal tumors (PNET or peripheral neuroepithelioma) and Askin's tumor.

[0462] In one preferred embodiment, agonistic antibodies of the invention are used to treat Ewing's tumor of bone. In one further preferred embodiment, agonistic antibodies of the invention are used to treat peripheral neuroepithelioma.

[0463] Antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of Ewing's sarcoma family tumors.

[0464] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more surgical and/or radiological procedures useful in the treatment of Ewing's sarcoma family tumors.

[0465] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of Ewing's sarcoma family tumors

including, but not limited to, Cyclophosphamide (Cytosar®, Neosar®, CTX), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Filgrastim (Neupogen®, G-CSF), Ifosfamide (IFEX®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), and Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®).

[0466] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of Ewing's sarcoma family tumors.

[0467] Preferred combinations of therapeutic agents useful in the treatment of Ewing's sarcoma family tumors which may be administered in combination with antibodies of the present invention include, but are not limited to, Cyclophosphamide+Topotecan, Cyclophosphamide+Doxorubicin+Vincristine, Cyclophosphamide+Doxorubicin+Vincristine, alternating with Ifosfamide+Etoposide and Cyclophosphamide+Doxorubicin+Vincristine, alternating with Filgrastim+Ifosfamide+Etoposide.

[0468] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of Ewing's sarcoma family tumors.

[0469] In further particular embodiments, antibodies of the present invention are used to treat, ameliorate and/or prevent hematological cancers. Antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent hematological cancers. Hematological cancers which may be treated using antibodies of the present invention include, but are not limited to, non-Hodgkin's lymphoma (e.g., small lymphocytic lymphoma, follicular center cell lymphoma, lymphoplasmacytoid lymphoma, marginal zone lymphoma, mantle cell lymphoma, immunoblastic lymphoma, burkitt's lymphoma, lymphoblastic lymphoma, peripheral T-cell lymphoma, anaplastic large cell lymphoma and intestinal T-cell lymphoma), leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia and plasma cell neoplasms including multiple myeloma.

[0470] In preferred embodiments, agonistic antibodies of the present invention are used to treat, ameliorate and/or prevent hematological cancers. Agonistic antibodies of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent hematological cancers. Hematological cancers which may be treated using agonistic antibodies of the present invention include, but are not limited to, non-Hodgkin's lymphoma (e.g., small lymphocytic lymphoma, follicular center cell lymphoma, lymphoplasmacytoid lymphoma, marginal zone lymphoma, mantle cell lymphoma, immunoblastic lymphoma, burkitt's lymphoma, lymphoblastic lymphoma, peripheral T-cell lymphoma, anaplastic large cell lymphoma and intestinal T-cell lymphoma), leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia and plasma cell neoplasms including multiple myeloma.

[0471] In one preferred embodiment, agonistic antibodies of the invention are used to treat plasma cell neoplasms. In a specific embodiment, that plasma cell neoplasm is multiple myeloma.

[0472] In another preferred embodiment, agonistic antibodies of the invention are used to treat non-Hodgkin's lymphoma.

[0473] In another preferred embodiment, agonistic antibodies of the invention are used to treat leukemia. In a specific embodiment, that leukemia is acute lymphocytic leukemia. In another specific embodiment, that leukemia is chronic lymphocytic leukemia.

[0474] Antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of hematological cancer including, but not limited to, bone marrow transplantation, external beam radiation and total body irradiation.

[0475] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more surgical and/or radiological procedures useful in the treatment of hematological cancer including, but not limited to, bone marrow transplantation, external beam radiation and total body irradiation.

[0476] In one preferred embodiment, agonistic antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of multiple myeloma including, but not limited to, allogeneic bone marrow transplantation and peripheral stem cell support.

[0477] In another preferred embodiment, agonistic antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of non-Hodgkin's lymphoma including, but not limited to, allogeneic bone marrow transplantation and peripheral stem cell support.

[0478] In further specific embodiments, agonistic antibodies of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of leukemia including, but not limited to, allogeneic bone marrow transplantation and peripheral stem cell support. In one specific preferred embodiment, agonistic antibodies of the invention are used to treat acute lymphocytic leukemia (ALL). In another specific preferred embodiment, agonistic antibodies of the invention are used to treat chronic lymphocytic leukemia (CLL).

[0479] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of multiple myeloma including, but not limited to, Alkylating agents, Anthracyclines, Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Dexamethasone (Decadron®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Methotrexate® (MTX, Mexate®, Folex®), Methylprednisolone (Solu-medrol®), Mitoxantrone (Novantrone®, DHAD), Ondansetron (Zofran®), Pentostatin (Nipent®, 2-deoxycoformycin), Perfosfamide (4-hydroperoxycyclophosphamide, 4-HC), Prednisone, Procarbazine (Matulane®), Rituximab® (Rituxan®, anti-CD20 MAb), Thiotepa (triethylenethiophosphoramido, Thioplex®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®D, VCR, Leurocristine®) and Vindesine (Eldisine®, Fildesin®).

[0480] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or

more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of multiple myeloma.

[0481] Preferred combinations of therapeutic agents useful in the treatment of multiple myeloma which may be administered in combination with antibodies of the present invention include, but are not limited to, Cyclophosphamide+Prednisone, Melphalan+Prednisone (MP), Vincristine+Adriamycin®+Dexamethasone (VAD), Vincristine+Carmustine+Melphalan+Cyclophosphamide+Prednisone (VBMCP; the M2 protocol), and Vincristine+Melphalan+Cyclophosphamide+Prednisone alternating with Vincristine+Carmustine+Doxorubicin+Prednisone (VMCP/VBAP).

[0482] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of multiple myeloma.

[0483] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of non-Hodgkin's lymphoma including, but not limited to, 2-chlorodeoxyadenosine, Amifostine (Ethylol®, Ethiofos®, WR-272), Bexarotene (Targretin®, Targretin gel®, Targretin oral®, LGD1069), Bleomycin (Blenoxane®), Busulfan (Busulfex®, Myleran®), Carboplatin (Paraplatin®, CBDCA), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Chlorambucil (Leukeran®), Cisplatin (Platinol®, CDDP), Cladribine (2-CdA, Leustatin®), Cyclophosphamide (Cytoxan®R, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®), Dacarbazine (DTIC), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Denileukin diftitox (Ontak®), Dexamethasone (Decadron®), Dolasetron mesylate (Anzemet®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Erythropoietin (EPO®, Epogen®, Procrit®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fludarabine (Fludara®, FAMP), Granisetron (Kytril®), Hydrocortisone, Idoarubicin (Idamycin®, DMDR, IDA), Ifosfamide (IFEX®), Interferon alpha (Alfaferone®, Alpha-IF®), Interferon alpha 2a (Intron A®), Mechlorethamine (Nitrogen Mustard, HN2, Mustargen®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Methotrexate® (MTX, Mexate®, Folex®), Methylprednisolone (Solu-medrol®), Mitoxantrone (Novantrone®, DHAD), Ondansetron (Zofran®), Pentostatin (Nipent®, 2-deoxycoformycin), Perfosfamide (4-hydroperoxycyclophosphamide, 4-HC), Prednisone, Procarbazine (Matulane®), Rituximab® (Rituxan®, anti-CD20 MAb), Thiotepa (triethylenethiophosphoramido, Thioplex®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®D, VCR, Leurocristine®) and Vindesine (Eldisine®, Fildesin®).

[0484] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of non-Hodgkin's lymphoma.

[0485] Preferred combinations of therapeutic agents useful in the treatment of non-Hodgkin's lymphoma which may be administered in combination with antibodies of the present invention include, but are not limited to,

Adriamycin®+V+Blenoxane+Vinblastine+Dacarbazine (ABVD), Anti-idiotype therapy (BsAb)+Interferon alpha, Anti-idiotype therapy (BsAb)+Chlorambucil, Anti-idiotype therapy (BsAb)+Interleukin-2, BCNU (Carmustine)+Etoposide+Ara-C (Cytarabine)+Melphalan (BEAM), Bleomycin+ Etoposide+Adriamycin+Cyclophosphamide+Vincristine+ Procarbazine+Prednisone (BEACOPP), Bryostatin+ Vincristine, Cyclophosphamide+BCNU (Carmustine)+VP-16 (Etoposide) (CBV), Cyclophosphamide+Vincristine+ Prednisone (CVP), Cyclophosphamide+Adriamycin® (Hydroxydaunomycin)+Vincristine (Oncovorin)+Prednisone (CHOP), Cyclophosphamide+Novantrone® (Mitoxantrone)+Vincristine (Oncovorin)+Prednisone (CNOP), Cyclophosphamide+Doxorubicin+Teniposide+Prednisone, Cyclophosphamide+Adriamycin® (Hydroxydaunomycin)+Vincristine (Oncovorin)+Prednisone+Rituximab (CHOP+Rituximab), Cyclophosphamide+Doxorubicin+Teniposide+Prednisone+Interferon alpha, Cytarabine+Bleomycin+Vincristine+Methotrexate (CytaBOM), Dexamethasone+ Cytarabine+Cisplatin (DHAP), Dexamethasone+Ifosfamide+Cisplatin+Etoposide (DICE), Doxorubicin+ Vinblastine+Mechlorethamine+Vincristine+Bleomycin+ Etoposide+Prednisone (Stanford V), Etoposide+Vinblastine+Adriamycin (EVA), Etoposide+Methylprednisone+Cytarabine+Cisplatin (ESHAP), Etoposide+Prednisone+Ifosfamide+Cisplatin (EPIC), Fludarabine, Mitoxantrone+Dexamethasone (FMD), Fludarabine, Dexamethasone, Cytarabine (ara-C),+Cisplatin (Platinol®) (FluDAP), Ifosfamide+Cisplatin+Etoposide (ICE), Mechlorethamine+Oncovin® (Vincristine)+Procarbazine+Prednisone (MOPP), Mesna+Ifosfamide+Idarubicin+Etoposide (MIZE), Methotrexate with leucovorin rescue+Bleomycin+Adriamycin+Cyclophosphamide+Oncovorin+Dexamethasone (m-BACOD), Prednisone+Methotrexate+Adriamycin+Cyclophosphamide+Etoposide (ProMACE), Thiotepa+Busulfan+Cyclophosphamide, Thiotepa+Busulfan+Melphalan, Topotecan+Paclitaxel, and Vincristine (Oncovin®)+Adriamycin®+Dexamethasone (VAD).

[0486] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of non-Hodgkin's lymphoma.

[0487] Further examples of therapeutic agents useful in the treatment of non-Hodgkin's lymphoma which may be administered in combination with antibodies of the present invention include, but are not limited to, A007 (4'-4-dihydroxybenzophenone-2,4-dinitrophenylhydrazone), AG-2034 (AG-2024, AG-2032, GARFT [glycinamide ribonucleoside transformylase] inhibitor), Aldesleukin (IL-2, Proleukin®), Alemtuzumab (Campath®), Alitretinoin (Panretin®, LGN-1057), Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Anti-CD19/CD3 MAb (anti-CD19/CD3 scFv, anti-NHL MAb), Anti-idiotype therapy (BsAb), Arabinosylguanine (Ara-G, GW506U78), Arsenic trioxide (Trisenox®, ATO), B43-Genistein (anti-CD19 Ab/genistein conjugate), B7 antibody conjugates, Betathine (Beta-LT), BLyS antagonists, Bryostatin-1 (Bryostatin2, BMY45618, NSC-339555), CHML (Cytotropic Heterogeneous Molecular Lipids), Clofarabine (chlorofluoro-araA), Daclizumab (Zenapax®), Depsipeptide (FR901228, FK228), Dolastatin-10 (DOLA-10, NSC-376128), Epirubicin (Ellence®, EPI, 4' epi-doxorubicin),

Epratuzumab (Lymphocide®, humanized anti-CD22, HAT), Fly3/flk2 ligand (Mobista®), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), HuLD10 (anti-HLA-DR MAb, SMART 1D10), HumaLYM (anti-CD20 MAb), Ibrutumomab tiuxetan (Zevalin®), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), Irinotecan (Camptosar®), CPT-11, Topotecin®, CaptoCPT-1), ISIS-2053, ISIS-3521 (PKC-alpha antisense), Lmb-2 immunotoxin (anti-CD25 recombinant immuno toxin, anti-Tac(Fv)-PE38), Leuvectin® (cytorectin+IL-2 gene, IL-2 gene therapy), Lym-1 (131-I LYM-1), Lymphoma vaccine (Genitope), Nelarabine (Compound 506, U78), Neugene compounds (Oncomyc-NG®, Resten-NG®, myc antisense), NovoMAb-G2 scFv (NovoMAb-G2 IgM), O6-benzylguanine (BG, Procept®), Oxaliplatin (Eloxatin®, Eloxatin®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Peldesine (BCX-34, PNP inhibitor), Rebeccamycin and Rebeccamycin analogues, SCH-66336, Sobuzoxane (MST-16, Perazolin®), SU5416 (Semaxanib®, VEGF inhibitor), TER-286, Thalidomide, TNP-470 (AGM-1470), Tositumomab (Bexxar®), Valspodar (PSC 833), Vaxid (B-cell lymphoma DNA vaccine), Vinorelbine (Navelbine®), WF10 (macrophage regulator) and XR-9576 (XR-9351, P-glycoprotein/MDR inhibitor).

[0488] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of non-Hodgkin's lymphoma.

[0489] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of acute lymphocytic leukemia including, but not limited to, Amsacrine, Carboplatin (Paraplatin®, CBDCA), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cholecaliferol, Cyclophosphamide (Cytosan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Dexamethasone (Decadron®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide (VP-16, Vepesid®), Filgrastim® (Neupogen®, G-CSF, Leukine®), Fludarabine (Fludara®, FAMP), Idarubicin (Idamycin®, DMMDR, IDA), Ifosfamide (IFEX®), Imatinib mesylate (STI-571, Imatinib®, Glivec®, Gleevec®, Abl tyrosine kinase inhibitor), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), L-asparaginase (Elspar®, Crastin®), Asparaginase medac®, Kidrolase®), Mercaptopurine (6-mercaptopurine, 6-MP), Methotrexate® (MTX, Mexate®, Folex®), Mitoxantrone (Novantrone®, DHAD), Pegasparagase® (Oncospars®), Prednisone, Retinoic acid, Teniposide (VM-26, Vumon®), Thioguanine (6-thioguanine, 6-TG), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Etoposide®), Tretinoin (Retin-A®, Atragen®, ATRA, Vesanoïd®) and Vincristine (Oncovorin®, Onco TCS®, VCR, Leurocristine®).

[0490] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of acute lymphocytic leukemia.

[0491] Further examples of therapeutic agents useful in the treatment of acute lymphocytic leukemia which may be

administered in combination with antibodies of the present invention include, but are not limited to, Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminopterin, Annamycin (AR-522, annamycin LF, Aronex®), Arabinosylguanine (Ara-G, GW506U78, Nelzarabine®), Arsenic trioxide (Trisenox®, ATO, Atrivex®), B43-Genistein (anti-CD 19 Ab/genistein conjugate), B43-PAP (anti-CD 19 Ab/pokeweed antiviral protein conjugate), Cordycepin, CS-682, Decitabine (5-aza-2'-deoxycytidine), Dolastatin-10 (DOLA-10, NSC-376128), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), MS-209, Phenylbutyrate, Quinine, TNP-470 (AGM-1470, Fumagillin), Trimetrexate (Neutrexin®), Troxacitabine (BCH-204, BCH-4556, Troxatyl®), UCN-01 (7-hydroxystaurosporine), WHI-Pi31 and WT1 Vaccine.

[0492] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of acute lymphocytic leukemia.

[0493] Preferred combinations of therapeutic agents useful in the treatment of acute lymphocytic leukemia which may be administered in combination with antibodies of the present invention include, but are not limited to, Carboplatin+Mitoxantrone, Carmustine+Cyclophosphamide+Etoposide, Cytarabine+Daunorubicin, Cytarabine+Doxorubicin, Cytarabine+Idarubicin, Cytarabine+Interferon gamma, Cytarabine+L-asparaginase, Cytarabine+Mitoxantrone, Cytarabine+Fludarabine and Mitoxantrone, Etoposide+Cytarabine, Etoposide+Ifosfamide, Etoposide+Mitoxantrone, Ifosfamide+Etoposide+Mitoxantrone, Ifosfamide+Teniposide, Methotrexate+Mercaptopurine, Methotrexate+Mercaptopurine+Vincristine+Prednisone, Phenylbutyrate+Cytarabine, Phenylbutyrate+Etoposide, Phenylbutyrate+Topotecan, Phenylbutyrate+Tretinoin, Quinine+Doxorubicin, Quinine+Mitoxantrone+Cytarabine, Thioguanine+Cytarabine+Amsacrine, Thioguanine+Etoposide+Idarubicin, Thioguanine+Retinoic acid+Cholecalciferol, Vincristine+Prednisone, Vincristine+Prednisone and L-asparaginase, Vincristine+Dexamethasone/Prednisone+Asparaginase+Daunorubicin/Doxorubicin, Vincristine+Dexamethasone/Prednisone+Asparaginase+Daunorubicin/Doxorubicin+Filgrastim, Vincristine+Dexamethasone/Prednisone+Asparaginase+Daunorubicin/Doxorubicin+ Cyclophosphamide+Methotrexate, and Vincristine+Dexamethasone/Prednisone+Asparaginase+Daunorubicin/Doxorubicin+Cyclophosphamide+Methotrexate+Filgrastim.

[0494] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of acute lymphocytic leukemia.

[0495] Antibodies of the present invention may be administered in combination with one or more therapeutic agents useful in the treatment of chronic lymphocytic leukemia including, but not limited to, Chlorambucil (Leukeran®), Cladribine (2-CdA, Leustatin®), Cyclophosphamide (Cytoxin®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®, cytarabine ocfosfate, ara-CMP), Doxorubicin (Adriamycin®, Doxil®, Rubex®),

Fludarabine (Fludara®, FAMP), Pentostatin (Nipent®, 2-deoxycoformycin), Prednisone and Vincristine (Oncovorin®, Onco TCS®, VCR, Leurocristine®).

[0496] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of chronic lymphocytic leukemia.

[0497] Further examples of therapeutic agents useful in the treatment of chronic lymphocytic leukemia which may be administered in combination with antibodies of the present invention include, but are not limited to, Alemtuzumab (Campath®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminopterin, Annamycin (AR-522, annamycin LF, Aronex®), Arabinosylguanine (Ara-G, GW506U78, Nelzarabine®, Compound 506U78), Arsenic trioxide (Trisenox®, ATO, Atrivex®), Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), CS-682, Dolastatin-10 (DOLA-10, NSC-376128), Filgrastim (Neupogen®, G-CSF, Leukine), Flavopiridol (NSC-649890, HMR-1275), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), MS-209, Phenylbutyrate, Rituximab® (Rituxan®, anti-CD20 MAb), Thalidomide, Theophylline, TNP-470 (AGM-1470, Fumagillin), UCN-01 (7-hydroxystaurosporine) and WHI-P131.

[0498] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of chronic lymphocytic leukemia.

[0499] Preferred combinations of therapeutic agents useful in the treatment of chronic lymphocytic leukemia which may be administered in combination with antibodies of the present invention include, but are not limited to, Fludarabine+Prednisone, and Cyclophosphamide+Doxorubicin+Vincristine+Prednisone (CHOP).

[0500] In preferred embodiments, agonistic antibodies of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of chronic lymphocytic leukemia.

[0501] Anti-DR5 antibodies may be utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines, tumor necrosis factors or TNF-related molecules (e.g., TNF- α , TNF- β , TNF- γ , TNF- γ - α , TNF- γ - β , and TRAIL), or hematopoietic growth factors (e.g., IL-2, IL-3 and IL-7). For example, agonistic anti-DR5 antibodies may be administered in conjunction with TRAIL when one seeks to induce DR5 mediated cell death in cells, which express DR5 receptors of the invention. Combination therapies of this nature, as well as other combination therapies, are discussed below in more detail.

[0502] The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment,

human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0503] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Polypeptide Assays

[0504] The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of DR5 protein, or the soluble form thereof, in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of DR5, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors, for example. Assay techniques that can be used to determine levels of a protein, such as a DR5 protein of the present invention, or a soluble form thereof, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis, and ELISA assays.

[0505] Assaying DR5 protein levels in a biological sample can occur using any art-known method. By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing DR5 receptor protein or mRNA. Preferred for assaying DR5 protein levels in a biological sample are antibody-based techniques. For example, DR5 protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M. et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M. et al., *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting DR5 protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

[0506] Suitable labels are known in the art and include enzyme labels, such as glucose oxidase, radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulphur (^{35}S), tritium (^3H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Therapeutics

[0507] The Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (Goeddel, D. V. et al., "Tumor Necrosis Factors: Gene Structure and Biological Activities," *Symp. Quant. Biol.* 51:597-609 (1986), Cold Spring Harbor; Beutler, B., and Cerami, A.,

Annu. Rev. Biochem. 57:505-518 (1988); Old, L. J., *Sci. Am.* 258:59-75 (1988); Fiers, W., *FEBS Lett.* 285:199-224 (1991)). The TNF-family ligands induce such various cellular responses by binding to TNF-family receptors, including the DR5 of the present invention.

[0508] DR5 polynucleotides, polypeptides, agonists and/or antagonists of the invention may be administered to a patient (e.g., mammal, preferably human) afflicted with any disease or disorder mediated (directly or indirectly) by defective, or deficient levels of, DR5. Alternatively, a gene therapy approach may be applied to treat and/or prevent such diseases or disorders. In one embodiment of the invention, DR5 polynucleotide sequences are used to detect mutein DR5 genes, including defective genes. Mutein genes may be identified in in vitro diagnostic assays, and by comparison of the DR5 nucleotide sequence disclosed herein with that of a DR5 gene obtained from a patient suspected of harboring a defect in this gene. Defective genes may be replaced with normal DR5-encoding genes using techniques known to one skilled in the art.

[0509] In another embodiment, the DR5 polypeptides, polynucleotides, agonists and/or antagonists of the present invention are used as research tools for studying the phenotypic effects that result from inhibiting TRAIL/DR5 interactions on various cell types. DR5 polypeptides and antagonists (e.g. monoclonal antibodies to DR5) also may be used in in vitro assays for detecting TRAIL or DR5 or the interactions thereof.

[0510] It has been reported that certain ligands of the TNF family (of which TRAIL is a member) bind to more than one distinct cell surface receptor protein. For example, a receptor protein designated DR4 reportedly binds TRAIL, but is distinct from the DR5 of the present invention (Pan et al., *Science* 276:111-113, (1997); hereby incorporated by reference). In another embodiment, a purified DR5 polypeptide, agonist and/or antagonist is used to inhibit binding of TRAIL to endogenous cell surface TRAIL. By competing for TRAIL binding, soluble DR5 polypeptides of the present invention may be employed to inhibit the interaction of TRAIL not only with cell surface DR5, but also with TRAIL receptor proteins distinct from DR5. Thus, in a further embodiment, DR5 polynucleotides, polypeptides, agonists and/or antagonists of the invention are used to inhibit a functional activity of TRAIL, in in vitro or in vivo procedures. By inhibiting binding of TRAIL to cell surface receptors, DR5 also inhibits biological effects that result from the binding of TRAIL to endogenous receptors. Various forms of DR5 may be employed, including, for example, the above-described DR5 fragments, derivatives, and variants that are capable of binding TRAIL. In a preferred embodiment, a soluble DR5, is employed to inhibit a functional activity of TRAIL, e.g., to inhibit TRAIL-mediated apoptosis of cells susceptible to such apoptosis. Thus, in an additional embodiment, DR5 is administered to a mammal (e.g., a human) to treat and/or prevent a TRAIL-mediated disorder. Such TRAIL-mediated disorders include conditions caused (directly or indirectly) or exacerbated by TRAIL.

[0511] Cells that express the DR5 polypeptide and are believed to have a potent cellular response to DR5 ligands include primary dendritic cells, endothelial tissue, spleen, chronic lymphocytic leukemia, and human thymus stromal

cells. By "a cellular response to a TNF-family ligand" is intended any genotypic, phenotypic, and/or morphologic change to a cell, cell line, tissue, tissue culture or patient that is induced by a TNF-family ligand. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis (programmed cell death) is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes (Ameisen, J. C., *AIDS* 8:1197-1213 (1994); Krammer, P. H. et al., *Curr. Opin. Immunol.* 6:279-289 (1994)).

[0512] Diseases associated with increased cell survival, or the inhibition of apoptosis, that may be treated, prevented, diagnosed and/or prognosed with the DR5 polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, DR5 polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

[0513] Additional diseases or conditions associated with increased cell survival that may be treated, prevented, diagnosed and/or prognosed with the DR5 polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endothelioma, lymphangiosarcoma, lymphangiomyoma, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarci-

noma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendrogioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

[0514] Diseases associated with increased apoptosis that may be treated, prevented, diagnosed and/or prognosed with the DR5 polynucleotides, polypeptides and/or agonists or antagonists of the invention include, but are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. In preferred embodiments, DR5 polynucleotides, polypeptides and/or agonists are used to treat and/or prevent the diseases and disorders listed above.

[0515] The state of immunodeficiency that defines AIDS is secondary to a decrease in the number and function of CD4⁺ T-lymphocytes. Recent reports estimate the daily loss of CD4⁺ T-cells to be between 3.5×10^7 and 2×10^9 cells (Wei X. et al., *Nature* 373:117-122 (1995)). One cause of CD4⁺ T-cell depletion in the setting of HIV infection is believed to be HIV-induced apoptosis (see, for example, Meyaard et al., *Science* 257:217-219, 1992; Groux et al., *J. Exp. Med.*, 175:331, 1992; and Oyaizu et al., in *Cell Activation and Apoptosis in HIV Infection*, Andrieu and Lu, Eds., Plenum Press, New York, 1995, pp. 101-114). Indeed, HIV-induced apoptotic cell death has been demonstrated not only in vitro but also, more importantly, in infected individuals (Ameisen, J. C., *AIDS* 8:1197-1213 (1994); Finkel, T. H., and Banda, N. K., *Curr. Opin. Immunol.* 6:605-615(1995); Muro-Cacho, C. A. et al., *J. Immunol.* 154:5555-5566 (1995)). Furthermore, apoptosis and CD4⁺ T-lymphocyte depletion is tightly correlated in different animal models of AIDS (Brunner, T., et al., *Nature* 373:441-444 (1995); Gougeon, M. L., et al., *AIDS Res. Hum. Retroviruses* 9:553-563 (1993)) and, apoptosis is not observed in those animal models in which viral replication does not result in AIDS (Gougeon, M. L. et al., *AIDS Res. Hum. Retroviruses* 9:553-563 (1993)). Further data indicates that uninfected but primed or activated T lymphocytes from HIV-infected individuals undergo apoptosis after encountering the TNF-family ligand FasL. Using monocytic cell lines that result in death following HIV infection, it has been demonstrated that infection of U937 cells with HIV results in the de novo expression of FasL and that FasL mediates HIV-induced apoptosis (Badley, A. D. et al., *J. Virol.* 70:199-206 (1996)). Further the TNF-family ligand was detectable in uninfected macrophages and its expression was upregulated following HIV infection resulting in selective killing of uninfected CD4 T-lymphocytes (Badley, A. D et al., *J. Virol.* 70:199-

206 (1996)). Further, additional studies have implicated Fas-mediated apoptosis in the loss of T-cells in HIV individuals (Katsikis et al., *J. Exp. Med.* 181:2029-2036,1995).

[0516] Thus, by the invention, a method for treating and/or preventing HIV⁺ individuals is provided which involves administering DR5, DR5 antagonists, and/or DR5 agonists of the present invention to reduce selective killing of CD4⁺ T-lymphocytes. Modes of administration and dosages are discussed in detail below.

[0517] In rejection of an allograft, the immune system of the recipient animal has not previously been primed to respond because the immune system for the most part is only primed by environmental antigens. Tissues from other members of the same species have not been presented in the same way that, for example, viruses and bacteria have been presented. In the case of allograft rejection, immunosuppressive regimens are designed to prevent the immune system from reaching the effector stage. However, the immune profile of xenograft rejection may resemble disease recurrence more than allograft rejection. In the case of disease recurrence, the immune system has already been activated, as evidenced by destruction of the native islet cells. Therefore, in disease recurrence the immune system is already at the effector stage. Agonists of the present invention are able to suppress the immune response to both allografts and xenografts because lymphocytes activated and differentiated into effector cells will express the DR5 polypeptide, and thereby are susceptible to compounds, which enhance apoptosis. Thus, the present invention further provides a method for creating immune privileged tissues.

[0518] DR5 antagonists or agonists of the invention may be useful for treating and/or preventing inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

[0519] In addition, due to lymphoblast expression of DR5, soluble DR5 agonist or antagonist mABs may be used to treat and/or prevent this form of cancer. Further, soluble DR5 or neutralizing mABs may be used to treat and/or prevent various chronic and acute forms of inflammation such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

[0520] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in the diagnosis, prognosis, treatment and/or prevention of a wide range of diseases and/or conditions. Such diseases and conditions include, but are not limited to, cancer (e.g., immune cell related cancers, breast cancer, prostate cancer, ovarian cancer, follicular lymphoma, glioblastoma, cancer associated with mutation or alteration of p53, brain tumor, bladder cancer, uterocervical cancer, colon cancer, colorectal cancer, non-small cell carcinoma of the lung, small cell carcinoma of the lung, stomach cancer, etc.), lymphoproliferative disorders (e.g., lymphadenopathy and lymphomas (e.g., Hodgkin's disease)), microbial (e.g., viral, bacterial, etc.) infection (e.g., HIV-1 infection, HIV-2 infection, herpesvirus infection (including, but not limited to, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7, EBV), adenovirus infection, poxvirus infection, human papilloma virus infection, hepatitis infection (e.g., HAV, HBV, HCV, etc.), *Helicobacter pylori* infection, invasive *Staphylococci*, etc.), parasitic infection, nephritis, bone disease (e.g., osteoporosis), atherosclerosis, pain, cardiovascular disorders (e.g.,

neovascularization, hypovascularization or reduced circulation (e.g., ischemic disease (e.g., myocardial infarction, stroke, etc.)), AIDS, allergy, inflammation, neurodegenerative disease (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, pigmentary retinitis, cerebellar degeneration, etc.), graft rejection (acute and chronic), graft vs. host disease, diseases due to osteomyelodysplasia (e.g., aplastic anemia, etc.), joint tissue destruction in rheumatism, liver disease (e.g., acute and chronic hepatitis, liver injury, and cirrhosis), autoimmune disease (e.g., multiple sclerosis, myasthenia gravis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, inflammatory autoimmune diseases, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, osteomyelitis, glomerulonephritis, septic shock, and ulcerative colitis.

[0521] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in promoting regulating hematopoiesis, regulating (e.g., promoting) angiogenesis, wound healing (e.g., wounds, burns, and bone fractures), and regulating bone formation.

[0522] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents and/or procedures in the treatment, prevention, amelioration and/or cure of cancers.

[0523] In preferred embodiments, agonists and/or antagonists of the invention may be administered in combination with one or more therapeutic agents and/or procedures in the treatment, prevention, amelioration and/or cure of cancers.

[0524] Therapeutic agents, useful in the treatment, prevention, amelioration and/or cure of cancers, with which polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered, include, for example, biological agents (e.g., inhibitors of signaling pathways, inhibitors of gene transcription, inhibitors of multi-drug resistance (MDR) mechanisms, inhibitors of angiogenesis, inhibitors of matrix metalloproteinases, hormones and hormone antagonists, and compounds of unknown mechanism), chemotherapeutic agents (e.g., alkylating agents, antimetabolites, farnesyl transferase inhibitors, mitotic spindle inhibitors (plant-derived alkaloids), nucleotide analogs, platinum analogs, and topoisomerase inhibitors), corticosteroids, gene therapies, immunotherapeutic agents (e.g., monoclonal antibodies, cytokines and vaccines), phototherapy, radiosensitizing agents, treatment support agents (e.g., anti-emetic agents, analgesic agents and hematopoietic agents), and other miscellaneous drug types. Therapeutic procedures, useful in the treatment, prevention, amelioration and/or cure of cancers, with which polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered, include, for example, but are not limited to, surgical procedures and radiation therapies.

[0525] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents and/or therapeutic procedures in the treatment, prevention, amelioration and/or cure of cancers.

[0526] In specific embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment, prevention, amelioration and/or cure of cancers including, but not limited to, 81C6 (Anti-tenascin monoclonal antibody), 2-chlorodeoxyadenosine, A007 (4-4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone), Abarelix® (Abarelix-Depot-M®, PPI-149, R-3827); Abiraterone acetate® (CB-7598, CB-7630), ABT-627 (ET-1 inhibitor), ABX-EGF (anti-EGFr MAb), Acetylinaline (CI-994, GOE-5549, GOR-5549, PD-130636), AG-2034 (AG-2024, AG-2032, GARFT [glycinamide ribonucleoside transformylase] inhibitor), Alanosine, Aldesleukin (IL-2, Proleukin®), Alemtuzumab® (Campath®), Alitretinoin (Panretin®, LGN-1057), Allopurinol (Aloprim®, Zyloprim®), Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Amifostine (Ethyol®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminoglutethimide (Cytadren®), Aminolevulinic acid (Levulan®, Kerastick®), Aminopterin, Amsacrine, Anastrozole (Arimidex®), Angiostatin, Annamycin (AR-522, annamycin LF, Aronex®), Anti-idiotype therapy (BsAb), Anti-CD19/CD3 MAb (anti-CD19/CD3 scFv, anti-NHL MAb), APC-8015 (Provence®, Dendritic cell therapy), Aplidine (Aplidin®, Aplidina®), Arabinosylguanine (Ara-G, GW506U78, Nelzarabine®, Compound 506U78), Arsenic trioxide (Trisenox®, ATO, Atrivex®), Avorelin® (Meterelin®, MF-6001, EP-23904), B43-Genistein (anti-CD19 Ab/genistein conjugate), B43-PAP (anti-CD19 Ab/pokeweed antiviral protein conjugate), B7 antibody conjugates, BAY 43-9006 (Raf kinase inhibitor), BBR 3464, Betathine (Beta-LT), Bevacizumab® (Anti-VEGF monoclonal antibody, rhuMAb-VEGF), Bexarotene (Targretin®, LGD 1069), BIBH-1 (Anti-FAP MAb), BIBX-1382, Bicutamide (Casodex®), Bircodar dicitrate (Incel®, Incel MDR Inhibitor), Bleomycin (Blenoxane®), BLP-25 (MUC-1 peptide), BLyS antagonists, BMS-214662 (BMS-192331, BMS-193269, BMS-206635), BNP-1350 (BNPI-1100, Karenitecins), Boronated Protoporphyrin Compound (PDIT, Photodynamic Immunotherapy), Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), Budenoside (Rhinocort®), Busulfan (Busulfex®, Myleran®), C225 (IMC-225, EGFR inhibitor, Anti-EGFr MAb, Cetuximab®), C242-DM1 (huC242-DM 1), Cabergoline (Dostinex®), Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Carbendazin® (FB-642), Carboplatin (Paraplatin®, CBDCA), Carboxyamidotriazole (NSC 609974, CAI, L-651582), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), CC49-zeta gene therapy, CEA-cide (Labetuzumab®, Anti-CEA monoclonal antibody, hMN-14), CeaVac® (MAb 3H1), Celecoxib (Celebrex®), CEP-701 (KT-5555), Cereport® (Lobradimil®, RMP-7), Chlorambucil (Leukeran®), CHML (Cytotropic Heterogeneous Molecular Lipids), Cholecaliferol, CI-1033 (Pan-erbB RTK inhibitor), Cilengitide (EMD-121974, integrin alphavbeta3 antagonist), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (Intra-Dose®, FocaCist®), Cisplatin-liposomal (SPI-077), 9-cis retinoic acid (9-cRA), Cladribine (2-CdA, Leustatin®), Clofarabine (chloro-fluoro-araA), Clonidine hydrochloride (Duraclon®), CMB-401 (Anti-PEM MAb/calicheamycin), CMT-3 (COL-3, Metastat®), Cordycepin, Cotara® (chTNT-1/B, [¹³¹I]-chTNT-1/B), CN-706, CP-358774 (Tarceva®, OSI-774, EGFR inhibitor), CP-609754, CP IL-4-toxin (IL-4 fusion toxin), CS-682, CT-2584 (Apra®, CT-2583,

CT-2586, CT-3536), CTP-37 (Avicine®, hCG blocking vaccine), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, Depo-Cyt®), D-limonene, DAB389-EGF (EGF fusion toxin), Dacarbazine (DTIC), Daclizumab® (Zenapax®), Dactinomycin (Cosmegen®), Daunomycin (Daunorubicin®, Cerubidine®), Daunorubicin (DaunoXome®, Daunorubicin®, Cerubidine®), DeaVac® (CEA anti-idiotype vaccine), Decitabine (5-aza-2'-deoxycytidine), Declopamide (Oxi-104), Denileukin diftitox (Ontak®), Depsipeptide (FR901228, FK228), Dexamethasone (Decadron®), Dexrazoxane (Zinecard®), Diethylnorspermine (DENSPM), Diethylstilbestrol (DES), Dihydro-5-azacytidine, Docetaxel (Taxotere®, Taxane®), Dolasetron mesylate (Anzemet®), Dolastatin-10 (DOLA-10, NSC-376128), Doxorubicin (Adriamycin®, Doxil®, Rubex®), DPPE, DX-8951f (DX-8951), Edatrexate, EGF-P64k Vaccine, Elliott's B Solution®, EMD-121974, Endostatin, Eniluracil (776c85), EO9 (EO1, EO4, EO68, EO70, EO72), Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Epratuzumab® (Lymphocide®, humanized anti-CD22, HAT), Erythropoietin (EPO®, EpoGen®, Procrit®), Estramustine (Emcyt®), Etanidazole (Radinyl®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Exemestane (Aromasin®, Nikidess®), Exetecan mesylate (DX-8951, DX-8951f), Exisulind (SAAND, Aptosyn®, cGMP-PDE2 and 5 inhibitor), F19 (Anti-FAP monoclonal antibody, iodinated anti-FAP MAb), Fadrozole (Afema®, Fadrozole hydrochloride, Arensin®), Fenretinide® (4HPR), Fentanyl citrate (Actiq®), Filgrastim (Neupogen®, G-CSF), FK-317 (FR-157471, FR-70496), Flavopiridol (HMR-1275), Fly3/flk2 ligand (Mobista®), Fluasterone, Fludarabine (Fludara®, FAMP), Fludeoxyglucose (F-18)), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®), Flutamide (Eulexin®), FMdC (KW-2331, MDL-101731), Formestane (Lentaron®), Fotemustine (Muphoran®, Mustophoran®), FUDR (Flouxuridine®), Fulvestrant (Faslodex®), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Gadolinium texaphyrin (Motexafin gadolinium, Gd-Tex®, Xcytrin®), Galarubicin hydrochloride (DA-125), GBC-590, Gastrimimmune® (Anti-gastrin-17 immunogen, anti-g17), Gemcitabine (Gemto®, Gemzar®), Gentuzumab-ozogamicin (Mylotarg®), GL331, Globo H hexasaccharide (Globo H-KLH®), Glufosamide® (β -D-glucosyl-isofosfamide mustard, D19575, INN), Goserelin acetate (Zoladex®), Granisetron (Kytril®), GVAX (GM-CSF gene therapy), Her-2/Neu vaccine, Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), HSPPC-96 (HSP cancer vaccine, gp96 heat shock protein-peptide complex), Hu1D10 (anti-HLA-DR MAb, SMART 1D10), HumaLYM (anti-CD20 MAb), Hydrocortisone, Hydroxyurea (Hydrea®), Hypericin® (VIMRxyn®), I-131 Lipiodol®, Ibrutinomab® tiuxetan (Zevalin®), Idarubicin (Idamycin®, DMDR, IDA), Ifosfamide (IFEX®), Imatinib mesylate (STI-571, Imatinib®, Glivec®, Gleevec®, Abl tyrosine kinase inhibitor), INGN-101 (p53 gene therapy/retrovirus), INGN-201 (p53 gene therapy/adenvirus), Interferon alpha (Alfaferone®, Alpha-IF®), Interferon alpha 2a (Intron A®), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), Interleukin-2 (ProleukinR®), Intoplicine (RP 60475), Irinotecan (Camptosar®, CPT-11, Topotecan®, CaptioCPT-1), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), ISIS-2053 (PKC-alpha antisense), ISIS-2503 (Ras antisense), ISIS-3521 (PKC-alpha antisense), ISIS-5132 (K-ras/raf antisense), Isotretinoin (13-CRA,

13-cis retinoic acid, Accutane®), Ketoconazole (Nizoral®), KRN-8602 (MX, MY-5, NSC-619003, MX-2), L-778123 (Ras inhibitors), L-asparaginase (Elspar®, Crastinin®, Asparaginase medac®, Kidrolase®), Leflunomide (SU-101, SU-0200), Letrozole (Femara®), Leucovorin (Leucovorin®, Wellcovorin®), Leuprolide acetate (Viadur®, Lupron®, Leuprorelin®, Eligard®), Leuvectin® (cytorectin+IL-2 gene, IL-2 gene therapy), Levamisole (Ergamisol®), Liarozole (Liazal, Liazol, R-75251, R-85246, Ro-85264), Lmb-2 immunotoxin (anti-CD25 recombinant immuno toxin, anti-Tac(Fv)-PE38), Lometrexol (T-64, T-904064), Lomustine (CCNU®, CeeNU®), LY-335979, Lym-1 (131-I LYM-1), Lymphoma vaccine (Genitope), Mannan-MUC1 vaccine, Marimastat® (BB-2516, TA-2516, MMP inhibitor), MDX-447 (MDX-220, BAB-447, EMD-82633, H-447, anti-EGFr/FcGammaRlr), Mechlorethamine (Nitrogen Mustard, HN2, Mustargen®), Megestrol acetate (Megace®, Pallace®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Mercaptopurine (6-mercaptopurine, 6-MP), Mesna (Mesnex®), Methotrexate® (MTX, Mexate®, Folex®), Methoxsalen (Uvadex®), 2-Methoxyestradiol (2-ME, 2-ME2), Methylprednisolone (Solumedrol®), Methyltestosterone (Android-10®, Testred®, Virilon®), MGV, Mitomycin C (Mitomycin®, Mutamycin®, Mito Extra®D), Mitoxantrone (Novantrone®, DHAD), Mitumomab® (BEC-2, EMD-60205), Mivobulin isethionate (CI-980), MN-14 (Anti-CEA immunoradiotherapy, ¹³¹I-MN-14, ¹⁸⁸Re-MN-14), Motexafin Lutetium (Lutrin®, Optrin®, Lu-Tex®, lutetium texaphyrin, Lucyn®, Antrin®), MPV-2213 ad (Finroazole®), MS-209, Muc-1 vaccine, NaPro Paclitaxel, Nclarabine (Compound 506, U78), Neovastat® (AE-941, MMP inhibitor), Neugene compounds (Oncomyc-NG, Res-tin-NG, myc antisense), Nilutamide (Nilandron®), NovoMAb-G2 scFv (NovoMAb-G2 IgM), O6-benzylguanine (BG, Procept®), Octreotide acetate (Sandostatin LAR® Depot), Odansetron (Zofran®), Onconase (Ranpirinase®), OncoVAX-CL, OncoVAX-CL Jenner (GA-733-2 vaccine), OncoVAX-P (OncoVAX-PrPSA), Onyx-015 (p53 gene therapy), Orelvekin (Neumage®), Orzel (Tegafur+Uracil+Leucovorin), Oxaliplatin (Eloxatin®, Eloxatin®), Pacis® (BCG, live), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Pamidronate (Aredia®), PC SPES, Pegademase (Adagen®, Pegademase bovine), Pegas-pargase® (Oncospair®), Peldesine (BCX-34, PNP inhibitor), Pemetrexed disodium (Alimta®, MTA, multitargeted antifolate, LY 231514), Pentostatin (Nipent®, 2-deoxycoformycin), Perfosfamide (4-hydroperoxycyclophosphamide, 4-HC), Perillyl alcohol (perilla alcohol, perillie alcohol, perillol, NSC-641066), Phenylbutyrate, Pirarubicin (THP), Pivaloyloxymethyl butyrate (AN-9, Pivanex®), Porfimer sodium (Photofrin®), Prednisone, Prinomastat® (AG-3340, MMP inhibitor), Procarbazine (Matulane®), PROSTVAC, Providence Portland Medical Center Breast Cancer Vaccine, PS-341 (LDP-341, 26S proteosome inhibitor), PSMA MAb (Prostate Specific Membrane Antigen monoclonal antibody), Pyrazoloacridine (NSC-366140, PD-115934), Quinine, R115777 (Zarnestra®), Raloxifene hydrochloride (Evista®, Keoxifene hydrochloride), Raltitrexed (Tomudex®, ZD-1694), Rebeccamycin, Retinoic acid, R-flurbiprofen (Flurizan®, E-7869, MPC-7869), RFS-2000 (9-nitrocamptothecan, 9-NC, rubitecan®), Rituximab® (Rituxan®, anti-CD20 MAb), RSR-13 (GSJ-61), Satraplatin (BMS-182751, JM-216), SCH 6636, SCH-66336, Sizofilan® (SPG, Sizofiran®, Schizophyllan®, Sonifilan®),

SKI-2053R (NSC-D644591), Sobuzoxane (MST-16, Perazolin®, Squalamine (MSI-1256F), SR-49059 (vasopressin receptor inhibitor, V1a), Streptozocin (Zanosar®), SU5416 (Semaxanib®, VEGF inhibitor), SU6668 (PDGF-TK inhibitor), T-67 (T-138067, T-607), Talc (Sclerosol®), Tamoxifen (Nolvadex®), Taurolidine (Taurolin®), Temozolamide (Temodar®, NSC 362856), Teniposide (VM-26, Vumon®), TER-286, Testosterone (Andro®, Androderm®, Testoderm TTS®, Testoderm®, Depo-Testosterone®, Androge®), depoAndro®), Tf-CRM107 (Transferrin-CRM-107), Thalidomide, Theratope, Thioguanine (6-thioguanine, 6-TG), Thiotapec (triethylenethiophosphoamido, Thioplex®), Thymosin alpha I (Zadaxin®, Thymalfasin®), Tiazofurin (Tiazole®), Tirapazamine (SR-259075, SR-4233, Tira-zone®, Win-59075), TNP-470 (AGM-1470, Fumagillin), Tocladesine (8-Cl-cAMP), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Toremifene (Estrimex®, Fareston®), Tositumomab® (Bexxar®), Tretinoïn (Retin-A®, Atragen®, ATRA, Vesanoïd®), TriAb® (anti-idiotype antibody immune stimulator), Trilostane (Modrefen®), Triptorelin pamoate (Trelstar Depot®, Decapeptyl®), Trimetrexate (Neutrexin®), Troxacitabine (BCH-204, BCH-4556, Troxaty®), TS-1, UCN-01 (7-hydroxystaurosporine), Valrubicin (Valstar®), Valspodar (PSC 833), Vapreotide® (BMY-41606), Vaxid (B-cell lymphoma DNA vaccine), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), Vindesine (Eldisine®, Fildesin®), Vinorelbine (Navelbine®), Vitaxin® (LM-609, integrin alphavbeta3 antagonistic MAb), WF 10 (macrophage regulator), WHI-P131, WT1 Vaccine, XR-5000 (DACA), XR-9576 (XR-9351, P-glycoprotein/MDR inhibitor), ZD-9331, ZD-1839 (IRESSA®), and Zoledronate (Zometa®).

[0527] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, prevention, amelioration and/or cure of cancers.

[0528] In further specific embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more combinations of therapeutic agents useful in the treatment, prevention, amelioration and/or cure of cancers including, but not limited to, 9-aminocamptothecin+G-CSF, Adriamycin+Blenoxane+Vinblastine+Dacarbazine (ABVD), BCNU (Carmustine)+Etoposide+Ara-C (Cytarabine)+Melphalen (BEAM), Bevacizumab®+Leucovorin, Bleomycin+Etoposide+Platinol® (Cisplatin) (BEP), Bleomycin+Etoposide+Adriamycin+Cyclophosphamide+Vincristine+Procarbazine+Prednisone (BEACOPP), Bryostatin+Vincristine, Busulfan+Melphalen, Carboplatin+Cereport®, Carboplatin+Cyclophosphamide, Carboplatin+Paclitaxel, Carboplatin+Etoposide+Bleomycin (CEB), Carboplatin+Etoposide+Thiotepa, Cisplatin+Cyclophosphamide, Cisplatin+Docetaxel, Cisplatin+Doxorubicin, Cisplatin+Etoposide, Cisplatin+Gemcitabine, Cisplatin+Interferon alpha, Cisplatin+Irinotecan, Cisplatin+Paclitaxel, Cisplatin+Teniposide, Cisplatin+Vinblastine, Cisplatin+Vindesine, Cisplatin+Vinorelbine, Cisplatin+Cytarabine+Ifosfamide, Cisplatin+Ifosfamide+Vinblastine, Cisplatin+Vinblastine+Mitomycin C, Cisplatin+Vincristine+Fluorouracil, Cisplatin+Vincristine+Lomustine, Cisplatin+Vinorelbine+Gemcitabine, Cisplatin+Carmustine+Dacarbazine+Tamoxifen, Cisplatin+Cyclophosphamide+Etoposide+Vincristine, Cisplatin (Platinol®)+Oncovin®+Doxorubicin (Adriamycin)

cin®)+Etoposide (CODE), Cisplatin+Cytarabine+Ifosfamide+Etoposide+Methotrexate, Cyclophosphamide+Adriamycin® (Doxorubicin), Cyclophosphamide+Melphalan, Cyclophosphamide+SCH 6636, Cyclophosphamide+Adriamycin®+Cisplatin (Platinol®) (CAP), Cyclophosphamide+Adriamycin®+Vincristine (CAV), Cyclophosphamide+Doxorubicin+Teniposide+Prednisone, Cyclophosphamide+Doxorubicin+Teniposide+Prednisone+Interferon alpha, Cyclophosphamide+Epirubicin+Cisplatin (Platinol®) (CEP), Cyclophosphamide+Epirubicin+Fluorouracil, Cyclophosphamide+Methotrexate+Fluoruracil (CMF), Cyclophosphamide+Methotrexate+Vincristine (CMV), Cyclophosphamide+Adriamycin®+Methotrexate+Fluorouracil (CAMF), Cyclophosphamide+Adriamycin®+Methotrexate+Procarbazine (CAMP), Cyclophosphamide+Adriamycin®+Vincristine+Etoposide (CAV-E), Cyclophosphamide+Adriamycin®+Vincristine+Prednisone (CHOP), Cyclophosphamide+Novantrone® (Mitoxantrone)+Vincristine (Oncovorin)+Prednisone (CNOP), Cyclophosphamide+Adriamycin®+Vincristine+Prednisone+Rituximab (CHOP+Rituximab), Cyclophosphamide+Adriamycin®+Vincristine+Teniposide (CAV-T), Cyclophosphamide+Adriamycin+Vincristine alternating with Platinol®+Etoposide (CAV/PE), Cyclophosphamide+BCNU (Carmustine)+VP-16 (Etoposide) (CBV), Cyclophosphamide+Vincristine+Prednisone (CVP), Cyclophosphamide+Oncovin®+Methotrexate+Fluorouracil (COMF), Cytarabine+Methotrexate, Cytarabine+Bleomycin+Vincristine+Methotrexate (CytaBOM), Dactinomycin+Vincristine, Dexamethasone+Cytarabine+Cisplatin (DHAP), Dexamethasone+Ifosfamide+Cisplatin+Etoposide (DICE), Docetaxel+Gemcitabine, Docetaxel+Vinorelbine, Doxorubicin+Vinblastine+Mechlorethamine+Vincristine+Bleomycin+Etoposide+Prednisone (Stanford V), Epirubicin+Gemcitabine, Estramustine+Docetaxel, Estramustine+Navelbine, Estramustine+Paclitaxel, Estramustine+Vinblastine, Etoposide (Vepesid®)+Ifosfamide+Cisplatin (Platinol®) (VIP), Etoposide+Vinblastine+Adriamycin (EVA), Etoposide (Vepesid®)+Ifosfamide+Cisplatin+Epirubicin (VIC-E), Etoposide+Methylprednisolone+Cytarabine+Cisplatin (ESHAP), Etoposide+Prednisone+Ifosfamide+Cisplatin (EPIC), Fludarabine+Mitoxantrone+Dexamethasone (FMD), Fludarabine+Dexamethasone+Cytarabine (ara-C)+Cisplatin (Platinol®) (FluDAP), Fluorouracil+Bevacizumab®, Fluorouracil+CeaVac®, Fluorouracil+Leucovorin, Fluorouracil+Levamisole, Fluorouracil+Oxaliplatin, Fluorouracil+Raltitrexed, Fluorouracil+SCH 6636, Fluorouracil+Trimetrexate, Fluorouracil+Leucovorin+Bevacizumab®, Fluorouracil+Leucovorin+Oxaliplatin, Fluorouracil+Leucovorin+Trimetrexate, Fluorouracil+Oncovin+Mitomycin C (FOMi), Hydrazine+Adriamycin®+Methotrexate (HAM), Ifosfamide+Docetaxel, Ifosfamide+Etoposide, Ifosfamide+Gemcitabine, Ifosfamide+Paclitaxel, Ifosfamide+Vinorelbine, Ifosfamide+Carboplatin+Etoposide (ICE), Ifosfamide+Cisplatin+Doxorubicin, Irinotecan+C225 (Cetuximab®), Irinotecan+Docetaxel, Irinotecan+Etoposide, Irinotecan+Fluorouracil, Irinotecan+Gemcitabine, Mechlorethamine+Oncovin (Vincristine)+Procarbazine (MOP), Mechlorethamine+Oncovin® (Vincristine)+Procarbazine+Prednisone (MOPP), Mesna+Ifosfamide+Idarubicin+Etoposide (MIZE), Methotrexate+Interferon alpha, Methotrexate+Vinblastine, Methotrexate+Cisplatin, Methotrexate with leucovorin rescue+Bleomycin+Adriamycin+Cyclophosphamide+Oncovorin+

Dexamethasone (m-BACOD), Mitomycin C+Ifosfamide+Cisplatin (Platinol®) (MIP), Mitomycin C+Vinblastine+Paraplatin® (MVP), Mitoxantrone+Hydrocortisone, Mitoxantrone+Prednisone, Oncovin®+V+SCH 6636, Oxaliplatin+Leucovorin, Paclitaxel+Doxorubicin, Paclitaxel+SCH 6636, Paraplatin®+Docetaxel, Paraplatin®+Etoposide, Paraplatin®+Gemcitabine, Paraplatin®+Interferon alpha, Paraplatin®+Irinotecan, Paraplatin®+Paclitaxel, Paraplatin®+Vinblastine, Carboplatin (Paraplatin®)+Vincristine, Paraplatin®+Vindesine, Paraplatin®+Vinorelbine, Pemetrexed disodium+Gemcitabine, Platinol® (Cisplatin)+Vinblastine+Bleomycin (PVB), Prednisone+Methotrexate+Adriamycin+Cyclophosphamide+Etoposide (ProMACE), Procarbazine+Lomustine, Procarbazine+Lomustine+Vincristine, Procarbazine+Lomustine+Vincristine+Thioguanine, Procarbazine+Oncovin®+CCNU®+Cyclophosphamide (POCC), Quinine+Doxorubicin, Quinine+Mitoxantrone+Cytarabine, Thiotepa+Etoposide, Thiotepa+Busulfan+Cyclophosphamide, Thiotepa+Busulfan+Melphalan, Thiotepa+Etoposide+Carmustine, Thiotepa+Etoposide+Carboplatin, Topotecan+Paclitaxel, Trimetrexate+Leucovorin, Vinblastine+Doxorubicin+Thiotepa, Vinblastine+Bleomycin+Etoposide+Carboplatin, Vincristine+Lomustine+Prednisone, Vincristine (Oncovin®)+Adriamycin®+Dexamethasone (VAD), Vincristine (Oncoving)+Adriamycin®+Procarbazine (VAP), Vincristine+Dactinomycin+Cyclophosphamide, and Vinorelbine+Gemcitabine.

[0529] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described combinations of therapeutic agents in the treatment, prevention, amelioration and/or cure of cancers.

[0530] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents described above to treat, prevent, ameliorate and/or cure cancers of any tissue known to express DR5 receptor.

[0531] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more therapeutic agents described above to treat, prevent, ameliorate and/or cure cancers of any tissue known to express DR5 receptor.

[0532] Tissues known to express DR5 receptor include, but are not limited to, heart, placenta, lung, liver, skeletal muscle, pancreas, spleen, thymus, prostate, testis, uterus, ovary, small intestine, colon, brain kidney, bone marrow, skin, pituitary, cartilage and blood.

[0533] In specific embodiments polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents, as described above, in the treatment, prevention, amelioration and/or cure of solid tissue cancers (e.g., skin cancer, prostate cancer, pancreatic cancer, hepatic cancer, lung cancer, ovarian cancer, colorectal cancer, head and neck tumors, breast tumors, endometrioma, osteoblastoma, osteoclastoma, Ewing's sarcoma, and Kaposi's sarcoma), as well as hematological cancers (e.g., leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, multiple myeloma).

[0534] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with

one or more therapeutic agents, as described above, in the treatment, prevention, amelioration and/or cure of solid tissue cancers (e.g., skin cancer, prostate cancer, pancreatic cancer, hepatic cancer, lung cancer, ovarian cancer, colorectal cancer, head and neck tumors, breast tumors, endothelioma, osteoblastoma, osteoclastoma, Ewing's sarcoma, and Kaposi's sarcoma), as well as hematological cancers (e.g., leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, multiple myeloma).

[0535] In specific embodiments polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used to treat, ameliorate and/or prevent skin cancers including, but not limited to, basal cell carcinoma, squamous cell carcinoma and malignant melanoma. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent skin cancers.

[0536] In preferred embodiments agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent skin cancers including, but not limited to, basal cell carcinoma, squamous cell carcinoma and malignant melanoma. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent skin cancers.

[0537] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of skin cancers including, but not limited to, Bleomycin (Blenoxane®), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cisplatin (Platinol®, CDDP), Dacarbazine (DTIC), Interferon alpha 2b (Intron A®), Interleukin-2 (ProleukinR®), Tamoxifen (Nolvadex®), Temozolamide (Temodar®, NSC 362856), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), and Vindesine (Eldisine®, Fildesin®).

[0538] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of skin cancers.

[0539] Preferred combinations of therapeutic agents useful in the treatment of skin cancers which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Cisplatin+Carmustine+Dacarbazine+Tamoxifen.

[0540] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of skin cancers.

[0541] In further specific embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent head and neck cancers including brain cancers. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological proce-

dures and/or therapeutic agents to treat, ameliorate and/or prevent head and neck cancers including brain cancers. Brain cancers which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, gliomas such as astrocytomas and oligodendromas, non-glial tumors such as neuronal, meningeal, ependymal and choroid plexus cell tumors, and metastatic brain tumors such as those originating as breast, lung, prostate and skin cancers.

[0542] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent head and neck cancers including brain cancers. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent head and neck cancers including brain cancers. Brain cancers which may be treated using agonists and/or antagonists of the present invention include, but are not limited to, gliomas such as astrocytomas and oligodendromas, non-glial tumors such as neuronal, meningeal, ependymal and choroid plexus cell tumors, and metastatic brain tumors such as those originating as breast, lung, prostate and skin cancers.

[0543] In preferred embodiments, agonists and/or antagonists of the invention are used to treat brain tumors. In one preferred embodiment, agonists of the invention are used to treat glioblastoma multiforme.

[0544] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more radiological procedures useful in the treatment of brain cancers including, but not limited to, external beam radiation therapy, stereotactic radiation therapy, conformal radiation therapy, intensity-modulated radiation therapy (IMRT), and radiosurgery.

[0545] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more radiological procedures useful in the treatment of brain cancers including, but not limited to, external beam radiation therapy, stereotactic radiation therapy, conformal radiation therapy, intensity-modulated radiation therapy (IMRT), and radiosurgery.

[0546] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of brain cancers including, but not limited to, Bleomycin (Blenoxane®), Busulfan (Busulfex®, Myleran®), Carboplatin (Paraplatin®, CBDCA), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (IntraDose®, FocaCist®), Cyclophosphamide (Cytoxan®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®), Dacarbazine (DTIC®), Dactinomycin (Cosmegen®), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Docetaxel (Taxotere®, Taxane®), Dexamethasone (Decadron®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fluorouracil (5-FU, Adrucil®), Hydroxyurea (Hydrea®), Ifosfamide (IFEX®), Lomustine (CCNU®, CeeNU®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Mercaptopurine (6-mercaptopurine, 6-MP), Meth-chlorethamine (Nitrogen Mustard, HN2, Mustargen®),

Methotrexate® (MTX, Mexate®, Folex®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Procarbazine (Matulane®), Temozolamide (Temodar®, NSC 362856), Teniposide (VM-26, Vumon®), Thioguanine (6-thioguanine, 6-TG), Thiotepa (triethylenethiophosphoramide), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), and Vincristine (Oncovin®, Onco TCS®V, VCR, Leurocristine®).

[0547] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of brain cancers.

[0548] Further examples of therapeutic agents useful in the treatment of brain cancers which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, 81C6 (Anti-tenascin monoclonal antibody), BIBX-1382, Cereport® (Lobradimil®, RMP-7), Cilengitide® (EMD-121974, integrin alphavbeta3 antagonist), CMT-3 (Metastat®), Cotara® (chTNT-1I/B, [¹³¹I]-chTNT-1/B), CP IL-4-toxin (IL-4 fusion toxin), Fenretinide® (4HPR), Fotemustine (Muphoran®, Mustophoran®), Gemcitabine (Gemto®, Gemzar®), Hypericin® (VIMRxyn®), Imatinib mesylate (STI-571, Imatinib®, Gleevec®, Gleevec®, Abl tyrosine kinase inhibitor), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Leflunomide (SU-101, SU-0200), Mivobulin isethionate (CI-980), 06-benzylguanine (BG, Procept®), Prinomastat® (AG-3340, MMP inhibitor), R115777 (Zamestra®), SU6668 (PDGF-TK inhibitor), T-67 (T-138067, T-607), Tamoxifen (Nolvadex®), Tf-CRM107 (Transferrin-CRM-107), Thalidomide, Tiazofurin (Tizazole®), Vapreotide® (BMY-41606), Vinorelbine (Navelbine®), and XR-5000 (DACA).

[0549] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of brain cancers.

[0550] Preferred combinations of therapeutic agents useful in the treatment of brain cancers which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Busulfan+Melphalan, Carboplatin+Cereport®, Carboplatin+Etoposide, Carboplatin+Etoposide+Thiotepa, Cisplatin+Etoposide, Cisplatin+Cytarabine+Ifosfamide, Cisplatin+Vincristine+Lomustine, Cisplatin+Cyclophosphamide+Etoposide+Vincristine, Cisplatin+Cytarabine+Ifosfamide+Etoposide+Methotrexate, Cyclophosphamide+Melphalan, Cytarabine+Methotrexate, Dactinomycin+Vincristine, Mechlorethamine+Oncovin® (Vincristine)+Procarbazine (MOP), Mechlorethamine+Oncovin® (Vincristine)+Procarbazine+Prednisone (MOPP), Carboplatin (Paraplatin®)+Etoposide, Carboplatin (Paraplatin®)+Vincristine, Procarbazine+Lomustine, Procarbazine+Lomustine+Vincristine, Procarbazine+Lomustine+Vincristine+Thioguanine, Thiotepa+Etoposide, Thiotepa+Etoposide+Carmustine, Thiotepa+Etoposide+Carboplatin, Vinblastine+Bleomycin+Etoposide+Carboplatin, and Vincristine+Lomustine+Prednisone.

[0551] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described combinations of therapeutic agents in the treatment, amelioration and/or prevention of brain cancers.

[0552] In further particular embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent breast cancer. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent breast cancer. Breast cancers which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, ductal carcinoma, stage I, stage II, stage III and stage IV breast cancers as well as invasive breast cancer and metastatic breast cancer.

[0553] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent breast cancer. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent breast cancer. Breast cancers which may be treated using agonists and/or antagonists of the present invention include, but are not limited to, ductal carcinoma, stage I, stage II, stage III and stage IV breast cancers as well as invasive breast cancer and metastatic breast cancer.

[0554] In preferred embodiment, agonists and/or antagonists of the invention are used to treat metastatic breast cancer.

[0555] In other preferred embodiments, agonists and/or antagonists of the present invention are administered in combination with one or more surgical and/or radiological procedures useful in the treatment of breast cancer.

[0556] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of breast cancer including, but not limited to, Amifostine (Ethylol®), Aminoglutethimide (Cytadren®), Anastrozole (Arimidex®), Bleomycin (Blenoxane®), Capecitabine (Xeloda®), Doxifluridine®, oral 5-FU), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (IntraDose®, FocaCist®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Docetaxel (Taxotere®, Taxane®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Exemestane (Aromasin®, Nikidess®), Fadrozole (Afema®, Fadrozole hydrochloride, Arens®), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®), Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), Ifosfamide (IFEX®), Letrozole (Femara®), Leucovorin (Leucovorin®, Wellcovorin®), Mechlorethamine (Nitrogen Mustard, HN2, Mustargen®), Megestrol acetate (Megace®, Pallace®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Methotrexate® (MTX, Mexate®, Folex®), Methyltestosterone (Android-10®, Testred®, Virilon®), Mitomycin C (Mitomycin®, Mutamycin®, Mito Extra®), Orzel® (Tegafur+Uracil+Leucovorin), Paclitaxel (Paxene®, Taxol®), Sobuzoxane (MST-16, Perazolin®), Tamoxifen (Nolvadex®), Testosterone (Andro®, Androderm®, Testoderm TTS®, Testoderm®, Depo-Testosterone®, Androgel®, depoAndro®), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), and Vinorelbine (Navelbine®).

[0557] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with

one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of breast cancers.

[0558] Further examples of therapeutic agents useful in the treatment of breast cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Aldesleukin (IL-2, Proleukin®), Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Angiostatin, Annamycin (AR-522, annamycin LF, Aronex®), Bircodar dicitrate (Incel®, Incel MDR Inhibitor), Boronated Protoporphyrin Compound (PDIT, Photodynamic Immunotherapy), Bryostatin-1 (Bryostatin, BMY-45618, NSC-339555), Busulfan (Busulfex®, Myleran®), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), D-limonene, Dacarbazine (DTIC), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Dolastatin-10 (DOLA-10, NSC-376128), DPPE, DX-8951f (DX-8951), EMD-121974, Endostatin, EO9 (EO1, EO4, EO68, EO70, EO72), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fluasterone, Fludarabine (Fludara®, FAMP), Flutamide (Eulexin®), Formestane (Lentaron®), Fulvestrant (Faslodex®), Galarubicin hydrochloride (DA-125), Gemcitabine (Gemto®, Gemzar®), Her-2/Neu vaccine, Hydroxyurea (Hydrea®), Idarubicin (Idamycin®, DMDR, IDA), Interferon alpha 2a (Intron A®), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Ketoconazole (Nizoral®), KRN-8602 (MX, MY-5, NSC-619003, MX-2), L-asparaginase (Elspar®), Leuprolide acetate (Viadur®, Lupron®), Lomustine (CCNU®, CeeNU®), LY-335979, Mannan-MUC1 vaccine, 2-Methoxyestradiol (2-ME, 2-ME2), Mitoxantrone (Novantrone®, DHAD), Motexafin Lutetium (Lutrin®, Optrin®, Lu-Tex®, lutetium texaphyrin, Lucyn®, Antrin®), MPV-2213ad (Finrozole®), MS-209, Muc-1 vaccine, NaPro Paclitaxel, Perillyl alcohol (perilla alcohol, perillic alcohol, perillol, NSC-641066), Pirarubicin (THP), Procarbazine (Matulane®), Providence Portland Medical Center Breast Cancer Vaccine, Pyrazoloacridine (NSC-366140, PD-115934), Raloxifene hydrochloride (Evista®, Keoxifene hydrochloride), Raltitrexed (Tomudex®, ZD-1694), Rebecamycin, Streptozocin (Zanosar®), Temozolamide (Temodar®, NSC 362856), Theratope, Thiotepa (triethylenethiophosphaoramide, Tmioplex®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Toremifene (Estrimex®, Fareston®), Trilostane (Modrefen®), and XR-9576 (XR-9351, P-glycoprotein/MDR inhibitor).

[0559] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of breast cancers.

[0560] Preferred combinations of therapeutic agents useful in the treatment of breast cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Cyclophosphamide+ Adriamycin® (Doxorubicin), Cyclophosphamide+Epirubicin+Fluorouracil, Cyclophosphamide+Methotrexate+Fluorouracil (CMF), Paclitaxel+Doxorubicin, and Vinblastine+ Doxorubicin+Thiotepa.

[0561] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with

one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of breast cancers.

[0562] In further specific embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent lung cancer. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent lung cancer. Lung cancer which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof includes, but is not limited to, non-small cell lung cancer (NSCLC) including early stage NSCLC (i.e., Stage IA/IB and Stage IIA/IIB), Stage IIIA NSCLC, Stage IIA(unresectable)/IIB NSCLC and Stage IV NSCLC, small cell lung cancer (SCLC) including limited stage SCLC and extensive stage SCLC as well as Malignant Pleural Mesothelioma.

[0563] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent lung cancer. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent lung cancer. Lung cancer which may be treated using agonists and/or antagonists of the present invention includes, but is not limited to, non-small cell lung cancer (NSCLC) including early stage NSCLC (i. e., Stage IA/IB and Stage IIA/ IIB), Stage IIIA NSCLC, Stage IIA(unresectable)/IIB NSCLC and Stage IV NSCLC, small cell lung cancer (SCLC) including limited stage SCLC and extensive stage SCLC as well as Malignant Pleural Mesothelioma.

[0564] In preferred embodiments, agonists and/or antagonists of the invention are used to treat non-small cell lung cancers.

[0565] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of lung cancer including, but not limited to, BAY 43-9006 (Raf kinase inhibitor), Carboplatin (Paraplatin®, CBDCA), Chlorambucil (Leukeran®), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (IntraDose®, FocaCist®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Docetaxel (Taxotere®, Taxane®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Edatrexate, Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Gemcitabine (Gemto®, Genzar®), Herceptint (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), Ifosfamide (IFEX®), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Lomustine (CCNUO, CeeNU®), Mechlorethamine (Nitrogen Mustard, HN2, Mustargen®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Methotrexate® (MTX, Mexatee, Folex®), Mitomycin C (Mitomycin®, Mutamycin®, Mito Extra®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Porfimer sodium (Photofrin®), Procarbazine (Matulane®), SKI-2053R (NSC-D644591), Teniposide (VM-26, Vumon®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Vinblastine (Velban®,

VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), Vindesine (Eldisine®, Fildesin®), and Vinorelbine (Navelbine®).

[0566] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of lung cancers.

[0567] Further examples of therapeutic agents useful in the treatment of lung cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, ABX-EGF (anti-EGFr MAb), Acetyl dinanine (CI-994), AG-2034 (AG-2024, AG-2032, GARFT [glycinamide ribonucleoside transformylase] inhibitor), Alanosine, Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Angiostatin, Aplidine (Aplidin®, Aplidinae), BBR 3464, Bexarotene (Targretin®, LGD1069), BIBH-1 (Anti-FAP MAb), BIBX-1382, BLP-25 (MUC-1 peptide), Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), Budesonide (Rhinocort®), C225 (IMC-225, EGFR inhibitor, Anti-EGFr MAb, Cetuximab®), Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Carboxyamidotriazole (NSC 609974, CAI, L-651582), CEA-cide® (Labetuzumab®, Anti-CEA monoclonal antibody, hMN-14), Cereport® (Lobradimil®, RMP-7), CI-1033 (Pan-erbB RTK inhibitor), Cilengitide® (EMD-121974, integrin alphavbeta3 antagonist), 9-cis retinoic acid (9-cRA), Cisplatin-liposomal (SPI-077), CMB-401 (Anti-PEM MAb/calicheamycin), CMT-3 (Metastat®), CP-358774 (Tarceva®, OSI-774, EGFR inhibitor), CT-2584 (Apra®), DAB389-EGF (EGF fusion toxin), Dea Vac® (CEA anti-idiotype vaccine), Decitabine (5-aza-2'-deoxyytidine), Diethylnor spermine (DENSPM), Dihydro-5-azacytidine, EGF-P64k Vaccine, Endostatin, Etanidazole (Radinyl®), Exetecan mesylate (DX-8951, DX-8951f), Exisulind (SAAND, Aptosyn®, cGMP-PDE2 and 5 inhibitor), FK-317 (FR-157471, FR-70496), Flavopiridol (HMR-1275), Fotemustine (Muphoran®, Mustophoran®), G3139 (Genasense®, GenetaAnticode®, Bcl-2 antisense), Gadolinium texaphyrin (Motexafin gadolinium, Gd-Tex®, Xcytrin®), GBC-590, GL331, Galarubicin hydrochloride (DA-125), Glufosfamide® (β -D-glucosyl-isofosfamide mustard, D19575, INN), GVAX (GM-CSF gene therapy), INGN-101 (p53 gene therapy/retrovirus), INGN-201 (p53 gene therapy/adenvirus), Irofulven (MGI-114), ISIS-2053, ISIS-3521 (PKC-alpha antisense), ISIS-5132 (K-ras/raf antisense), Isotretinoin (13-CRA, 13-cis retinoic acid, Accutane®), Lometrexol (T-64, T-904064), Marimastat® (BB-2516, TA-2516, MMP inhibitor), MDX-447 (BAB-447, EMD-82633, H-447, anti-EGFr/FcGammaR1r), MGV, Mitumomab® (BEC-2, EMD-60205), Mivobulin isethionate (CI-980), Neovastat® (AE-941, MMP inhibitor), Onconase (Ranpirnase®), Onyx-015 (p53 gene therapy), Pemetrexed disodium (Alimta®, MTA, multitargeted antifolate, LY 231514), Pivaloyloxyethyl butyrate (AN-9, Pivanex®), Prinomastat® (AG-3340, MMP inhibitor), PS-341 (LD-341, 26S proteosome inhibitor), Pyrazoloacridine (NSC-366140, PD-115934), R115777 (Zarnestra®), Raltitrexed (Tomudex®, ZD-1694), R-flurbiprofen (Flurizan®, E-7869, MPC-7869), RFS-2000 (9-nitrocamptothecan, 9-NC, rubitecan®), RSR-13 (GSJ-61), Satraplatin (BMS-182751, JM-216), SCH-66336, Sizofilan® (SPG, Sizofiran®, Schizophyllan®, Sonifilan®), Squalamine (MSI-1256F), SR-49059 (vasopressin receptor inhibitor, V1a), SU5416

(Semaxanib®, VEGF inhibitor), Taurolidine (Taurolin®), Temozolamide (Temodar®, NSC 362856), Thalidomide, Thymosin alpha I (Zadaxin®, Thymalfasin®), Tirapazamine (SR-259075, SR-4233, Tirazone®, Win-59075), TNP-470 (AGM-1470), TriAb® (anti-idiotype antibody immune stimulator), Tretinoin (Retin-A®, Atragen®, ATRA, Vesanoïd®), Troxacitabine (BCH-204, BCH-4556, Troxatyl®), Vitaxin® (LM-609, integrin alphavbeta3 antagonistic MAb), XR-9576 (P-glycoprotein/MDR inhibitor), and ZD-1839 (IRESSA®).

[0568] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of lung cancers.

[0569] Preferred combinations of therapeutic agents useful in the treatment of lung cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Cisplatin+Docetaxel, Cisplatin+Etoposide, Cisplatin+Gemcitabine, Cisplatin+Interferon alpha, Cisplatin+Irinotecan, Cisplatin+Paclitaxel, Cisplatin+Teniposide, Cisplatin+Vinblastine, Cisplatin+Vindesine, Cisplatin+Vinorelbine, Cisplatin+Vinblastine+Mitomycin C, Cisplatin+Vinorelbine+Gemcitabine, Cisplatin (Platinol®)+Oncovin®+Doxorubicin (Adriamycin®)+Etoposide (CODE), Cyclophosphamide+Adriamycin®+Cisplatin (Platinol®) (CAP), Cyclophosphamide+Adriamycin®+Vincristine (CAV), Cyclophosphamide+Epirubicin+Cisplatin (Platinol®) (CEP), Cyclophosphamide+Methotrexate+Vincristine (CMV), Cyclophosphamide+Adriamycin®D, Methotrexate+Fluorouracil (CAMF), Cyclophosphamide+Adriamycin®, Methotrexate+Procarbazine (CAMP), Cyclophosphamide+Adriamycin®, Vincristine+Etoposide (CAV-E), Cyclophosphamide+Adriamycin®, Vincristine+Teniposide (CAV-T), Cyclophosphamide+Oncovin®, Methotrexate+Fluorouracil (COMF), Cyclophosphamide+Adriamycin®+Vincristine, alternating with Cisplatin+Etoposide (CAV/PE), Docetaxel+Gemcitabine, Docetaxel+Vinorelbine, Etoposide (Vepesid®)+Ifosfamide+Cisplatin (Platinol®) (VIP), Etoposide (Vepesid®)+Ifosfamide, Cisplatin+Epirubicin (VIC-E), Fluorouracil+Oncovin®+Mitomycin C (FOMi), Hydrazine+Adriamycin+Methotrexate (HAM), Ifosfamide+Docetaxel, Ifosfamide+Etoposide, Ifosfamide+Gemcitabine, Ifosfamide+Paclitaxel, Ifosfamide+Vinorelbine, Ifosfamide+Carboplatin+Etoposide (ICE), Irinotecan+Docetaxel, Irinotecan+Etoposide, Irinotecan+Gemcitabine, Methotrexate+Cisplatin, Methotrexate+Interferon alpha, Methotrexate+Vinblastine, Mitomycin C+Ifosfamide+Cisplatin (Platinol®) (MIP), Mitomycin C+Vinblastine+Paraplatin® (MVP), Paraplatin®V+Docetaxel, Paraplatin®V+Etoposide, Paraplatin+Gemcitabine, Paraplatin®V+Interferon alpha, Paraplatin®+Irinotecan, Paraplatin+Paclitaxel, Paraplatin®+Vinblastine, Paraplatin+Vindesine, Paraplatin®+Vinorelbine, Procarbazine+Oncovin®+CCNU® (Lomustine)+Cyclophosphamide (POCC), Vincristine (Oncovin®)+Adriamycin+Procarbazine (VAP), and Vinorelbine+Gemcitabine.

[0570] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of lung cancers.

[0571] In further particular embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent colorectal cancer. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent colorectal cancer. Colorectal cancers which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, colon cancer (e.g., early stage colon cancer (stage I and II), lymph node positive colon cancer (stage III), metastatic colon cancer (stage IV)) and rectal cancer.

[0572] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent colorectal cancer. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent colorectal cancer. Colorectal cancers which may be treated using agonists and/or antagonists of the present invention include, but are not limited to, colon cancer (e.g., early stage colon cancer (stage I and II), lymph node positive colon cancer (stage III), metastatic colon cancer (stage IV)) and rectal cancer.

[0573] In preferred embodiments, agonists and/or antagonists of the invention are used to treat colon cancer.

[0574] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of colorectal cancer including, but not limited to, Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Fluorouracil (5-FU, Adrucil®, Fluropoplex®, Efudex®), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Leucovorin (Leucovorin®, Wellcovorin®), and Levamisole (Ergamisol®).

[0575] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of colorectal cancers.

[0576] Preferred combinations of therapeutic agents useful in the treatment of colorectal cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Fluorouracil+Leucovorin and Fluorouracil+Levamisole.

[0577] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of colorectal cancers.

[0578] Further examples of therapeutic agents useful in the treatment of colorectal cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aplidine (Aplidin®, Aplidina®), Bevacizumab® (Anti-VEGF monoclonal antibody, rhuMAb-VEGF), C225 (IMC-225, EGFR

inhibitor, Anti-EGFr MAb, Cetuximab®), C242-DM1 (huC242-DM1), CC49-zeta gene therapy, CEA-cide® (Labetuzumab®, Anti-CEA monoclonal antibody, hMN-14), CeaVac® (MAb 3H1), CP-609754, CTP-37 (Avicine®, hCG blocking vaccine), Declopamide (Oxi-104), Eniluracil (776c85), F19 (Anti-FAP monoclonal antibody, iodinated anti-FAP MAb), FMdC (KW-2331, MDL-101731), FUDR (Flouxuridine®D), Gemcitabine (Gemto®, Gemzar®), Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), Intoplicine (RP 60475), L-778123 (Ras inhibitors), Leuvectin® (cytotoxin+IL-2 gene, IL-2 gene therapy), MN-14 (Anti-CEA immunoradiotherapy, ¹³¹I-MN14, ¹⁸⁸Re-MN-14), OncoVAX-CL, OncoVAX-CL-Jenner (GA-733-2 vaccine), Orzel® (Tegafur+Uracil+Leucovorin), Oxaliplatin (Eloxatin®, Eloxatin®), Paclitaxel-DHA (Taxoprexin®), Pemetrexed disodium (Alimta®, MTA, multitargeted antifolate, LY 231514), R115777 (Zamestra®), Raltitrexed (Tomudex®, ZD-1694), SCH 66336, SU5416 (Semaxanib®, VEGF inhibitor), Tocladesine (8-Cl-cAMP), Trimetrexate (Neutrexin®), TS-1, and ZD-9331.

[0579] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of colorectal cancers.

[0580] Further exemplary combinations of therapeutic agents useful in the treatment of colorectal cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Aminocamptothecin+G-CSF, Bevacizumab®+Fluorouracil, Bevacizumab®+Leucovorin, Bevacizumab®+Fluorouracil+Leucovorin, Cyclophosphamide+SCH 6636, Fluorouracil+CeaVac®, Fluorouracil+Oxaliplatin, Fluorouracil+Raltitrexed, Fluorouracil+SCH 6636, Fluorouracil+Trimetrexate, Fluorouracil+Leucovorin+Oxaliplatin, Fluorouracil+Leucovorin+Trimetrexate, Irinotecan+C225 (Cetuximab®), Oncovin®+SCH 6636, Oxaliplatin+Leucovorin, Paclitaxel+SCH 6636, Pemetrexed disodium+Gemcitabine, and Trimetrexate+Leucovorin.

[0581] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of colorectal cancers.

[0582] In further specific embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent prostate cancer. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent prostate cancer. Prostate cancer which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof includes, but is not limited to, benign prostatic hyperplasia, malignant prostate cancer (e.g., stage I, stage II, stage III or stage IV) and metastatic prostate cancer.

[0583] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate

and/or prevent prostate cancer. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent prostate cancer. Prostate cancer which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof includes, but is not limited to, benign prostatic hyperplasia, malignant prostate cancer (e.g., stage I, stage II, stage III or stage IV) and metastatic prostate cancer.

[0584] In preferred embodiments, agonists and/or antagonists of the invention are used to treat malignant prostate cancer. In other preferred embodiments, agonists and/or antagonists of the invention are used to treat metastatic prostate cancer.

[0585] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more surgical, radiological and/or hormonal procedures useful in the treatment of prostate cancer including, but not limited to, prostatectomy (e.g., radical retropubic prostatectomy), external beam radiation therapy, brachytherapy, orchietomy and hormone treatment (e.g., LHRH agonists, androgen receptor inhibitors).

[0586] In preferred embodiments, agonists and/or antagonists of the present invention may be administered in combination with one or more surgical, radiological and/or hormonal procedures useful in the treatment of prostate cancer including, but not limited to, prostatectomy (e.g., radical retropubic prostatectomy), external beam radiation therapy, brachytherapy, orchietomy and hormone treatment (e.g., LHRH agonists, androgen receptor inhibitors).

[0587] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of prostate cancer including, but not limited to, Aminoglutethimide (Cytadren®), Bicalutamide (Casodex®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Diethylstilbestrol (DES), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Flutamide (Eulexin®), Hydrocortisone, Ketoconazole (Nizoral®), Leuprolide acetate (Viadur®, Lupron®, Leuprorel®, Eligard®), Mitoxantrone (Novantrone®, DHAD), Nilutamide (Nilandron®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), PC SPES, Prednisone, Triptorelin pamoate (Trelstar Depot®, Decapeptyl®), and Vinblastine (Velban®, VLB).

[0588] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of prostate cancers.

[0589] Further examples of therapeutic agents useful in the treatment of prostate cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Abarelix® (Abarelix-Depot-M®, PPI-149, R-3827); Abiraterone acetate(& (CB-7598, CB-7630), ABT-627 (ET-1 inhibitor), APC-8015 (Provence®, Dendritic cell therapy), Avorelin® (Meterelin®, MF-6001, EP-23904), CEP-701 (KT-5555), CN-706,

CT-2584 (Apra®, CT-2583, CT-2586, CT-3536), GBC-590, Globo H hexasaccharide (Globo H-KLH®), Interferon alpha 2a (Intron A®), Liarozole (Liazal, Liazol, R-75251, R-85246, Ro-85264), MDX-447 (MDX-220, BAB-447, EMD-82633, H-447, anti-EGFr/FcGammaR1r), OncoVAX-P (OncoVAX-PrPSA), PROSTVAC, PS-341 (LDP-341, 26S proteosome inhibitor), PSMA MAb (Prostate Specific Membrane Antigen monoclonal antibody), and R-flurbiprofen (Flurizan®, E-7869, MPC-7869).

[0590] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of prostate cancers.

[0591] Preferred combinations of therapeutic agents useful in the treatment of prostate cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Docetaxel+Estramustine, Mitoxantrone+Hydrocortisone, Mitoxantrone+Prednisone, Navelbine+Estramustine, Paclitaxel+Estramustine, and Vinblastine+Estramustine.

[0592] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of prostate cancers.

[0593] In further specific embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent pancreatic cancer. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent pancreatic cancer. Pancreatic cancers which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, adenocarcinoma, endocrine (islet cell) tumors, tumors confined to the pancreas, locally advanced pancreatic cancer and metastatic pancreatic cancer.

[0594] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent pancreatic cancer. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent pancreatic cancer. Pancreatic cancers which may be treated using agonists and/or antagonists of the present invention include, but are not limited to, adenocarcinoma, endocrine (islet cell) tumors, tumors confined to the pancreas, locally advanced pancreatic cancer and metastatic pancreatic cancer.

[0595] In preferred embodiments, agonists and/or antagonists of the invention are used to treat locally advanced pancreatic cancer. In other preferred embodiments, agonists and/or antagonists of the invention are used to treat metastatic pancreatic cancer.

[0596] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more surgical

and/or radiological procedures useful in the treatment of pancreatic cancer including, but not limited to, pancreaticoduodenectomy (Whipple resection).

[0597] In preferred embodiments, agonists and/or antagonists of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of pancreatic cancer including, but not limited to, pancreaticoduodenectomy (Whipple resection).

[0598] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of pancreatic cancer including, but not limited to, Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Cisplatin (Platinol®, CDDP), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®), Gemcitabine (Gemto®, Gemzar®), and Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1).

[0599] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of pancreatic cancers.

[0600] Preferred combinations of therapeutic agents useful in the treatment of pancreatic cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Cisplatin+Gemcitabine, CP-358774+Gemcitabine, Docetaxel+Gemcitabine, Irinotecan+Fluorouracil, Irinotecan+Gemcitabine, and Paclitaxel+Gemcitabine.

[0601] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of pancreatic cancers.

[0602] Further examples of therapeutic agents useful in the treatment of pancreatic cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, ABX-EGF (anti-EGFr MAb), Acetylindoline (CI-994, GOE-5549, GOR-5549, PD-130636), BMS-214662 (BMS-192331, BMS-193269, BMS-206635), BNP-1350 (BNPI-1100, Karenitecins), C225 (IMC-225, EGFR inhibitor, Anti-EGFr MAb, Cetuximab®), C242-DM1 (huC242-DM1, SB-408075), Carbendazin® (FB-642), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), CMT-3 (COL-3, Metastat®), CP-358774 (Tarseva®, OSI-774, EGFR inhibitor), Docetaxel (Taxotere®, Taxane®), Exetecan mesylate (DX-8951, DX-8951f), Flavopiridol (HMR-1275), Gasuimmune® (Anti-gastrin-17 immunogen, anti-g17), GBC-590, Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), HSPPC-96 (HSP cancer vaccine, gp96 heat shock protein-peptide complex), Irofulven (MGI-114), ISIS-2503 (Ras antisense), Onyx-015 (p53 gene therapy), Paclitaxel (Paxene®, Taxol®), Pemetrexed disodium (Alimta®, MTA, multitargeted antifolate, LY 231514), Perillyl alcohol (perilla alcohol, perillic alcohol, perillol, NSC-641066), RFS-2000 (9-nitrocamptothecan, 9-NC, rubitecan®), and Rituximab® (Rituxan®, anti-CD20 MAb).

[0603] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of pancreatic cancers.

[0604] In further particular embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent hepatic cancer. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent hepatic cancer. Hepatic cancers which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, hepatocellular carcinoma, malignant hepatoma, cholangiocarcinoma, mixed hepatocellular cholangiocarcinoma or hepatoblastoma.

[0605] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent hepatic cancer. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent hepatic cancer. Hepatic cancers which may be treated using agonists and/or antagonists of the present invention include, but are not limited to, hepatocellular carcinoma, malignant hepatoma, cholangiocarcinoma, mixed hepatocellular cholangiocarcinoma or hepatoblastoma.

[0606] In preferred embodiments, agonists and/or antagonists of the invention are used to treat hepatoblastoma. In other preferred embodiments, agonists and/or antagonists of the invention are used to treat hepatocellular carcinoma.

[0607] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of hepatic cancers including, but not limited to, partial hepatectomy, liver transplant, radiofrequency ablation, laser therapy, microwave therapy, cryosurgery, percutaneous ethanol injection, hepatic arterial infusion, hepatic artery ligation, chemoembolization and external beam radiation therapy.

[0608] In preferred embodiments, agonists and/or antagonists of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of hepatic cancers including, but not limited to, partial hepatectomy, liver transplant, radiofrequency ablation, laser therapy, microwave therapy, cryosurgery, percutaneous ethanol injection, hepatic arterial infusion, hepatic artery ligation, chemoembolization and external beam radiation therapy.

[0609] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of hepatic cancer including, but not limited to, Aldesleukin (IL-2, Proleukin®), Cisplatin (Platinol®, CDDP), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fluorouracil (5-FU, Adrucil®, Flu-

roplex®, Efudex®), I-131 Lipidiol®, Ifosfamide (IFEX®), Megestrol acetate (Megace®, Pallace®), Pravastatin sodium (Pravachol®), and Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®).

[0610] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of hepatic cancers.

[0611] Preferred combinations of therapeutic agents useful in the treatment of hepatic cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Cisplatin+Doxorubicin, Cisplatin+Etoposide, Cisplatin+Vincristine+Fluorouracil, and Ifosfamide+Cisplatin+Doxorubicin.

[0612] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of hepatic cancers.

[0613] In further particular embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent ovarian cancer. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent ovarian cancer. Ovarian cancers which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, epithelial carcinoma, germ cell tumors and stromal tumors.

[0614] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent ovarian cancer. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent ovarian cancer. Ovarian cancers which may be treated using agonists and/or antagonists of the present invention include, but are not limited to, epithelial carcinoma, germ cell tumors and stromal tumors.

[0615] In preferred embodiments, agonists and/or antagonists of the invention are used to treat germ cell tumors. In other preferred embodiments, agonists and/or antagonists of the invention are used to treat epithelial carcinoma.

[0616] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of ovarian cancer including, but not limited to, hysterectomy, oophorectomy, hysterectomy with bilateral salpingo-oophorectomy, omentectomy, tumor debulking, external beam radiation therapy and intraperitoneal radiation therapy.

[0617] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described surgical and/or radiological procedures in the treatment, amelioration and/or prevention of ovarian cancers.

[0618] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of ovarian cancer including, but not limited to, Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Bleomycin (Blenoxane®), Carboplatin (Paraplatin®, CBDCA), Cisplatin (Platinol®, CDDP), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Dactinomycin (Cosmegen®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fluorouracil (5-FU, Adrucil®, Fluoroplex®, Efudex®), Gemcitabine (Gemto®, Gemzar®), Ifosfamide (IFEX®), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Leucovorin (Leucovorin®, Wellcovorin®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Paclitaxel (Paxene®, Taxol®), Tamoxifen (Nolvadex®), Vinblastine (Velban®, VLB) and Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®).

[0619] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of ovarian cancers.

[0620] Preferred combinations of therapeutic agents useful in the treatment of ovarian cancer which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Bleomycin+Etoposide+Platinol® (Cisplatin) (BEP), Carboplatin+Cyclophosphamide, Carboplatin+Paclitaxel, Carboplatin+Etoposide+Bleomycin (CEB), Cisplatin+Cyclophosphamide, Cisplatin+Etoposide, Cisplatin+Paclitaxel, Cisplatin+Ifosfamide+Vinblastine, Fluorouracil+Leucovorin, Platinol® (Cisplatin)+Vinblastine+Bleomycin (PVB), and Vincristine+Dactinomycin+Cyclophosphamide.

[0621] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of ovarian cancers.

[0622] In further particular embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent Ewing's sarcoma. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent Ewing's sarcoma. Ewing's sarcoma family tumors which may be treated using polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Ewing's tumor of bone (ETB), extraosseus Ewing's (EOE), primitive neuroectodermal tumors (PNET or peripheral neuroepithelioma) and Askin's tumor.

[0623] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent Ewing's sarcoma. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent Ewing's sarcoma. Ewing's sarcoma family tumors which

may be treated using agonists and/or antagonists of the present invention include, but are not limited to, Ewing's tumor of bone (ETB), extraosseus Ewing's (EOE), primitive neuroectodermal tumors (PNET or peripheral neuroepithelioma) and Askin's tumor.

[0624] In preferred embodiments, agonists and/or antagonists of the invention are used to treat Ewing's tumor of bone. In other preferred embodiments, agonists and/or antagonists of the invention are used to treat peripheral neuroepithelioma.

[0625] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of Ewing's sarcoma family tumors.

[0626] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more surgical and/or radiological procedures useful in the treatment of Ewing's sarcoma family tumors.

[0627] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of Ewing's sarcoma family tumors including, but not limited to, Cyclophosphamide (Cytoxan®, Neosar®, CTX), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Filgrastim (Neupogen®, G-CSF), Ifosfamide (IFEX®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), and Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®).

[0628] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of Ewing's sarcoma family tumors.

[0629] Preferred combinations of therapeutic agents useful in the treatment of Ewing's sarcoma family tumors which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Cyclophosphamide+Topotecan, Cyclophosphamide+Doxorubicin+Vincristine, Cyclophosphamide+Doxorubicin+Vincristine, alternating with Ifosfamide+Etoposide and Cyclophosphamide+Doxorubicin+Vincristine, alternating with Filgrastim+Ifosfamide+Etoposide.

[0630] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of Ewing's sarcoma family tumors.

[0631] In further specific embodiments, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are used to treat, ameliorate and/or prevent hematological cancers. Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent hematological cancers. Hematological cancers which may be treated using polynucleotides and/or polypeptides of the invention and/or

agonists and/or antagonists thereof include, but are not limited to, non-Hodgkin's lymphoma (e.g., small lymphocytic lymphoma, follicular center cell lymphoma, lymphoplasmacytoid lymphoma, marginal zone lymphoma, mantle cell lymphoma, immunoblastic lymphoma, burkitt's lymphoma, lymphoblastic lymphoma, peripheral T-cell lymphoma, anaplastic large cell lymphoma and intestinal T-cell lymphoma), leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia and plasma cell neoplasms including multiple myeloma.

[0632] In preferred embodiments, agonists and/or antagonists of the present invention are used to treat, ameliorate and/or prevent hematological cancers. Agonists and/or antagonists of the present invention may be used in combination with one or more surgical and/or radiological procedures and/or therapeutic agents to treat, ameliorate and/or prevent hematological cancers. Hematological cancers which may be treated using agonists and/or antagonists of the present invention include, but are not limited to, non-Hodgkin's lymphoma (e.g., small lymphocytic lymphoma, follicular center cell lymphoma, lymphoplasmacytoid lymphoma, marginal zone lymphoma, mantle cell lymphoma, immunoblastic lymphoma, burkitt's lymphoma, lymphoblastic lymphoma, peripheral T-cell lymphoma, anaplastic large cell lymphoma and intestinal T-cell lymphoma), leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia and plasma cell neoplasms including multiple myeloma.

[0633] In preferred embodiments, agonists and/or antagonists of the invention are used to treat plasma cell neoplasms. In certain preferred embodiments, that plasma cell neoplasm is multiple myeloma.

[0634] In other preferred embodiment, agonists and/or antagonists of the invention are used to treat non-Hodgkin's lymphoma.

[0635] In other preferred embodiments, agonists and/or antagonists of the invention are used to treat leukemia. In certain preferred embodiments, that leukemia is acute lymphocytic leukemia. In certain preferred embodiments, that leukemia is chronic lymphocytic leukemia.

[0636] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of hematological cancer including, but not limited to, bone marrow transplantation, external beam radiation and total body irradiation.

[0637] In specific embodiments, agonists and/or antagonists of the invention are administered in combination with one or more surgical and/or radiological procedures useful in the treatment of hematological cancer including, but not limited to, bone marrow transplantation, external beam radiation and total body irradiation.

[0638] In preferred embodiments, agonists and/or antagonists of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of multiple myeloma including, but not limited to, allogeneic bone marrow transplantation and peripheral stem cell support.

[0639] In other preferred embodiments, agonists and/or antagonists of the present invention may be administered in

combination with one or more surgical and/or radiological procedures useful in the treatment of non-Hodgkin's lymphoma including, but not limited to, allogeneic bone marrow transplantation and peripheral stem cell support.

[0640] In other preferred embodiments, agonists and/or antagonists of the present invention may be administered in combination with one or more surgical and/or radiological procedures useful in the treatment of leukemia including, but not limited to, allogeneic bone marrow transplantation and peripheral stem cell support. In specific embodiments, agonists and/or antagonists of the invention are used to treat acute lymphocytic leukemia (ALL). In other specific embodiments, agonists and/or antagonists of the invention are used to treat chronic lymphocytic leukemia (CLL).

[0641] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of multiple myeloma including, but not limited to, Alkylating agents, Anthracyclines, Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Dexamethasone (Decadron®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Methotrexate® (MTX, Mexate®, Folex®), Methylprednisolone (Solumedrol®), Mitoxantrone (Novantrone®, DHAD), Ondansetron (Zofran®), Pentostatin (Nipent®, 2-deoxycoformycin), Perfosfamide (4-hydroperoxycyclophosphamide, 4-HC), Prednisone, Procarbazine (Matulane®), Rituximab® (Rituxan®, anti-CD20 MAB), Thiotapec (triethylenethiophosphoaramide, Thioplex®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Etoposide®), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®) and Vindesine (Eldisine®, Fildesin®).

[0642] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of multiple myeloma.

[0643] Preferred combinations of therapeutic agents useful in the treatment of multiple myeloma which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Cyclophosphamide+Prednisone, Melphalan+Prednisone (MP), Vincristine+Adriamycin®+Dexamethasone (VAD), Vincristine+Carmustine+Melphalan+Cyclophosphamide+Prednisone (VBMC; the M2 protocol), and Vincristine+Melphalan+Cyclophosphamide+Prednisone alternating with Vincristine+Carmustine+Doxorubicin+Prednisone (VMCP/VBAP).

[0644] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of multiple myeloma.

[0645] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of non-Hodgkin's lymphoma including, but not limited to, 2-chlorodeoxyadenosine, Amifostine (Ethylol®, Ethiofos®, WR-272), Bexarotene (Targretin®, Targretin gel®, Targretin oral®, LGD1069), Bleomycin (Blenoxane®), Busulfan (Busulfex®, Myleran®), Carboplatin (Paraplatin®, CBDCA), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Chlorambucil (Leukeran®), Cisplatin (Platinol®, CDDP), Cladribine (2-CdA, Leustatin®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®), Dacarbazine (DTIC), Daunorubicin

(Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Denileukin diftitox (Ontak®), Dexamethasone (Decadron®), Dolasetron mesylate (Anzemet®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Erythropoietin (EPO®, Epogen®, Procrit®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fludarabine (Fludara®, FAMP), Granisetron (Kytril®), Hydrocortisone, Idarubicin (Idamycin®, DMDR, IDA), Ifosfamide (IFEX®), Interferon alpha (Alfaferone®, Alpha-IF®), Interferon alpha 2a (Intron A®), Mechlorethamine (Nitrogen Mustard, HN₂, Mustargen®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Methotrexate® (MTX, Mexate®, Folex®), Methylprednisolone (Solumedrol®), Mitoxantrone (Novantrone®, DHAD), Ondansetron (Zofran®), Pentostatin (Nipent®, 2-deoxycoformycin), Perfosfamide (4-hydroperoxycyclophosphamide, 4-HC), Prednisone, Procarbazine (Matulane®), Rituximab® (Rituxan®, anti-CD20 MAB), Thiotapec (triethylenethiophosphoaramide, Thioplex®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Etoposide®), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®) and Vindesine (Eldisine®, Fildesin®).

[0646] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of non-Hodgkin's lymphoma.

[0647] Preferred combinations of therapeutic agents useful in the treatment of non-Hodgkin's lymphoma which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Adriamycin®+Blenoxane+Vinblastine+Dacarbazine (ABVD), Anti-idiotype therapy (BsAb)+Interferon alpha, Anti-idiotype therapy (BsAb)+Chlorambucil, Anti-idiotype therapy (BsAb)+Interleukin-2, BCNU (Carmustine)+Etoposide+Ara-C (Cytarabine)+Melphalen (BEAM), Bleomycin+Etoposide+Adriamycin+Cyclophosphamide+Vincristine+Procarbazine+Prednisone (BEACOPP), Bryostatin+Vincristine, Cyclophosphamide+BCNU (Carmustine)+VP-16 (Etoposide) (CBV), Cyclophosphamide+Vincristine+Prednisone (CVP), Cyclophosphamide+Adriamycin® (Hydroxydaunomycin)+Vincristine (Oncovin)+Prednisone (CHOP), Cyclophosphamide+Novantrone® (Mitoxantrone)+Vincristine (Oncovin)+Prednisone (CNOP), Cyclophosphamide+Doxorubicin+Teniposide+Prednisone, Cyclophosphamide+Adriamycin® (Hydroxydaunomycin)+Vincristine (Oncovin)+Prednisone+Rituximab (CHOP+Rituximab), Cyclophosphamide+Doxorubicin+Teniposide+Prednisone+Interferon alpha, Cytarabine+Bleomycin+Vincristine+Methotrexate (Cytarabine+Bleomycin+Vincristine+Methotrexate) (Cytarabine+Bleomycin+Vincristine+Methotrexate), Dexamethasone+Cytarabine+Cisplatin (DHAP), Dexamethasone+Ifosfamide+Cisplatin+Etoposide (DICE), Doxorubicin+Vinblastine+Mechlorethamine+Vincristine+Bleomycin+Etoposide+Prednisone (Stanford V), Etoposide+Vinblastine+Adriamycin (EVA), Etoposide+Methylprednisolone+Cytarabine+Cisplatin (ESHAP), Etoposide+Prednisone+Ifosfamide+Cisplatin (EPIC), Fludarabine, Mitoxantrone+Dexamethasone (FMD), Fludarabine, Dexamethasone, Cytarabine (ara-C),+Cisplatin (Platinol®) (FluDAP), Ifosfamide+Cisplatin+Etoposide (ICE), Mechlorethamine+Oncovin (Vincristine)+Procarbazine+Prednisone (MOPP), Mesna+Ifosfamide+Idarubicin+Etoposide (MIZE), Methotrexate with leucovorin rescue+Bleomycin

cin+Adriamycin+Cyclophosphamide+Oncovorin+ Dexamethasone (m-BACOD), Prednisone+Methotrexate+ Adriamycin+Cyclophosphamide+Etoposide (ProMACE), Thiotepa+Busulfan+Cyclophosphamide, Thiotepa+Busulfan+Melphalan, Topotecan+Paclitaxel, and Vincristine (Oncovin®)+Adriamycin(O+Dexamethasone (VAD).

[0648] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of non-Hodgkin's lymphoma.

[0649] Further examples of therapeutic agents useful in the treatment of non-Hodgkin's lymphoma which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, A007 (4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone), AG-2034 (AG-2024, AG-2032, GARFT [glycinamide ribonucleoside transformylase] inhibitor), Aldesleukin (IL-2, Proleukin®), Alemtuzumab (Campath®), Altretinoin (Panretin®, LGN-1057), Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Anti-CD19/CD3 MAb (anti-CD19/CD3 scFv, anti-NHL MAb), Anti-idiotype therapy (BsAb), Arabinosylguanine (Ara-G, GW506U78), Arsenic trioxide (Trisenox®, ATO), B43-Genistein (anti-CD19 Ab/genistein conjugate), B7 antibody conjugates, Betathine (Beta-LT), BLyS antagonists, Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), CHML (Cytotropic Heterogeneous Molecular Lipids), Clofarabine (chlorofluoro-araA), Daclizumab (Zenapax®), Depsipeptide (FR901228, FK228), Dolastatin-10 (DOLA-10, NSC-376128), Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Epratuzumab (Lymphocide®, humanized anti-CD22, HAT), Fly3/flk2 ligand (Mobista®), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Hu1D10 (anti-HLA-DR MAb, SMART 1D10), HumaLYM (anti-CD20 MAb), Ibrutumomab tiuxetan (Zevalin®), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), ISIS-2053, ISIS-3521 (PKC-alpha antisense), Lmb-2 immunotoxin (anti-CD25 recombinant immuno toxin, anti-Tac(Fv)-PE38), Leuvelectin® (cytorectin+IL-2 gene, IL-2 gene therapy), Lym-1 (131-I LYM-1), Lymphoma vaccine (Genitope), Nelarabine (Compound 506, U78), Neugene compounds (Oncomyc-NG®, Resten-NG®, myc antisense), NovoMAb-G2 scFv (NovoMAb-G2 IgM), O6-benzylguanine (BG, Procept®), Oxaliplatin (Eloxatine®, Eloxatin®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Peldesine (BCX-34, PNP inhibitor), Rebeccamycin and Rebeccamycin analogues, SCH-66336, Sobuzoxane (MST-16, Perazolin®), SU5416 (Semaxanib®, VEGF inhibitor), TER-286, Thalidomide, TNP-470 (AGM-1470), Tositumomab (Bexxar®), Valspodar (PSC 833), Vaxid (B-cell lymphoma DNA vaccine), Vinorelbine (Navelbine®), WF10 (macrophage regulator) and XR-9576 (XR-9351, P-glycoprotein/MDR inhibitor).

[0650] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of non-Hodgkin's lymphoma.

[0651] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of acute lymphocytic leukemia including, but not limited to, Amsacrine, Carboplatin (Paraplatin®, CBDCA), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cholecaliferol, Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, arac, cytosine arabinoside, DepoCyt®), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Dexamethasone (Decadron®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide (VP-16, Vepesid®), Filgrastam® (Neupogen®, G-CSF, Leukine®), Fludarabine (Fludara®, FAMP), Idarubicin (Idamycin®, DMDR, IDA), Ifosfamide (IFEX®), Imatinib mesylate (STI-571, Imatinib®, Glivec®, Gleevec®, Abl tyrosine kinase inhibitor), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), L-asparaginase (Elspar®, Crastin®), Asparaginase medac®, Kidrolase®), Mercaptopurine (6-mercaptopurine, 6-MP), Methotrexate® (MTX, Mexate®, Folex®), Mitoxantrone (Novantrone®, DHAD), Pegaspargase® (Oncospars®), Prednisone, Retinoic acid, Teniposide (VM-26, Vumon®), Thioguanine (6-thioguanine, 6-TG), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Etoposide®), Tretinoin (Retin-A®, Atragen®, ATRA, Vesano®) and Vincristine (Oncovorin®, Onco TCS®, VCR, Leurocristine®).

[0652] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of acute lymphocytic leukemia.

[0653] Further examples of therapeutic agents useful in the treatment of acute lymphocytic leukemia which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminopterin, Annamycin (AR-522, annamycin LF, Aronex®), Arabinosylguanine (Ara-G, GW506U78, Nelzarabine®), Arsenic trioxide (Trisenox®, ATO, Atrivex®), B43-Genistein (anti-CD19 Ab/genistein conjugate), B43-PAP (anti-CD19 Ab/pokeweed antiviral protein conjugate), Cordycepin, CS-682, Decitabine (5-aza-2'-deoxycytidine), Dolastatin-10 (DOLA-10, NSC-376128), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), MS-209, Phenylbutyrate, Quinine, TNP-470 (AGM-1470, Fumagillin), Trimetrexate (Neutrexin®), Troxacitabine (BCH-204, BCH-4556, Troxatyl®), UCN-01 (7-hydroxystaurosporine), WHI-P131 and WT1 Vaccine.

[0654] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of acute lymphocytic leukemia.

[0655] Preferred combinations of therapeutic agents useful in the treatment of acute lymphocytic leukemia which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Carboplatin+Mitoxantrone, Carmustine+Cyclophosphamide+Eto-

poside, Cytarabine+Daunorubicin, Cytarabine+Doxorubicin, Cytarabine+Idarubicin, Cytarabine+Interferon gamma, Cytarabine+L-asparaginase, Cytarabine+Mitoxantrone, Cytarabine+Fludarabine and Mitoxantrone, Etoposide+Cytarabine, Etoposide+Ifosfamide, Etoposide+Mitoxantrone, Ifosfamide+Etoposide+Mitoxantrone, Ifosfamide+Teniposide, Methotrexate+Mercaptopurine, Methotrexate+Mercaptopurine+Vincristine+Prednisone, Phenylbutyrate+Cytarabine, Phenylbutyrate+Etoposide, Phenylbutyrate+Topotecan, Phenylbutyrate+Tretinoin, Quinine+Doxorubicin, Quinine+Mitoxantrone+Cytarabine, Thioguanine+Cytarabine+Amsacrine, Thioguanine+Etoposide+Idarubicin, Thioguanine+Retinoic acid+Cholecalciferol, Vincristine+Prednisone, Vincristine+Prednisone and L-asparaginase, Vincristine+Dexamethasone/Prednisone+Asparaginase+Daunorubicin/Doxorubicin, Vincristine+Dexamethasone/Prednisone+Asparaginase+Daunorubicin/Doxorubicin+Filgrastim, Vincristine+Dexamethasone/Prednisone+Asparaginase+Daunorubicin/Doxorubicin+Cyclophosphamide+Methotrexate, and Vincristine+Dexamethasone/Prednisone+Asparaginase+Daunorubicin/Doxorubicin+Cyclophosphamide+Methotrexate+Filgrastim.

[0656] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of acute lymphocytic leukemia.

[0657] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof may be administered in combination with one or more therapeutic agents useful in the treatment of chronic lymphocytic leukemia including, but not limited to, Chlorambucil (Leukeran®), Cladribine (2-CdA, Leustatin®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®, cytarabine ocfosfate, ara-CMP), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Fludarabine (Fludara®, FAMP), Pentostatin (Nipent®, 2-deoxycoformycin), Prednisone and Vincristine (Oncovorin®, Onco TCS®, VCR, Leurocristine®).

[0658] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of chronic lymphocytic leukemia.

[0659] Further examples of therapeutic agents useful in the treatment of chronic lymphocytic leukemia which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Alemtuzumab (Campath®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminopterin, Annamycin (AR-522, annamycin LF, Aronex®), Arabinosylguanine (Ara-G, GW506U78, Nelzarabine®, Compound 506U78), Arsenic trioxide (Trisenox®, ATO, Atrivex®), Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), CS-682, Dolastatin-10 (DOLA-10, NSC-376128), Filgrastim @Neupogen®, G-CSF, Leukine, Flavopiridol (NSC-649890, HMR-1275), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), MS-209, Phenylbutyrate, Rituximab® (Rituxan(V, anti-

CD20 MAb), Thalidomide, Theophylline, TNP-470 (AGM-1470, Fumagillin), UCN-01 (7-hydroxystauroporine) and WHI-P131.

[0660] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agents in the treatment, amelioration and/or prevention of chronic lymphocytic leukemia.

[0661] Preferred combinations of therapeutic agents useful in the treatment of chronic lymphocytic leukemia which may be administered in combination with polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof include, but are not limited to, Fludarabine+Prednisone, and Cyclophosphamide+Doxorubicin+Vincristine+Prednisone (CHOP).

[0662] In preferred embodiments, agonists and/or antagonists of the invention are administered in combination with one or more of the above-described therapeutic agent combinations in the treatment, amelioration and/or prevention of chronic lymphocytic leukemia.

[0663] DR5 polynucleotides or polypeptides, or agonists of DR5, can be used in the treatment and/or prevention of infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B-cells in response to an infectious agent, infectious diseases may be treated and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, DR5 polynucleotides or polypeptides, or agonists or antagonists of DR5, may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

[0664] Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated and/or prevented by DR5 polynucleotides or polypeptides, or agonists of DR5. Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue virus, HIV-1, HIV-2, Flaviviridae, Hepadnaviridae (e.g., hepatitis B virus), Herpesviridae (e.g., cytomegalovirus, herpes simplex viruses 1 and 2, varicella-zoster virus, Epstein-Barr virus (EBV), herpes B virus, and human herpes viruses 6, 7, and 8), Morbillivirus, Rhabdoviridae (e.g., rabies virus), Orthomyxoviridae (e.g., influenza A virus, and influenza B), Paramyxoviridae (e.g., parainfluenza virus), papilloma virus, Papovaviridae, Parvoviridae, Picornaviridae (e.g., EMCV and poliovirus), Poxviridae (e.g., areola or vaccinia virus), Reoviridae (e.g., rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). These viruses and virus families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory diseases, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, smallpox, opportunistic infections (e.g. AIDS, Kaposi's sarcoma), pneumonia, Burkitt's lymphoma, chickenpox, zoster, hemorrhagic fever, measles, mumps, parainfluenza, rabies, the common cold, polio, leukemia, rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and

viremia. DR5 polynucleotides or polypeptides, or agonists or antagonists of DR5, can be used to treat, prevent, and/or detect any of these symptoms or diseases. In specific embodiments, DR5 polynucleotides, polypeptides, or agonists are used to treat and/or prevent: meningitis, Dengue, EBV, and/or hepatitis. In an additional specific embodiment DR5 polynucleotides, polypeptides, or agonists are used to treat patients non-responsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment, DR5 polynucleotides, polypeptides, or agonists are used to treat AIDS.

[0665] Similarly, bacteria and fungi that can cause disease or symptoms and that can be treated and/or prevented by DR5 polynucleotides or polypeptides, or agonists or antagonists of DR5, include, but are not limited to the following organisms. Bacteria include, but are not limited to *Actinomyces*, *Bacillus* (e.g., *B. anthracis*), *Bacteroides*, *Bordetella*, *Bartonella*, *Borrelia* (e.g., *B. burgdorferi*), *Brucella*, *Campylobacter*, *Capnocytophaga*, *Chlamydia*, *Clostridium*, *Corynebacterium*, *Coxiella*, *Dermatophilus*, *Enterococcus*, *Ehrlichia*, *Escherichia* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), *Francisella*, *Fusobacterium*, *Haemobartonella*, *Haemophilus* (e.g., *H. influenzae* type b), *Helicobacter*, *Klebsiella*, L-form bacteria, *Legionella*, *Lepospira*, *Listeria*, *Mycobacteria* (e.g., *M. leprae* and *M. tuberculosis*), *Mycoplasma*, *Neisseria* (e.g., *N. gonorrhoeae* and *N. meningitidis*), *Neorickettsia*, *Nocardia*, *Pasteurella*, *Peptococcus*, *Peptostreptococcus*, *Pneumococcus*, *Proteus*, *Pseudomonas*, *Rickettsia*, *Rochalimaea*, *Salmonella* (e.g., *S. typhimurium* and *S. typhi*), *Serratia*, *Shigella*, *Staphylococcus* (e.g., *S. aureus*), *Streptococcus* (e.g., *S. pyogenes*, *S. pneumoniae*, and Group B *streptococcus*), *Streptomyces*, *Treponema*, *Vibrio* (e.g., *Vibrio cholerae*) and *Yersinia* (e.g., *Y. pestis*). Fungi include, but are not limited to: *Absidia*, *Acremonium*, *Alternaria*, *Aspergillus*, *Basidiobolus*, *Bipolaris*, *Blastomycetes*, *Candida* (e.g., *C. albicans*), *Coccidioides*, *Conidiobolus*, *Cryptococcus* (e.g., *C. neoformans*), *Curvularia*, *Erysipelothrrix*, *Epidermophyton*, *Exophiala*, *Geotrichum*, *Histoplasma*, *Madurella*, *Malassezia*, *Microsporum*, *Moniliella*, *Mortierella*, *Mucor*, *Paecilomyces*, *Penicillium*, *Phialemonium*, *Phialophora*, *Prototheca*, *Pseudallescheria*, *Pseudomicrodochium*, *Pythium*, *Rhinosporidium*, *Rhizopus*, *Scolecosbasidium*, *Sporothrix*, *Stemphylium*, *Trichophyton*, *Trichosporon*, and *Xylohypha*. These and other bacteria or fungi can cause diseases or symptoms including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prostheses-related infections, Reiter's Disease, respiratory tract infections, such as whooping cough or emphysema, sepsis, Lyme Disease, cat-scratch disease, dysentery, paratyphoid fever, food poisoning, typhoid, pneumonia, gonorrhea, meningitis, chlamydia, syphilis, diphtheria, leprosy, paratuberculosis, tuberculosis, lupus, botulism, gangrene, tetanus, impetigo, rheumatic fever, scarlet fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocytoses), toxemia, urinary tract infections, and wound infections. DR5 polynucleotides or polypeptides, or agonists or antagonists of DR5, can be used to treat, prevent and/or detect any of these symptoms or diseases. In specific embodiments, DR5 polynucleotides, polypeptides, or agonists thereof are used to treat and/or prevent: tetanus, diphtheria, botulism, and/or meningitis type B.

[0666] Moreover, parasites causing parasitic diseases or symptoms that can be treated and/or prevented by DR5 polynucleotides or polypeptides, or agonists of DR5, include, but are not limited to: protozoan parasites including, but not limited to, *Babesia*, *Balantidium*, *Besnoitia*, *Cryptosporidium*, *Emerita*, *Encephalitozoon*, *Entamoeba*, *Giardia*, *Hammondia*, *Hepatozoon*, *Isospora*, *Leishmania*, *Microsporidia*, *Neospora*, *Nosema*, *Pentatrichomonas*, *Plasmodium* (e.g., *Plasmodium virax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*), *Pneumocystis*, *Sarcocystis*, *Schistosoma*, *Theileria*, *Toxoplasma*, and *Trypanosoma*; and helminth parasites including, but not limited to, *Acanthocheilonema*, *Aelurostrongylus*, *Ancylostoma*, *Angiostrongylus*, *Ascaris*, *Brugia*, *Bunostomum*, *Capillaria*, *Chabertia*, *Cooperia*, *Crenosoma*, *Dictyocaulus*, *Diectophyme*, *Dipetalonema*, *Diphyllobothrium*, *Diplydium*, *Dirofilaria*, *Dracunculus*, *Enterobius*, *Filaroides*, *Haemonchus*, *Lagochilascaris*, *Loa*, *Mansonella*, *Muellerius*, *Nanophyetus*, *Necator*, *Nematodirus*, *Oesophagostomum*, *Onchocerca*, *Opisthorchis*, *Ostertagia*, *Parafilaria*, *Paragonimus*, *Parascaris*, *Physaloptera*, *Protostongylus*, *Setaria*, *Spirocera*, *Spirometra*, *Stephanofilaria*, *Strongyloides*, *Strongylus*, *Thelazia*, *Toxascaris*, *Toxocara*, *Trichinella*, *Trichostrongylus*, *Trichuris*, *Uncinaria*, and *Wuchereria*. These parasites can cause a variety of diseases or symptoms, including, but not limited to: scabies, trombiculiasis, eye infections (e.g., river blindness), elephantiasis, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. DR5 polynucleotides or polypeptides, or agonists or antagonists of DR5, can be used to treat, prevent and/or detect any of these symptoms or diseases. In specific embodiments, DR5 polynucleotides, polypeptides, or agonists thereof are used to treat and/or prevent malaria.

[0667] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are also useful as a vaccine adjuvant to enhance immune responsiveness to specific antigen, tumor-specific, and/or anti-viral immune responses.

[0668] An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex virus, and yellow fever.

[0669] Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to

enhance an immune response to a bacterium or fungus, disease, or symptom selected from the group consisting of: tetanus, diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, Group B *streptococcus*, *Shigella* spp., Enterotoxigenic *E. coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

[0670] Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to *Plasmodium* spp. (malaria).

[0671] More generally, DR5 polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in regulating (i.e., elevating or reducing) immune response. For example, polynucleotides and/or polypeptides of the invention may be useful in preparation or recovery from surgery, trauma, radiation therapy, chemotherapy, and transplantation. Further, polynucleotides and/or polypeptides of the invention may be used to boost immune response and/or accelerate recovery in the elderly and immunocompromised individuals, or as an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies. Also, polynucleotides and/or polypeptides of the invention may be useful as an agent to induce higher affinity antibodies, or to increase serum immunoglobulin concentrations.

[0672] In one embodiment, DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may be used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogenic or xenogenic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T-cell populations, but prior to full recovery of B-cell populations.

[0673] In another embodiment, DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may be used as an agent to boost immunoresponsiveness among B-cell immunodeficient individuals. B-cell immunodeficiencies that may be ameliorated or treated and/or prevented by administering the DR5 polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, severe combined immune deficiency (SCID), congenital agammaglobulinemia, common variable immunodeficiency, Wiskott-Aldrich Syndrome, and X-linked immunodeficiency with hyper IgM.

[0674] Additionally, DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may be

used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B-cell function. Conditions resulting in an acquired loss of B-cell function that may be ameliorated, treated, and/or prevented by administering the DR5 polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B-cell chronic lymphocytic leukemia (CLL).

[0675] Furthermore, DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may be used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated, treated, and/or prevented by administering the DR5 polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery.

[0676] DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may also be used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, DR5 (in soluble, membrane-bound or transmembrane forms) enhances antigen presentation or antagonizes antigen presentation in vitro or in vivo.

[0677] In related embodiments, said enhancement or antagonization of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system. For example, DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may be used as an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response. Also, DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may be used as a stimulator of B-cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

[0678] In another embodiment, DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may be used as a means to induce tumor proliferation and thus make the tumor more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

[0679] Other embodiments where DR5 polynucleotides and/or polypeptides of the invention and/or agonists thereof may be used include, but are not limited to: as a stimulator of B-cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency; as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect; as a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients; as an antigen for the generation of antibodies to inhibit or enhance DR5 mediated responses; as a means of activating T-cells; as pretreatment of bone marrow samples prior to transplant (such treatment would increase B-cell representation and thus accelerate recovery); as a means of regulating secreted cytokines that

are elicited by DR5; to modulate IgE concentrations in vitro or in vivo; and to treat and/or prevent IgE-mediated allergic reactions including, but are not limited to, asthma, rhinitis, and eczema.

[0680] Alternatively, DR5 polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful as immunosuppressive agents, for example in the treatment and/or prevention of autoimmune disorders. In specific embodiments, polynucleotides and/or polypeptides of the invention are used to treat and/or prevent chronic inflammatory, allergic or autoimmune conditions, such as those described herein or are otherwise known in the art.

[0681] Preferably, treatment using DR5 polynucleotides or polypeptides, or agonists of DR5, could either be by administering an effective amount of DR5 polypeptide to the patient, or by removing cells from the patient, supplying the cells with DR5 polynucleotide, and returning the engineered cells to the patient (ex vivo therapy). Moreover, as further discussed herein, the DR5 polypeptide or polynucleotide can be used as an adjuvant in a vaccine to raise an immune response against infectious disease.

[0682] Additional preferred embodiments of the invention include, but are not limited to, the use of DR5 polypeptides and functional agonists in the following applications: administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response; or administration to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO96/34096, WO96/33735, and WO91/10741).

[0683] Antagonists of DR5 include binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the DR5 receptor(s). These would be expected to reverse many of the activities of herein, as well as find clinical or practical application including, but not limited to the following applications. DR5 antagonists may be used as a means of blocking various aspects of immune responses to foreign agents or self, for example, autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens. Although our current data speaks directly to the potential role of DR5 in B-cell and T-cell related pathologies, it remains possible that other cell types may gain expression or responsiveness to DR5. Thus, DR5 may, like CD40 and its ligand, may be regulated by the status of the immune system and the microenvironment in which the cell is located. DR5 antagonists may be used as a therapy for preventing the B-cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus

erythematosus and; as an inhibitor of graft versus host disease or transplant rejection; as a therapy for B-cell malignancies such as ALL, Hodgkin's disease, non-Hodgkin's lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases; as a therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonal gammopathies, and plasmacytomas; as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas; as a means of decreasing the involvement of B-cells and Ig associated with Chronic Myelogenous Leukemia; or as an immunosuppressive agent.

[0684] Furthermore, DR5 polypeptides or polynucleotides of the invention, or antagonists thereof may be used to modulate IgE concentrations in vitro or in vivo, or to treat and/or prevent IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.

[0685] All of the therapeutic applications of DR5 polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof described herein may, in addition to their uses in human medicine, be used in veterinary medicine. The present invention includes treatment of companion animals, including, but not limited to dogs, cats, ferrets, birds, and horses; food animals, including, but not limited to cows, pigs, chickens, and sheep; and exotic animals, e.g., zoo animals.

[0686] The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

[0687] DR5 polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof described herein may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

[0688] In one aspect, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR5 polypeptide an effective amount of DR5 ligand, analog or an agonist capable of increasing DR5 mediated signaling. Preferably, DR5 mediated signaling is increased to treat and/or prevent a disease wherein decreased apoptosis or decreased cytokine and adhesion molecule expression is exhibited. An agonist can include soluble forms of DR5 and monoclonal antibodies directed against the DR5 polypeptide.

[0689] In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR5 polypeptide an effective amount of an antagonist capable of decreasing DR5 mediated signaling. Preferably, DR5 mediated signaling is decreased to treat and/or prevent a disease wherein increased apoptosis or NF- κ B expression is exhibited. An antagonist can include

soluble forms of DR5 (e.g., polypeptides containing all or a portion of the DR5 extracellular domain) and monoclonal antibodies directed against the DR5 polypeptide.

[0690] The present invention further encompasses methods and compositions for killing of cells expressing DR5 on their surface, comprising, or alternatively consisting of, contacting agonists of the invention with such cells expressing DR5 on their surface.

[0691] In preferred embodiments, the present invention further encompasses methods and compositions for killing of cells expressing DR5 on their surface, comprising, or alternatively consisting of, contacting agonistic anti-DR5 antibodies of the invention with such cells expressing DR5 on their surface.

[0692] In specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing DR5 on their surface, comprising, or alternatively consisting of, contacting agonists of the invention with such cells expressing DR5 on their surface.

[0693] In preferred embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing DR5 on their surface, comprising, or alternatively consisting of, contacting agonistic anti-DR5 antibodies of the invention with such cells expressing DR5 on their surface.

[0694] In further specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising amino acids about 52 to about 184 of SEQ ID NO:2 on their surface, comprising, or alternatively consisting of, contacting agonists of the invention with such cells expressing said polypeptide on their surface.

[0695] In preferred embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising amino acids about 52 to about 184 of SEQ ID NO:2 on their surface, comprising, or alternatively consisting of, contacting agonistic anti-DR5 antibodies of the invention with such cells expressing said polypeptide on their surface.

[0696] In further specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920 on their surface, comprising, or alternatively consisting of, contacting agonists of the invention with such cells expressing said polypeptide on their surface.

[0697] In preferred embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920 on their surface, comprising, or alternatively consisting of, contacting agonistic anti-DR5 antibodies of the invention with such cells expressing said polypeptide on their surface.

[0698] The present invention further encompasses methods and compositions for killing of cells expressing DR5 on their surface, comprising, or alternatively consisting of, administering to an animal, agonists of the invention in an amount effective to kill such DR5 expressing cells.

[0699] In preferred embodiments, the present invention further encompasses methods and compositions for killing of cells expressing DR5 on their surface, comprising, or alternatively consisting of, administering to an animal, agonistic anti-DR5 antibodies of the invention in an amount effective to kill such DR5 expressing cells.

[0700] In specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing DR5 on their surface, comprising, or alternatively consisting of, administering to an animal, agonists of the invention in an amount effective to induce apoptosis in such DR5 expressing cells.

[0701] In preferred embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing DR5 on their surface, comprising, or alternatively consisting of, administering to an animal, agonistic anti-DR5 antibodies of the invention in an amount effective to induce apoptosis in such DR5 expressing cells.

[0702] In further specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising amino acids about 52 to about 184 of SEQ ID NO:2 on their surface, comprising, or alternatively consisting of, administering to an animal, agonists of the invention in an amount effective to induce apoptosis in such cells expressing said polypeptide on their surface.

[0703] In preferred embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising amino acids about 52 to about 184 of SEQ ID NO:2 on their surface, comprising, or alternatively consisting of, administering to an animal, agonistic anti-DR5 antibodies of the invention in an amount effective to induce apoptosis in such cells expressing said polypeptide on their surface.

[0704] In further specific embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920 on their surface, comprising, or alternatively consisting of, administering to an animal, agonists of the invention in an amount effective to induce apoptosis such cells expressing said polypeptide on their surface.

[0705] In preferred embodiments, the present invention encompasses methods and compositions for inducing apoptosis in cells expressing a polypeptide comprising the extracellular domain of the polypeptide encoded by the cDNA clone contained in ATCC Deposit No. 97920 on their surface, comprising, or alternatively consisting of, administering to an animal, agonistic anti-DR5 antibodies of the invention in an amount effective to induce apoptosis such cells expressing said polypeptide on their surface.

[0706] By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating apoptosis. By "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting apoptosis. Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below.

[0707] One such screening procedure involves the use of melanophores, which are transfected to express the receptor of the present invention. Such a screening technique is described in PCT WO 92/01810, published Feb. 6, 1992. Such an assay may be employed, for example, for screening for a compound that inhibits (or enhances) activation of the receptor polypeptide of the present invention by contacting the melanophore cells that encode the receptor with both a TNF-family ligand and the candidate antagonist (or agonist). Inhibition or enhancement of the signal generated by the ligand indicates that the compound is an antagonist or agonist of the ligand/receptor signaling pathway.

[0708] Other screening techniques include the use of cells that express the receptor (for example, transfected CHO cells) in a system, which measures extracellular pH changes caused by receptor activation. For example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g., signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

[0709] Another such screening technique involves introducing RNA encoding the receptor into *Xenopus oocytes* to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds, which are thought to inhibit activation of the receptor.

[0710] Another screening technique involves expressing in cells a construct wherein the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as herein above described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase signal.

[0711] Another method involves screening for compounds (antagonists) that inhibit activation of the receptor polypeptide of the present invention by determining inhibition of binding of labeled ligand to cells, which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand, which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

[0712] Further screening assays for agonist and antagonist of the present invention are described in Tartaglia, L. A., and Goeddel, D. V., *J. Biol. Chem.* 267:4304-4307(1992).

[0713] Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the DR5 polypeptide with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a

standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or a TNF-family ligand (e.g., determining or estimating an increase or decrease in T-cell or B-cell proliferation, or tritiated thymidine labeling). By the invention, a cell expressing the DR5 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

[0714] Agonist according to the present invention include naturally occurring and synthetic compounds such as, for example, TNF family ligand peptide fragments, transforming growth factor, neurotransmitters (such as glutamate, dopamine, N-methyl-D-aspartate), tumor suppressors (p53), cytolytic T-cells and antimetabolites. Preferred agonists include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and β -amyloid peptide. (*Science* 267:1457-1458 (1995)). Further preferred agonists include polyclonal and monoclonal antibodies raised against the DR5 polypeptide, or a fragment thereof. Such agonist antibodies raised against a TNF-family receptor are disclosed in Tartaglia, L. A., et al., *Proc. Natl. Acad. Sci. USA* 88:9292-9296 (1991); and Tartaglia, L. A., and Goeddel, D. V., *J. Biol. Chem.* 267 (7):4304-4307 (1992) See, also, PCT Application WO 94/09137.

[0715] Antagonist according to the present invention include naturally occurring and synthetic compounds such as, for example, the CD40 ligand, neutral amino acids, zinc, estrogen, androgens, viral genes (such as Adenovirus E1B, Baculovirus p35 and L4P, Cowpox virus crmA, Epstein-Barr virus BHRF1, LMP-1, African swine fever virus LMW5-HL, and Herpesvirus y1 34.5), calpain inhibitors, cysteine protease inhibitors, and tumor promoters (such as PMA, Phenobarbital, and alpha-Hexachlorocyclohexane).

[0716] Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem.* 56:560 (1991); *Oligodeoxyribonucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, Fla. (1988). Triple helix formation is discussed in, for instance Lee et al., *Nucleic Acids Research* 6:3073 (1979); Cooney et al., *Science* 241:456 (1988); and Dervan et al., *Science* 251:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

[0717] For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into

receptor polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of the DR5 receptor.

[0718] In one embodiment, the DR5 antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the DR5 antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding DR5, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or a constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature* 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of *Rous sarcoma* virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., *Nature* 296:39-42 (1982)), etc.

[0719] The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a DR5 gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded DR5 antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a DR5 RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0720] Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature* 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of the DR5 shown in FIG. 1 could be used in an antisense approach to inhibit translation of endogenous DR5 mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5', 3', or coding region of DR5 mRNA, antisense nucleic acids should be at

least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0721] The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci.* 84:648-652 (1987); PCT Publication No. WO88/098 10, published Dec. 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published Apr. 25, 1989), hybridization-triggered cleavage agents. (See, e.g., Krol et al., *BioTechniques* 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, *Pharm. Res.* 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0722] The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl)uracil, (acp3)w, and 2,6-diaminopurine.

[0723] The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylose, and hexose.

[0724] In yet another embodiment, the antisense oligonucleotide comprises at least one-modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

[0725] In yet another embodiment, the antisense oligonucleotide is an -anomeric oligonucleotide. An -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual -units, the strands run parallel to each other (Gautier et al., *Nucl. Acids Res.* 15:6625-6641 (1987)). The oligonucleotide is a

2-0-methylribonucleotide (Inoue et al., *Nucl. Acids Res.* 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., *FEBS Lett.* 215:327-330 (1987)).

[0726] Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published Oct. 4, 1990; Sarver et al, *Science* 247:1222-1225 (1990). While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy DR5 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, *Nature* 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of DR5 (FIG. 1). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the DR5 mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. Since ribozymes, unlike antisense molecules are catalytic, a lower intracellular concentration is required for efficiency.

[0727] Further antagonists according to the present invention include soluble forms of DR5, i.e., DR5 fragments that include the ligand-binding domain from the extracellular region of the full-length receptor. Such soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize DR5 mediated signaling by competing with the cell surface DR5 for binding to TNF-family ligands. Thus, soluble forms of the receptor that include the ligand-binding domain are novel cytokines capable of inhibiting apoptosis induced by TNF-family ligands. These may be expressed as monomers, but, are preferably expressed as dimers or trimers, since these have been shown to be superior to monomeric forms of soluble receptor as antagonists, e.g., IgGFc-TNF receptor family fusions. Other such cytokines are known in the art and include Fas B (a soluble form of the mouse Fas receptor) that acts physiologically to limit apoptosis induced by Fas ligand (Hughes, D. P. and Crispe, I. N., *J. Exp. Med.* 182:1395-1401 (1995)).

[0728] As discussed above, the term "antibody" (Ab) or "monoclonal antibody" (mAb) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab, and F(ab')₂ fragments) which are capable of binding an antigen. Fab, Fab', and F (ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).

[0729] Antibodies according to the present invention may be prepared by any of a variety of standard methods using DR5 immunogens of the present invention. As indicated, such DR5 immunogens include the full length DR5 polypeptide (which may or may not include the leader sequence) and DR5 polypeptide fragments such as the ligand binding domain, the transmembrane domain, the intracellular domain and the death domain.

[0730] Antibodies of the invention can be used in methods known in the art relating to the localization and activity of the polypeptide sequences of the invention, e.g., for imaging these polypeptides, measuring levels thereof in appropriate physiological samples, etc. The antibodies also have use in immunoassays and in therapeutics as agonists and antagonists of DR5.

[0731] Proteins and other compounds that bind the DR5 domains are also candidate agonist and antagonist according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature* 340:245-246 (1989)). A modified version of the yeast two-hybrid system has been described by Roger Brent and his colleagues (Gyuris, J. et al., *Cell* 75:791-803 (1993); Zervos, A. S. et al., *Cell* 72:223-232 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds that bind to either the DR5 ligand-binding domain or to the DR5 intracellular domain. Such compounds are good candidate agonist and antagonist of the present invention.

[0732] By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing the ligand/receptor-signaling pathway. Members of the TNF ligand family include, but are not limited to, DR5 ligands, TRAIL, TNF- α , lymphotoxin- α (LT- α , also known as TNF- β), LT- β (found in complex heterotrimer LT- α 2- β), FasL, CD40, CD27, CD30, 4-IBB, OX40 and nerve growth factor (NGF). An example of an assay that can be performed to determine the ability of DR5 and derivatives (including fragments) and analogs thereof to bind TRAIL is described below in Example 6.

Gene Therapy

[0733] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit and/or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0734] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0735] For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11 (5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

[0736] In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic

acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody nucleic acids (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

[0737] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

[0738] In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Pat. No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, micro-particles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180 dated Apr. 16, 1992 (Wu et al.); WO 92/22635 dated Dec. 23, 1992 (Wilson et al.); WO92/20316 dated Nov. 26, 1992 (Findeis et al.); WO93/14188 dated Jul. 22, 1993 (Clarke et al.), WO 93/20221 dated Oct. 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

[0739] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., 1993, *Meth. Enzymol.* 217:581-599). These retroviral vectors have been to delete retroviral sequences that are not necessary for packaging of the viral

genome and integration into host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitate delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., 1994, *Biotherapy* 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, *J. Clin. Invest.* 93:644-651; Kiem et al., 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

[0740] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431-434; Rosenfeld et al., 1992, *Cell* 68:143-155; Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225-234; PCT Publication WO94/12649; and Wang et al., 1995, *Gene Therapy* 2:775-783. In a preferred embodiment, adenovirus vectors are used.

[0741] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300; U.S. Pat. No. 5,436,146).

[0742] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0743] In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599-618; Cohen et al., 1993, *Meth. Enzymol.* 217:618-644; Cline, 1985, *Pharmac. Ther.* 29:69-92) and maybe used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0744] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0745] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T-lymphocytes, B-lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0746] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0747] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see, e.g., PCT Publication WO 94/08598, dated Apr. 28, 1994; Stemple and Anderson, 1992, *Cell* 71:973-985; Rheinwald, 1980, *Meth. Cell Bio.* 21A:229; and Pittelkow and Scott, 1986, *Mayo Clinic Proc.* 61:771).

[0748] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Modes of Administration

[0749] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0750] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0751] The agonist or antagonists described herein can be administered *in vitro*, *ex vivo*, or *in vivo* to cells which express the receptor of the present invention. By administration of an "effective amount" of an agonist or antagonist is intended an amount of the compound that is sufficient to enhance or inhibit a cellular response to a TNF-family ligand and include polypeptides. In particular, by administration of

an "effective amount" of an agonist or antagonists is intended an amount effective to enhance or inhibit DR5 mediated apoptosis. Of course, where it is desired for apoptosis is to be enhanced, an agonist according to the present invention can be co-administered with a TNF-family ligand. One of ordinary skill will appreciate that effective amounts of an agonist or antagonist can be determined empirically and may be employed in pure form or in pharmaceutically acceptable salt, ester or prodrug form. The agonist or antagonist may be administered in compositions in combination with one or more pharmaceutically acceptable excipients (i.e., carriers).

[0752] It will be understood that, when administered to a human patient, the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon factors well known in the medical arts.

[0753] As a general proposition, the total pharmaceutically effective amount of DR5 polypeptide administered parenterally per dose will be in the range of about 1 $\mu\text{g}/\text{kg}/\text{day}$ to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the DR5 agonists or antagonists is typically administered at a dose rate of about 1 $\mu\text{g}/\text{kg}/\text{hour}$ to about 50 $\mu\text{g}/\text{kg}/\text{hour}$, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

[0754] Dosaging may also be arranged in a patient specific manner to provide a predetermined concentration of an agonist or antagonist in the blood, as determined by the RIA technique. Thus patient dosaging may be adjusted to achieve regular on-going trough blood levels, as measured by RIA, on the order of from 50 to 1000 ng/ml, preferably 150 to 500 ng/ml.

[0755] Pharmaceutical compositions are provided comprising an agonist or antagonist (including DR5 polynucleotides and polypeptides of the invention) and a pharmaceutically acceptable carrier or excipient, which may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray. Importantly, by co-administering an agonist and a TNF-family ligand, clinical side effects can be reduced by using lower doses of both the ligand and the agonist. It will be understood that the agonist can be "co-administered" either before, after, or simultaneously with the TNF-family ligand, depending on the exigencies of a particular therapeutic application. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In a specific embodiment, "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers include

sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0756] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0757] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0758] The term "parenteral" as used herein refers to modes of administration, which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0759] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules,

recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0760] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0761] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, 1990, *Science* 249:1527-1533; Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, N.Y., pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

[0762] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:201; Buchwald et al., 1980, *Surgery* 88:507; Saudek et al., 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, N.Y. (1984); Ranger and Peppas, J., 1983, *Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, *J. Neurosurg.* 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

[0763] Other controlled release systems are discussed in the review by Langer (1990, *Science* 249:1527-1533).

[0764] DR5 compositions of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

[0765] Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D-(α)-3-hydroxybutyric acid (EP 133,988).

[0766] Sustained-release compositions also include liposomally entrapped compositions of the invention (see generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, N.Y., pp. 317-327 and 353-365 (1989)). Liposomes containing DR5 polypeptide may be prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal DR5 polypeptide therapy.

[0767] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0768] In yet an additional embodiment, the compositions of the invention are delivered by way of a pump (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

[0769] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0770] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see

U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0771] Pharmaceutical compositions of the present invention for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use.

[0772] In addition to soluble DR5 polypeptides, DR5 polypeptide containing the transmembrane region can also be used when appropriately solubilized by including detergents, such as CHAPS or NP-40, with buffer.

[0773] The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

[0774] The compositions of the invention may be administered alone or in combination with other adjuvants. Adjuvants that may be administered with the compositions of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, compositions of the invention are administered in combination with alum. In another specific embodiment, compositions of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the compositions of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the compositions of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, Hepatitis A, Hepatitis B, *Haemophilus influenzae* type B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the

combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0775] The compositions of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the compositions of the invention, include but are not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, antivirals, steroid and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines, chemokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0776] In one embodiment, the compositions of the invention are administered in combination with other members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the compositions of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), TRAIL, AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), TR12, and soluble forms of CD154, CD70, and CD153.

[0777] In another embodiment, the compositions of the invention are administered in combination with CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVRENDE□), biologically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0778] In yet another embodiment, the compositions of the invention are administered in combination with one, two, three, four, five, or more of the following compositions: tacrolimus (Fujisawa), thalidomide (e.g., Celgene), anti-Tac(Fv)-PE40 (e.g., Protein Design Labs), inolimomab (Biotest), MAK-195F (Knoll), ASM-981 (Novartis), interleukin-1 receptor (e.g., Immunex), interleukin-4 receptor (e.g., Immunex), ICM3 (ICOS), BMS-188667 (Bristol-Myers Squibb), anti-TNF Ab (e.g., Therapeutic antibodies),

CG-1088 (Celgene), anti-B7 monoclonal antibody (e.g., Innogetics), MEDI-507 (BioTransplant), ABX-CBL (Abgenix).

[0779] According to the invention, a patient susceptible to both Fas ligand (Fas-L) mediated and TRAIL mediated cell death may be treated with both an agent that inhibits TRAIL/TRAIL-R interactions and an agent that inhibits Fas-L/Fas interactions. Suitable agents for blocking binding of Fas-L to Fas include, but are not limited to, soluble Fas polypeptides; oligomeric forms of soluble Fas polypeptides (e.g., dimers of sFas/Fc); anti-Fas antibodies that bind Fas without transducing the biological signal that results in apoptosis; anti-Fas-L antibodies that block binding of Fas-L to Fas; and muteins of Fas-L that bind Fas but do not transduce the biological signal that results in apoptosis. Preferably, the antibodies employed according to this method are monoclonal antibodies. Examples of suitable agents for blocking Fas-L/Fas interactions, including blocking anti-Fas monoclonal antibodies, are described in WO 95/10540, hereby incorporated by reference.

[0780] In certain embodiments, compositions of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, RETROVIR® (zidovudine/AZT), VIDEX® (didanosine/ddI), HIVID® (zalcitabine/ddC), ZERIT® (stavudine/d4T), EPIVIR® (lamivudine/3TC), and COMBIVIR® (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, VIRAMUNE® (nevirapine), DESCRIPTOR® (delavirdine), and SUSTIVA® (efavirenz). Protease inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, CRIXIVAN® (indinavir), NORVIR® (ritonavir), INVIRASE® (saquinavir), and VIRACEPT® (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with compositions of the invention to treat AIDS and/or to prevent or treat HIV infection.

[0781] In other embodiments, compositions of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the compositions of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE®, DAPSONE®, PENTAMIDINE®, ATOVAQUONE®, ISONIAZIDE®, RIFAMPIN®, PYRAZINAMIDE®, ETHAMBUTOL®, RIFABUTIN®, CLARITHROMYCIN®, AZITHROMYCIN®, GANCICLOVIR®, FOSCARNET®, CIDOVIR®, FLUCONAZOLE®, ITRACONAZOLE®, KETOCONAZOLE®, ACYCLOVIR®, FAMCICOLVIR®, PYRIMETHAMINE®, LEUCOVORIN®, NEUPOGEN® (filgrastim/G-CSF), and LEUKINE® (sargramostim/GM-CSF). In a specific embodiment, compositions of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE®, DAPSONE®, PENTAMIDINE®, and/or ATOVAQUONE® to prophylactically treat and/or prevent an opportunistic *Pneumocystis*

carinii pneumonia infection. In another specific embodiment, compositions of the invention are used in any combination with ISONIAZID®, RIFAMPIN®, PYRAZINAMIDE®, and/or ETHAMBUTOL® to prophylactically treat and/or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, compositions of the invention are used in any combination with RIFABUTIN®, CLARITHROMYCIN®, and/or AZITHROMYCIN® to prophylactically treat and/or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, compositions of the invention are used in any combination with GANCICLOVIR®, FOSCARNET®, and/or CIDOFOVIR® to prophylactically treat and/or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, compositions of the invention are used in any combination with FLUCONAZOLE®, ITRACONAZOLE®, and/or KETOCONAZOLE® to prophylactically treat and/or prevent an opportunistic fungal infection. In another specific embodiment, compositions of the invention are used in any combination with ACYCLOVIR® and/or FAMCICOLVIR® to prophylactically treat and/or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, compositions of the invention are used in any combination with PYRIMETHAMINE® and/or LEUCOVORIN® to prophylactically treat and/or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, compositions of the invention are used in any combination with LEUCOVORIN® and/or NEUPOGEN® to prophylactically treat and/or prevent an opportunistic bacterial infection.

[0782] In a further embodiment, the compositions of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the compositions of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0783] In a further embodiment, the compositions of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the compositions of the invention include, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

[0784] Conventional nonspecific immunosuppressive agents, that may be administered in combination with the compositions of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisolone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T-cells.

[0785] In specific embodiments, compositions of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the compositions of the invention include, but are not limited to, ORTHOCLONE® (OKT3), SANDIMMUNE®/NEORAL®/SANGDYA® (cyclosporin), PROGRAF® (tacrolimus), CELLCEPT® (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE®

(sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0786] In an additional embodiment, compositions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the compositions of the invention include, but not limited to, GAMMAR®, IVEEGAM®, SANDOGLOBULIN®, GAMMAGARD S/D®, and GAMIMUNE®. In a specific embodiment, compositions of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0787] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the compositions of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotoin, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0788] In one embodiment, the compositions of the invention are administered in combination with steroid therapy. Steroids that may be administered in combination with the compositions of the invention, include, but are not limited to, oral corticosteroids, prednisone, and methylprednisolone (e.g., IV methylprednisolone). In a specific embodiment, compositions of the invention are administered in combination with prednisone. In a further specific embodiment, the compositions of the invention are administered in combination with prednisone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the compositions of the invention and prednisone are those described herein, and include, but are not limited to, azathioprine, cyclophosphamide, and cyclophosphamide IV. In another specific embodiment, compositions of the invention are administered in combination with methylprednisolone. In a further specific embodiment, the compositions of the invention are administered in combination with methylprednisolone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the compositions of the invention and methylprednisolone are those described herein, and include, but are not limited to, azathioprine, cyclophosphamide, and cyclophosphamide IV.

[0789] In another embodiment, the compositions of the invention are administered in combination with an antimalarial. Antimalarials that may be administered with the compositions of the invention include, but are not limited to, hydroxychloroquine, chloroquine, and/or quinacrine.

[0790] In yet another embodiment, the compositions of the invention are administered in combination with an NSAID.

[0791] In a nonexclusive embodiment, the compositions of the invention are administered in combination with one,

two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecaserrnin (Chiron), T-614 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000 (Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-IRa gene therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIIL-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-663 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

[0792] In yet another embodiment, the compositions of the invention are administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL® (Etanercept), anti-TNF antibody, and prednisolone. In a more preferred embodiment, the compositions of the invention are administered in combination with an antimalarial, methotrexate, anti-TNF antibody, ENBREL® and/or sulfasalazine. In one embodiment, the compositions of the invention are administered in combination with methotrexate. In another embodiment, the compositions of the invention are administered in combination with anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with methotrexate and anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with methotrexate, anti-TNF antibody, and sulfasalazine. In another specific embodiment, the compositions of the invention are administered in combination with methotrexate, anti-TNF antibody, and sulfasalazine. In another embodiment, the compositions of the invention are administered in combination ENBREL®. In another embodiment, the compositions of the invention are administered in combination with ENBREL® and methotrexate. In another embodiment, the compositions of the invention are administered in combination with ENBREL®, methotrexate and sulfasalazine. In another embodiment, the compositions of the invention are administered in combination with ENBREL®, methotrexate and sulfasalazine. In other embodiments, one or more antimalarials are combined with one of the above-recited combinations. In a specific embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL®, methotrexate and sulfasalazine. In another specific embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), sulfasalazine, anti-TNF antibody, and methotrexate.

[0793] In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the compositions of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic

acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., methoxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotriamisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); triterpenoids (e.g. oleanic acid and urosolic acid); cyclohexamide; casein kinase inhibitors; and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

[0794] In specific embodiments, compositions of the present invention are administered in combination with one or more chemotherapeutic agents including, but not limited to, 81 C6 (Anti-tenascin monoclonal antibody), 2-chlorodeoxyadenosine, A007 (4-4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone), Abarelix® (Abarelix-Depot-M®, PPI-149, R-3827); Abiraterone acetate® (CB-7598, CB-7630), ABT-627 (ET-1 inhibitor), ABX-EGF (anti-EGFr MAb), Acetylinaline (CI-994, GOE-5549, GOR-5549, PD-130636), AG-2034 (AG-2024, AG-2032, GARFT [glycinamide ribonucleoside transformylase] inhibitor), Alanosine, Aldesleukin (IL-2, Proleukin®), Alemtuzumab® (Campath®), Altretinoin (Panretin®, LGN-1057), Allopurinol (Aloprim®, Zyloprim®), Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Amifostine (Ethyol®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminoglutethimide (Cytadren®), Aminolevulinic acid (Levulan®, Kerastick®), Aminopterin, Amsacrine, Anastrozole (Arimidex®), Angiostatin, Annamycin (AR-522, annamycin LF, Aronex®), Anti-idiotype therapy (BsAb), Anti-CD19/CD3 MAb (anti-CD19/CD3 scFv, anti-NHL MAb), APC-8015 (Provenges, Dendritic cell therapy), Aplidine (Aplidin®, Aplidina®), Arabinosylguanine (Ara-G, GW506U78, Nelzarabine®, Compound 506U78), Arsenic trioxide (Trisenox®, ATO, Atrivex®), Avorelin® (Meterelin®, MF-6001, EP-23904), B43-Genistein (anti-CD19 Ab/genistein conjugate), B43-PAP (anti-CD19 Ab/pokeweed antiviral protein conjugate), B7 antibody conjugates, BAY 43-9006 (Raf kinase inhibitor), BBR 3464, Betathine (Beta-LT), Bevacizumab® (Anti-VEGF monoclonal antibody, rhuMAb-VEGF), Bexarotene (Targretin®, LGD1069), BIBH-1 (Anti-FAP MAb), BIBX-1382, Biclitamide (Casodex®), Bircodar dicitrate (Incel®, Incel MDR Inhibitor), Bleomycin (Blenoxane®), BLP-25 (MUC-1 peptide), BLyS antagonists, BMS-214662 (BMS-192331, BMS-193269, BMS-206635), BNP-1350 (BNPI-1100, Karenitecins), Boronated Protoporphyrin Compound (PDIT, Photodynamic Immunotherapy), Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), Budesonide (Rhinocort®), Busulfan (Busulfex®, Myleran®), C225 (IMC-225, EGFR inhibitor, Anti-EGFr MAb, Cetuximab®), C242-DM1 (huC242-DM1), Cabergoline (Dostinex®), Capecitabine (Xeloda®, Doxifluridine®, oral 5-FU), Carbendazin® (FB-642), Carboplatin (Paraplatin®, CBDCA), Carboxyamidotriazole (NSC 609974, CAI, L-651582), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), CC49-zeta gene therapy, CEA-cide® (Labetuzumab®, Anti-CEA monoclonal antibody, hMN-14), CeaVac® (MAb 3H1), Celecoxib (Celebrex®), CEP-701 (KT-5555), Cereport®

(Lobradimil®, RMP-7), Chlorambucil (Leukeran®), CHML (Cytotropic Heterogeneous Molecular Lipids), Cholecalciferol, CI-1033 (Pan-erbB RTK inhibitor), Cilengitide (EMD-121974, integrin alphavbeta3 antagonist), Cisplatin (Platinol®, CDDP), Cisplatin-epinephrine gel (IntraDose®, FocaCist®), Cisplatin-liposomal (SPI-077), 9-cis retinoic acid (9-cRA), Cladribine (2-CdA, Leustatin®), Clofarabine (chloro-fluoro-araA), Clonidine hydrochloride (Duraclon®), CMB-401 (Anti-PEM MAb/calicheamycin), CMT-3 (COL-3, Metastat®), Cordycepin, Cotara® (chTNT-1/B, [¹³¹I]-chTNT-1/B), CN-706, CP-358774 (Tarceva®, OSI-774, EGFR inhibitor), CP-609754, CP IL-4-toxin (IL-4 fusion toxin), CS-682, CT-2584 (Apra®, CT-2583, CT-2586, CT-3536), CTP-37 (Avicine®, hCG blocking vaccine), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, Depo-Cyt®), D-limonene, DAB389-EGF (EGF fusion toxin), Dacarbazine (DTIC), Daclizumab® (Zenapax®), Dactinomycin (Cosmegen®), Daunomycin (Daunorubicin®, Cerubidine®), Daunorubicin (DaunoXome®, Daunorubicin®, Cerubidine®), DeaVac® (CEA anti-idiotype vaccine), Decitabine (5-aza-2'-deoxytidine), Declopamride (Oxi-104), Denileukin ditox (Ontak®), Depsipeptide (FR901228, FK228), Dexamethasone (Decadron®), Dexrazoxane (Zinecard®), Diethylstilbestrol (DES), Dihydro-5-azacytidine, Docetaxel (Taxotere®, Taxane®), Dolasetron mesylate (Anzemet®), Dolastatin-10 (DOLA-10, NSC-376128), Doxorubicin (Adriamycin®, Doxil®, Rubex®), DPPE, DX-8951f (DX-8951), Edatrexate, EGF-P64k Vaccine, Elliott's B Solution®, EMD-121974, Endostatin, Eniluracil (776c85), EO9 (EO1, EO4, EO68, EO70, EO72), Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Epratuzumab® (Lymphocide®, humanized anti-CD22, HAT), Erythropoietin (EPO®, EpoGen®, Procrit®), Estramustine (Emcyt®), Etanidazole (Radanyl®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Exemestane (Aromasin®, Nikides®), Exetecan mesylate (DX-8951, DX-8951f), Exisulind (SAAND, Aptosyn®, cGMP-PDE2 and 5 inhibitor), F19 (Anti-FAP monoclonal antibody, iodinated anti-FAP MAb), Fadrozole (Afema®, Fadrozole hydrochloride, Arensin®), Fenretinide® (4HPR), Fentanyl citrate (Actiq®), Filgrastim (Neupogen®, G-CSF), FK-317 (FR-157471, FR-70496), Flavopiridol (HMR-1275), Fly3/flk2 ligand (Mobista®), Fluasterone, Fludarabine (Fludara®, FAMP), Fludeoxyglucose (F-18®), Fluorouracil (5-FU, Adrucil®, Fluropoplex®, Efudex®), Flutamide (Eulexin®), FMdC (KW-2331, MDL-101731), Formestane (Lentaron®), Fotemustine (Muphoran®, Mustophoran®), FUDR (Flouxuridine®), Fulvestrant (Faslodex®), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Gadolinium texaphyrin (Motexafin gadolinium, Gd-Tex®, Xcytrin®), Galarubicin hydrochloride (DA-125), GBC-590, Gastrimmune® (Anti-gastrin-17 immunogen, anti-g17), Gemcitabine (Gemto®, Gemzar®), Gentuzumab-ozogamicin (Mylotarg®), GL331, Globo H hexasaccharide (Globo H-KLH®), Glufosfamide® (β -D-glucosyl-isofosfamide mustard, D19575, INN), Goserelin acetate (Zoladex®), Granisetron (Kytril®), GVAX (GM-CSF gene therapy), Her-2/Neu vaccine, Herceptin® (Trastuzumab®, Anti-HER-2 monoclonal antibody, Anti-EGFR-2 MAb), HSPPC-96 (HSP cancer vaccine, gp96 heat shock protein-peptide complex), Hu1D10 (anti-HLA-DR MAb, SMART 1D10), HumaLYM (anti-CD20 MAb), Hydrocortisone, Hydroxyurea (Hydrea®), Hypericin® (VIMRxyn®), I-131

Lipidiol®, Ibrutinomab® tiuxetan (Zevalin®), Idarubicin (Idamycin®, DMDR, IDA), Ifosfamide (IFEX®), Imatinib mesylate (STI-571, Imatinib, Gleevec®, Abl tyrosine kinase inhibitor), INGN-101 (p53 gene therapy/retrovirus), INGN-201 (p53 gene therapy/adenovirus), Interferon alpha (Alfaferone®, Alpha-IF®g), Interferon alpha 2a (Intron A®), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), Interleukin-2 (ProleukinR®), Intoplicine (RP 60475), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), ISIS-2053 (PKC-alpha antisense), ISIS-2503 (Ras antisense), ISIS-3521 (PKC-alpha antisense), ISIS-5132 (K-ras/raf antisense), Isotretinoin (13-CRA, 13-cis retinoic acid, Accutane®), Ketoconazole (Nizoral®), KRN-8602 (MX, MY-5, NSC-619003, MX-2), L-778123 (Ras inhibitors), L-asparaginase (Elspar®, Crastin®), Asparaginase medac®, Kidrolase®, Leflunomide (SU-101, SU-0200), Letrozole (Femara®), Leucovorin (Leucovorin®, Wellcovorin®), Leuprolide acetate (Viadur®, Lupron®, LeuprogeL®, Eligard®), Leuvectin® (cytorectin+IL-2 gene, IL-2 gene therapy), Levamisole (Ergamisol®), Liarozole (Liazol, Liazol, R-75251, R-85246, Ro-85264), Lmb-2 immunotoxin (anti-CD25 recombinant immuno toxin, anti-Tac(Fv)-PE38), Lometrexol (T-64, T-904064), Lomustine (CCNU®, CeeNU®), LY-335979, Lym-1 (131-I LYM-1), Lymphoma vaccine (Genitope), Mannan-MUC I vaccine, Marimastat® (BB-2516, TA-2516, MMP inhibitor), MDX-447 (MDX-220, BAB-447, EMD-82633, H-447, anti-EGFr/FcGammaR1), Mechlorethamine (Nitrogen Mustard, HN₂, Mustargen®), Megestrol acetate (Megace®, Pallace®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Mercaptapurine (6-mercaptopurine, 6-MP), Mesna (Mesnex®), Methotrexate® (MTX, Mexate®, Folex®), Methoxsalen (Uvadex®), 2-Methoxyestradiol (2-ME, 2-ME2), Methylprednisolone (Solumedrol®), Methyltestosterone (Android-10®, Testred®, Virilon®), MGV, Mitomycin C (Mitomycin®, Mutamycin®, Mito Extra®), Mitoxantrone (Novantrone®, DHAD), Mitumomab® (BEC-2, EMD-60205), Mivobulin isethionate (CI-980), MN-14 (Anti-CEA immunoradiotherapy, ¹³¹I-MN-14, ¹⁸⁸Re-MN-14), Motexafin Lutetium (Lutrin®, Optrin®, LuTex®, lutetium texaphyrin, Lucyn®, Antrin®), MPV-2213ad (Finrozole®), MS-209, Muc-1 vaccine, NaPro Paclitaxel, Nclarabine (Compound 506, U78), Neovastat® (AE-941, MMP inhibitor), Neugene compounds (Oncomycin-NG, Resten-NG, myc antisense), Nilutamide (Nilandron®), NovoMAb-G2 scFv (NovoMAb-G2 IgM), O6-benzylguanine (BG, Procept®), Octreotide acetate (Sandostatin LAR® Depot), Odansetron (Zofran®), Onconase (Ranpirimidine®), OncoVAX-CL, OncoVAX-CL Jenner (GA-733-2 vaccine), OncoVAX-P (OncoVAX-PrPSA), Onyx-015 (p53 gene therapy), Oprelvekin (Neumage®), Orzel (Tegafur+Uracil+Leucovorin), Oxaliplatin (Eloxatin®, Eloxatin®), Pacis® (BCG, live), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Pamidronate (Aredia®), PC SPES, Pegademase (Adagen®, Pegademase bovine), Pegasparagase® (Oncospars®), Peldesine (BCX-34, PNP inhibitor), Pemetrexed disodium (Alimta®, MTA, multitargeted anti-folate, LY 231514), Pentostatin (Nipent®, 2-deoxycoformycin), Perfosfamide (4-hydroperoxycyclophosphamide, 4-HC), Perillyl alcohol (perilla alcohol, perillie alcohol, perillol, NSC-641066), Phenylbutyrate, Pirarubicin (THP), Pivaloyloxymethyl butyrate (AN-9, Pivanex®), Porfimer sodium (Photofrin®), Prednisone, Prinomastat® (AG-3340,

MMP inhibitor), Procarbazine (Matulane®), PROSTVAC, Providence Portland Medical Center Breast Cancer Vaccine, PS-341 (LDP-341, 26S proteosome inhibitor), PSMA MAb (Prostate Specific Membrane Antigen monoclonal antibody), Pyrazoloacridine (NSC-366140, PD-115934), Quinine, R115777 (Zarnestra®), Raloxifene hydrochloride (Evista®, Keoxifene hydrochloride), Raltitrexed (Tomudex®, ZD-1694), Rebeccamycin, Retinoic acid, R-flurbiprofen (Flurizan®, E-7869, MPC-7869), RFS-2000 (9-nitrocamptothecan, 9-NC, rubitecan®), Rituximab® (Rituxan®, anti-CD20 MAb), RSR-13 (GSJ-61), Satraplatin (BMS-182751, JM-216), SCH 6636, SCH-66336, Sizofilan® (SPG, Sizofiran®, Schizophyllan®, Sonifilan®), SKI-2053R (NSC-D644591), Sobuzoxane (MST-16, Perazolin®), Squalamine (MSI-1256F), SR-49059 (vasopressin receptor inhibitor, V1a), Streptozocin (Zanosar®), SU5416 (Semaxanib®, VEGF inhibitor), SU6668 (PDGF-TK inhibitor), T-67 (T-138067, T-607), Talc (Sclerosol®), Tamoxifen (Nolvadex®), Taurolidine (Taurolin®), Temozolamide (Temodar®, NSC 362856), Teniposide (VM-26, Vumon®), TER-286, Testosterone (Andro®, Androderm®, Testoderm TTS®, Testoderm®, Depo-Testosterone®, Androgel®, depoAndro®), Tf-CRM107 (Transferrin-CRM-107), Thalidomide, Theratope, Thioguanine (6-thioguanine, 6-TG), Thiotepa (triethylenethiophosphoramide, Thioplex®), Thymosin alpha I (Zadaxin®, Thymalfasin®), Tiazofuirin (Tiazole®), Tirapazamine (SR-259075, SR-4233, Tira-zone®, Win-59075), TNP-470 (AGM-1470, Fumagillin), Tocladesine (8-Cl-cAMP), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Toremifene (Estrimex®, Fareston®), Tositumomab® (Bexxar®), Tretinoin (Retin-A®, Atragen®, ATRA, Vesanoïd®), TriAb® (anti-idiotype antibody immune stimulator), Trilostane (Modrefen®), Triptorelin pamoate (Trelstar Depot®, Decapeptyl®), Trimetrexate (Neutrexin®), Troxacitabine (BCH-204, BCH-4556, Troxaty®), TS-1, UCN-01 (7-hydroxystauroporine), Valrubicin (Valstar®), Valspodar (PSC 833), Vapreotide® (BMY-41606), Vaxid (B-cell lymphoma DNA vaccine), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®), Vindesine (Eldisine®, Fildesin®), Vinorelbine (Navelbine®), Vitaxin® (LM-609, integrin alphavbeta3 antagonistic MAb), WF10 (macrophage regulator), WHI-P131, WT1 Vaccine, XR-5000 (DACA), XR-9576 (XR-9351, P-glycoprotein/MDR inhibitor), ZD-9331, ZD-1839 (IRESSA®), and Zoledronate (Zometa®).

[0795] In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of the components of CHOP.

[0796] In further specific embodiments, compositions of the present invention are administered in combination with one or more combinations of chemotherapeutic agents including, but not limited to, 9-aminocamptothecin+G-CSF, Adriamycin®+Blenoxane+Vinblastine+Dacarbazine (ABVD), BCNU (Carmustine)+Etoposide+Ara-C (Cytarabine)+Melphalan (BEAM), Bevacizumab®+Leucovorin, Bleomycin+Etoposide+Platinol® (Cisplatin) (BEP), Bleomycin+Etoposide+Adriamycin+Cyclophosphamide+Vinc-

ristine+Procarbazine+Prednisone (BEACOPP), Bryostatin+Vinceristine, Busulfan+Melphalan, Carboplatin+Cereport®, Carboplatin+Cyclophosphamide, Carboplatin+Paclitaxel, Carboplatin+Etoposide+Bleomycin (CEB), Carboplatin+Etoposide+Thiotepa, Cisplatin+Cyclophosphamide, Cisplatin+Docetaxel, Cisplatin+Doxorubicin, Cisplatin+Etoposide, Cisplatin+Gemcitabine, Cisplatin+Interferon alpha, Cisplatin+Irinotecan, Cisplatin+Paclitaxel, Cisplatin+Teniposide, Cisplatin+Vinblastine, Cisplatin+Vindesine, Cisplatin+Vinorelbine, Cisplatin+Cytarabine+Ifosfamide, Cisplatin+Ifosfamide+Vinblastine, Cisplatin+Vinblastine+Mitomycin C, Cisplatin+Vincristine+Fluorouracil, Cisplatin+Vincristine+Lomustine, Cisplatin+Vinorelbine+Gemcitabine, Cisplatin+Carmustine+Dacarbazine+Tamoxifen, Cisplatin+Cyclophosphamide+Etoposide+Vincristine, Cisplatin (Platinol®)+Oncovin®+Doxorubicin (Adriamycin®)+Etoposide (CODE), Cisplatin+Cytarabine+Ifosfamide+Etoposide+Methotrexate, Cyclophosphamide+Adriamycin® (Doxorubicin), Cyclophosphamide+Melphalan, Cyclophosphamide+Sch 6636, Cyclophosphamide+Adriamycin®+Cisplatin (Platinol®) (CAP), Cyclophosphamide+Adriamycin+Vincristine (CAV), Cyclophosphamide+Doxorubicin+Teniposide+Prednisone, Cyclophosphamide+Doxorubicin+Teniposide+Prednisone+Interferon alpha, Cyclophosphamide+Epirubicin+Cisplatin (Platinol®) (CEP), Cyclophosphamide+Epirubicin+Fluorouracil, Cyclophosphamide+Methotrexate+Fluorouracil (CMF), Cyclophosphamide+Methotrexate+Vincristine (CMV), Cyclophosphamide+Adriamycin®+Methotrexate+Fluorouracil (CAMF), Cyclophosphamide+Adriamycin®+Methotrexate+Procarbazine (CAMP), Cyclophosphamide+Adriamycin®+Vincristine+Etoposide (CAV-E), Cyclophosphamide+Adriamycin®+Vincristine+Prednisone (CHOP), Cyclophosphamide+Novantrone® (Mitoxantrone)+Vincristine (Oncovin)+Prednisone (CNOP), Cyclophosphamide+Adriamycin®+Vincristine+Prednisone+Rituximab (CHOP+Rituximab), Cyclophosphamide+Adriamycin®+Vincristine+Teniposide (CAV-T), Cyclophosphamide+Adriamycin®+Vincristine alternating with Platinol®+Etoposide (CAV/PE), Cyclophosphamide+BCNU (Carmustine)+VP-16 (Etoposide) (CBV), Cyclophosphamide+Vincristine+Prednisone (CVP), Cyclophosphamide+Oncovin®+Methotrexate+Fluorouracil (COMF), Cytarabine+Methotrexate, Cytarabine+Bleomycin+Vincristine+Methotrexate (CytaBOM), Dactinomycin+Vincristine, Dexamethasone+Cytarabine+Cisplatin (DHAP), Dexamethasone+Ifosfamide+Cisplatin+Etoposide (DICE), Docetaxel+Gemcitabine, Docetaxel+Vinorelbine, Doxorubicin+Vinblastine+Mechlorethamine+Vincristine+Bleomycin+Etoposide+Prednisone (Stanford V), Epirubicin+Gemcitabine, Estramustine+Docetaxel, Estramustine+Navelbine, Estramustine+Paclitaxel, Estramustine+Vinblastine, Etoposide (Vepesid®)+Ifosfamide+Cisplatin (Platinol®) (VIP), Etoposide+Vinblastine+Adriamycin (EVA), Etoposide (Vepesid®)+Ifosfamide+Cisplatin+Epirubicin (VIC-E), Etoposide+Methylprednisolone+Cytarabine+Cisplatin (ESHAP), Etoposide+Prednisone+Ifosfamide+Cisplatin (EPIC), Fludarabine+Mitoxantrone+Dexamethasone (FMD), Fludarabine+Dexamethasone+Cytarabine (ara-C)+Cisplatin (Platinol®) (FluDAP), Fluorouracil+Bevacizumab®, Fluorouracil+CeaVac®, Fluorouracil+Leucovorin, Fluorouracil+Levamisole, Fluorouracil+Oxaliplatin, Fluorouracil+Raltitrexed, Fluorouracil+Sch 6636, Fluorouracil+Trimetrexate, Fluorouracil+

Leucovorin+Bevacizumab®, Fluorouracil+Leucovorin+Oxaliplatin, Fluorouracil+Leucovorin+Trimetrexate, Fluorouracil+Oncovin®+Mitomycin C (FOMi), Hydrazine+Adriamycin®+Methotrexate (HAM), Ifosfamide+Docetaxel, Ifosfamide+Etoposide, Ifosfamide+Gemcitabine, Ifosfamide+Paclitaxel, Ifosfamide+Vinorelbine, Ifosfamide+Carboplatin+Etoposide (ICE), Ifosfamide+Cisplatin+Doxorubicin, Irinotecan+C225 (Cetuximab®), Irinotecan+Docetaxel, Irinotecan+Etoposide, Irinotecan+Fluorouracil, Irinotecan+Gemcitabine, Mechlorethamine+Oncovin® (Vincristine)+Procarbazine (MOP), Mechlorethamine+Oncovin® (Vincristine)+Procarbazine+Prednisone (MOPP), Mesna+Ifosfamide+Idarubicin t Etoposide (MIZE), Methotrexate+Interferon alpha, Methotrexate+Vinblastine, Methotrexate+Cisplatin, Methotrexate with leucovorin rescue+Bleomycin+Adriamycin+Cyclophosphamide+Oncovorin+Dexamethasone (m-BACOD), Mitomycin C+Ifosfamide+Cisplatin (Platinol®) (MIP), Mitomycin C+Vinblastine+Paraplatin® (MVP), Mitoxantrone+Hydrocortisone, Mitoxantrone+Prednisone, Oncovin®+SCH 6636, Oxaliplatin+Leucovorin, Paclitaxel+Doxorubicin, Paclitaxel+SCH 6636, Paraplatin®+Docetaxel, Paraplatin®+Etoposide, Paraplatin®+Gemcitabine, Paraplatin®+Interferon alpha, Paraplatin®+Irinotecan, Paraplatin®+Paclitaxel, Paraplatin®+Vinblastine, Carboplatin (Paraplatin®)+Vincristine, Paraplatin®+Vindesine, Paraplatin®+Vinorelbine, Pemetrexed disodium+Gemcitabine, Platinol® (Cisplatin)+Vinblastine+Bleomycin (PVB), Prednisone+Methotrexate+Adriamycin+Cyclophosphamide+Etoposide (ProMACE), Procarbazine+Lomustine, Procarbazine+Lomustine+Vincristine, Procarbazine+Lomustine+Vincristine+Thioguanine, Procarbazine+Oncovin®+CCNU(G+Cyclophosphamide (POCC), Quinine+Doxorubicin, Quinine+Mitoxantrone+Cytarabine, Thiotepa+Etoposide, Thiotepa+Busulfan+Cyclophosphamide, Thiotepa+Busulfan+Melphalan, Thiotepa+Etoposide+Carmustine, Thiotepa+Etoposide+Carboplatin, Topotecan+Paclitaxel, Trimetrexate+Leucovorin, Vinblastine+Doxorubicin+Thiotepa, Vinblastine+Bleomycin+Etoposide+Carboplatin, Vincristine+Lomustine+Prednisone, Vincristine (Oncovin®)+Adriamycin®+Dexamethasone (VAD), Vincristine (Oncovin®)+Adriamycin®+Procarbazine (VAP), Vincristine+Dactinomycin+Cyclophosphamide, and Vinorelbine+Gemcitabine.

[0797] In an additional embodiment, the compositions of the invention are administered in combination with cytokines. Cytokines that may be administered with the compositions of the invention include, but are not limited to, GM-CSF, G-CSF, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, anti-CD40, CD40L, IFN-alpha, IFN-beta, IFN-gamma, TNF-alpha, and TNF-beta.

[0798] In an additional embodiment, the compositions of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the compositions of the invention included, but are not limited to, LEUKINE® (SARGRAMOSTIM®) and NEUPOGEN® (FILGRASTIM®).

[0799] In an additional embodiment, the compositions of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be adminis-

tered with the compositions of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., *Growth Factors*, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor-B-186 (VEGF-B 186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above-mentioned references are incorporated herein by reference herein.

[0800] In an additional embodiment, the compositions of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the compositions of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0801] In one embodiment, the compositions of the invention are administered in combination with one or more chemokines. In specific embodiments, the compositions of the invention are administered in combination with an α (CxC) chemokine selected from the group consisting of gamma-interferon inducible protein-10 (γ IP-10), interleukin-8 (IL-8), platelet factor-4 (PF4), neutrophil activating protein (NAP-2), GRO- α , GRO- β , GRO- γ , neutrophil-activating peptide (ENA-78), granulocyte chemoattractant protein-2 (GCP-2), and stromal cell-derived factor-1 (SDF-1, or pre-B-cell stimulatory factor (PBSF)); and/or a β (CC) selected from the group consisting of: RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory protein-1 alpha (MIP-1 α), macrophage inflammatory protein-1 beta (MIP-1 β), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-2 (MCP-2), monocyte chemotactic protein-3 (MCP-3), monocyte chemotactic protein-4 (MCP-4) macrophage inflammatory protein-1 gamma (MIP-1 γ), macrophage inflammatory protein-3 alpha (MIP-3 α), macrophage inflammatory protein-3 beta (MIP-3 β), macrophage inflammatory protein-4 (MIP-4/DC-CK-1/PARC), eotaxin, Exodus, and I-309; and/or the γ (C) chemokine, lymphotactin.

[0802] In additional embodiments, the compositions of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

[0803] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceu-

tically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[0804] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0805] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[0806] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

[0807] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or disorders associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

[0808] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the

polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[0809] Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, M. et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M. et al., *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (¹²⁵I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (¹¹²In), and technetium (⁹⁹Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0810] One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of the interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

[0811] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells, which contain the specific protein. In vivo tumor imaging is described in S. W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S. W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0812] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0813] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0814] Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0815] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Pat. No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

[0816] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope, which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody, which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0817] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope, which is specifically immunoreactive with at least

one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0818] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0819] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0820] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme, which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, Mo.).

[0821] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0822] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Chromosome Assays

[0823] The nucleic acid molecules of the present invention are also valuable for chromosome identification. The

sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

[0824] In certain preferred embodiments in this regard, the cDNA and/or polynucleotides herein disclosed is used to clone genomic DNA of a DR5 gene. This can be accomplished using a variety of well-known techniques and libraries, which generally are available commercially. The genomic DNA is then used for *in situ* chromosome mapping using well-known techniques for this purpose.

[0825] In addition, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

[0826] Fluorescence *in situ* hybridization ("FISH") of a cDNA to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60 bp. For a review of this technique, see Verma et al, *Human Chromosomes: a Manual of Basic Techniques*, Pergamon Press, New York (1988).

[0827] Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

[0828] Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

[0829] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLE 1

[0830] Expression and Purification in *E. coli*

[0831] The DNA sequence encoding the mature DR5 protein in the deposited cDNA (ATCC No. 97920) is amplified using PCR oligonucleotide primers specific to the amino terminal sequences of the DR5 protein and to vector sequences 3' to the gene. Additional nucleotides containing restriction sites to facilitate cloning are added to the 5' and 3' sequences respectively.

[0832] The following primers are used for expression of DR5 extracellular domain in *E. coli*: The 5' primer has the sequence: 5'-CGCCCCATGGAGTCTGCTCTGACAC-3'

(SEQ ID NO:8) and contains the underlined NcoI site; and the 3' primer has the sequence: 5'-CGCAAGCTTTAGC-CTGATTCTTGAC-3' (SEQ ID NO:9) and contains the underlined HindIII site.

[0833] The restriction sites are convenient to restriction enzyme sites in the bacterial expression vector pQE60, which are used for bacterial expression in this example. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, Calif., 91311). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, and a ribosome-binding site ("RBS").

[0834] The amplified DR5 DNA and the vector pQE60 both are digested with NcoI and HindIII and the digested DNAs are then ligated together. Insertion of the DR5 protein DNA into the restricted pQE60 vector places the DR5 protein coding region downstream of and operably linked to the vector's IPTG-inducible promoter and in-frame with an initiating AUG appropriately positioned for translation of DR5 protein.

[0835] The ligation mixture is transformed into competent *E. coli* cells using standard procedures. Such procedures are described in Sambrook et al., Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses lac repressor and confers kanamycin resistance ("Kan"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing DR5 protein, is available commercially from Qiagen, supra.

[0836] Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR, and DNA sequencing.

[0837] Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from lac repressor sensitive promoters, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours.

[0838] Cells then are harvested by centrifugation and disrupted, by standard methods. Inclusion bodies are purified from the disrupted cells using routine collection techniques, and protein is solubilized from the inclusion bodies into 8M urea. The 8M urea solution containing the solubilized protein is passed over a PD-10 column in 2x phosphate-buffered saline ("PBS"), thereby removing the urea, exchanging the buffer and refolding the protein. The protein is purified by a further step of chromatography to remove endotoxin. Then, it is sterile filtered. The sterile filtered protein preparation is stored in 2xPBS at a concentration of 95 µl/ml.

EXAMPLE 2

[0839] Expression in Mammalian Cells

[0840] A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g RSV, HTLV, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular signals can also be used (e.g. the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC67109). Mammalian host cells that could be used include, human HeLa 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

[0841] Alternatively, the gene of interest can be expressed in stable cell lines that contain the gene integrated into a chromosome. Co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

[0842] The transfected gene can also be amplified to express large amounts of the encoded protein. The dihydrofolate reductase (DHFR) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem. J.* 227:277-279 (1991); Bebbington et al., *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) cells are often used for the production of proteins.

[0843] The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology* 5:438-447 (March 1985)), plus a fragment of the CMV-enhancer (Boshart et al., *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g. with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

[0844] Cloning and Expression in CHO Cells

[0845] The vector pC4 is used for the expression of the DR5 polypeptide. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids, can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate (MTX). The amplification of the DHFR genes in cells

resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., *J. Biol. Chem.* 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., *Biochem. et Biophys. Acta* 1097:107-143 (1990); Page, M. J. and Sydenham, M. A. 1991, *Biotechnology* 9:64-68(1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

[0846] Plasmid pC4 contains, for expressing the gene of interest, the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology* 5:438-447 (March 1985), plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart et al., *Cell* 41:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, XbaI, and Asp718. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for expression, e.g., the human β -actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the DR5 polypeptide in a regulated way in mammalian cells (Gossen, M., & Bujard, H., *Proc. Natl. Acad. Sci. USA* 89:5547-5551 (1992)). For the polyadenylation of the mRNA, other signals, e.g., from the human growth hormone or globin genes, can be used as well.

[0847] Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418, or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

[0848] The plasmid pC4 is digested with the restriction enzyme BamHI and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

[0849] The DNA sequence encoding the complete polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the desired portion of the gene. The 5' primer containing the underlined BamHI site, a Kozak sequence, and an AUG start codon, has the following sequence: 5'-CGCGGATCCGCCATCATG-GAACAACGGGGACAGAAC-3' (SEQ ID NO:10). The 3' primer, containing the underlined Asp718 site, has the following sequence: 5'-CGCGGTACCTTAGGACATG-GCAGAGTC-3' (SEQ ID NO:11).

[0850] The amplified fragment is digested with the endonuclease BamHI and Asp718 and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria

are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

[0851] Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. Five pg of the expression plasmid pC4 is cotransfected with 0.5 μ g of the plasmid pSVneo using the lipofectin method (Felgner et al., supra). The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days, single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μ M, 2 μ M, 5 μ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200 μ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

[0852] Cloning and Expression in COS Cells

[0853] The expression plasmid, pDR5-HA, is made by cloning a cDNA encoding the soluble extracellular domain of the DR5 protein into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.). The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 and a polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. A DNA fragment encoding the extracellular domain of the DR5 polypeptide and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson et al., *Cell* 37:767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope.

[0854] The plasmid construction strategy is as follows. The DR5 cDNA of the deposited plasmid is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of DR5 in *E. coli*.

[0855] To facilitate detection, purification and characterization of the expressed DR5, one of the primers contains a hemagglutinin tag ("HA tag") as described above. Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined BamHI site has the following sequence: 5'-CGCGGATCCGGCAT-CATGGAACAACGGGACAGAAC-3' (SEQ ID NO:10).

The 3' primer, containing the underlined Asp718 restriction sequence has the following sequence: 5'-CGCGGTACCT-TAGCCTGATTCTTTGGAC-3' (SEQ ID NO:12).

[0856] The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI and Asp718 and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, Calif. 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the fragment encoding the extracellular domain of the DR5 polypeptide.

[0857] For expression of recombinant DR5, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook et al., *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, N.Y. (1989). Cells are incubated under conditions for expression of DR5 by the vector.

[0858] Expression of the DR5-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow et al., *Antibodies: A Laboratory Manual*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing 35 S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson et al., cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

[0859] The primer sets used for expression in this example are compatible with pC4 used for CHO expression in this example, pcDNAI/Amp for COS expression in this example, and pA2 used for baculovirus expression in the following example. Thus, for example, the complete DR5 encoding fragment amplified for CHO expression could also be ligated into pcDNAI/Amp for COS expression or pA2 for baculovirus expression.

EXAMPLE 3

[0860] Protein Fusions of DR5

[0861] DR5 polypeptides of the invention are optionally fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of DR5 polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See EP A 394,827; Traunecker, et al., *Nature* 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to DR5 polypeptides can target the protein to a specific sub-cellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules

having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made using techniques known in the art or by using or routinely modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

[0862] Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described in SEQ ID NO:13. These primers also preferably contain convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

[0863] For example, if the pC4 (Accession No. 209646) expression vector is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and DR5 polynucleotide, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

[0864] If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

EXAMPLE 4

[0865] Cloning and Expression of the Soluble Extracellular Domain of DR5 in a Baculovirus Expression System

[0866] In this illustrative example, the plasmid shuttle vector pA2 is used to insert the cDNA encoding the complete DR5 protein, including its naturally associated signal sequence, into a baculovirus to express the DR5 protein, using standard methods, such as those described in Summers et al., *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No.1555 (1987). This expression vector contains the strong polyhedron promoter of the *Autographa californica* nuclear polyhedrosis virus (ACM-NPV) followed by convenient restriction sites. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

[0867] Many other baculovirus vectors could be used in place of pA2, such as pAc373, pVL941 and pAcIM1 provided, as one skilled in the art would readily appreciate, that construction provides appropriately located signals for transcription, translation, secretion, and the like, such as an in-frame AUG and a signal peptide, as required. Such vectors are described, for example, in Luckow et al., *Virolgy* 170:31-39 (1989).

[0868] The cDNA sequence encoding the soluble extracellular domain of DR5 protein in the deposited plasmid

(ATCC Deposit No. 97920) is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

[0869] The 5' primer for DR5 has the sequence: 5'-CGCG-GATCCGCCATCATGGAACACGGGGACAGAAC-3' (SEQ ID NO:10) containing the underlined BamHI restriction enzyme site. Inserted into an expression vector, as described below, the 5' end of the amplified fragment encoding DR5 provides an efficient cleavage signal peptide. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol.* 196:947-950 (1987) is appropriately located in the vector portion of the construct.

[0870] The 3' primer for DR5 has the sequence: 5'-CGCG-GTACCTTAGCCTGATTCTTGAC-3' (SEQ ID NO:12) containing the underlined Asp718 restriction followed by nucleotides complementary to the DR5 nucleotide sequence in FIG. 1, followed by the stop codon.

[0871] The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Calif.) The fragment then is digested with BamHI and Asp718 and again is purified on a 1% agarose gel. This fragment is designated "F1."

[0872] The plasmid is digested with the restriction enzymes BamHI and Asp718 and optionally can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Calif.). The vector DNA is designated herein "V1."

[0873] Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 cells, or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, Calif.) cells, are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid with the human DR5 are identified using the PCR method, in which one of the primers used to amplify the gene is directed to the DR5 sequence and the second primer is from well within the vector so that only those bacterial colonies containing the DR5 gene fragment will show amplification of the DNA. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac DR5.

[0874] 5 µg of the plasmid pBac DR5 is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, Calif.), using the lipofectin method described by Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987). 1 µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBac DR5 are mixed in a sterile well of a microtiter plate containing 50 µl of serum free Grace's medium (Life Technologies Inc., Gaithersburg, Md.). Afterwards 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to SF9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27° C. After 5 hours, the transfection solution is removed from

the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27° C. for four days.

[0875] After four days, the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, cited above. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg, Md.) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, Md., pages 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipette (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later, the supernatants of these culture dishes are harvested and then they are stored at 4° C. The recombinant virus is called V-DR5.

[0876] To verify expression of the DR5 gene, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-DR5 at a multiplicity of infection ("MOI") of about 2 (about 1 to about 3). Six hours later, the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Gaithersburg, Md.). If radiolabeled proteins are desired, 42 hours later, 5 μ Ci of 35 S-methionine and 5 μ Ci 35 S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the mature protein and thus the cleavage point and length of the secretory signal peptide.

EXAMPLE 5

[0877] DR5 Induced Apoptosis in Mammalian Cells

[0878] Overexpression of Fas/APO-1 and TNFR-1 in mammalian cells mimics receptor activation (M. Muzio et al., *Cell* 85: 817-827 (1996); M. P. Boldin et al., *Cell* 85:803-815 (1996)). Thus, this system was utilized to study the functional role of DR5 in inducing apoptosis. This example demonstrates that overexpression of DR5 induced apoptosis in both MCF7 human breast carcinoma cells and in human epitheloid carcinoma (HeLa) cells.

[0879] Experimental Design

[0880] Cell death assays were performed essentially as previously described (A. M. Chinnaiyan, et al., *Cell* 81:505-12 (1995); M. P. Boldin, et al., *J Biol Chem* 270: 7795-8 (1995); F. C. Kischkel, et al., *EMBO* 14:5579-5588 (1995); A. M. Chinnaiyan, et al., *J Biol Chem* 271: 4961-4965 (1996)). Briefly, MCF-7 human breast carcinoma clonal cell lines and HeLa cells were co-transfected with vector, DR5, DR5 Δ (52-411), or TNFR-1, together with a beta-galactosidase reporter construct.

[0881] MCF7 and HeLa cells were transfected using the lipofectamine procedure (GIBCO-BRL), according to the manufacturer's instructions. 293 cells were transfected using CaPO₄ precipitation. Twenty-four hours following transfection, cells were fixed and stained with X-Gal as previously described (A. M. Chinnaiyan, et al., *Cell* 81:505-12 (1995); M. P. Boldin, et al., *J Biol Chem* 270:7795-8 (1995); F. C. Kischkel, et al., *EMBO* 14:5579-5588 (1995)), and examined microscopically. The data (mean \pm SD) presented in FIG. 5 represents the percentage of round, apoptotic cells as a function of total beta-galactosidase positive cells (n=3). Overexpression of DR5 induced apoptosis in both MCF7 (FIG. 5A) and HeLa cells (FIG. 5B).

[0882] MCF7 cells were also transfected with a DR5 expression construct in the presence of z-VAD-fmk (20 μ l) (Enzyme Systems Products, Dublin, Calif.) or co-transfected with a three-fold excess of CrmA (M. Tewari et al., *J Biol Chem* 270:3255-60 (1995)), or FADD-DN expression construct, or vector alone. The data presented in FIG. 5C shows that apoptosis induced by DR5 was attenuated by caspase inhibitors, but not by dominant negative FADD.

[0883] As depicted in FIG. 5D, DR5 did not associate with FADD or TRADD in vivo. 293 cells were co-transfected with the indicated expression constructs using calcium phosphate precipitation. After transfection (at 40 hours), cell lysates were prepared and immunoprecipitated with Flag M2 antibody affinity gel (IBI, Kodak), and the presence of FADD or myc-tagged TRADD (myc-TRADD) was detected by immunoblotting with polyclonal antibody to FADD or horseradish peroxidase (HRP) conjugated antibody to myc (BMB)(Baker, S. J. et al., *Oncogene* 12:1 (1996); Chinnaiyan, A. M. et al., *Science* 274:990 (1996)).

[0884] As depicted in FIG. 5E, FLICE 2-DN blocks DR5-induced apoptosis. 293 cells were co-transfected with DR5 or TNFR-1 expression construct and a fourfold excess of CrmA, FLICE-DN, FLICE 2-DN, or vector alone in the presence of a beta-galactosidase reporter construct as indicated. Cells were stained and examined 25-30 hours later.

[0885] Results

[0886] Overexpression of DR5, induced apoptosis in both MCF7 human breast carcinoma cells (FIG. 5A) and in human epitheloid carcinoma (HeLa) cells (FIG. 5B). Most of the transfected cells displayed morphological changes characteristic of cells undergoing apoptosis (Earnshaw, W. C., *Curr. Biol.* 7:337 (1995)), becoming rounded, condensed and detaching from the dish. Deletion of the death domain abolished killing ability. Like DR4, DR5-induced apoptosis was blocked by caspase inhibitors, CrmA and z-VAD-fmk, but dominant negative FADD was without effect (FIG. 5C). Consistent with this, DR5 did not interact with FADD and TRADD in vivo (FIG. SD). A dominant negative version of a newly identified FLICE-like molecule, FLICE2 (Vincenz, C. et al., *J. Biol. Chem.* 272:6578 (1997)), efficiently blocked DR5-induced apoptosis, while dominant negative FLICE had only partial effect under conditions it blocked. TNFR-1 induced apoptosis effectively (FIG. 5E). Taken together, the evidence suggests that DR5 engages an apoptotic program that involves activation of FLICE2 and downstream caspases, but is independent of FADD.

EXAMPLE 6

[0887] The Extracellular Domain of DR5 Binds the Cytotoxic Ligand, TRAIL, and Blocks TRAIL-Induced Apoptosis

[0888] As discussed above, TRAIL/Apo2L is a cytotoxic ligand that belongs to the tumor necrosis factor (TNF) ligand family and induces rapid cell death of many transformed cell lines, but not normal tissues, despite its death domain containing receptor, DR4, being expressed on both cell types. This example shows that the present receptor, DRS, also binds TRAIL.

[0889] Given the similarity of the extracellular ligand binding cysteine-rich domains of DRS and DR4, the present inventors theorized that DRS would also bind TRAIL. To confirm this, the soluble extracellular ligand binding domains of DRS were expressed as fusions to the Fc portion of human immunoglobulin (IgG). cDNA encoding the amino acids 1 to 129 in SEQ ID NO:2 was obtained by polymerase chain reaction and cloned into a modified pCMVFLAG vector that allowed for in-frame fusion with the Fc portion of human IgG.

[0890] As shown in FIG. 6A, DRS-Fc specifically bound TRAIL, but not the related cytotoxic ligand TNF α . In this experiment, the Fc-extracellular domains of DRS, DR4, TRID, or TNFR-1 and the corresponding ligands were prepared and binding assays performed as described in Pan et al., *Science* 276:111 (1997). The respective Fc-fusions were precipitated with protein G-Sepharose and co-precipitated soluble ligands were detected by immunoblotting with anti-Flag (Babco) or anti-myc-HRP (BMB). The bottom panel of FIG. 6A shows the input Fc-fusions present in the binding assays.

[0891] Additionally, DR5-Fc blocked the ability of TRAIL to induce apoptosis (FIG. 6B). MCF7 cells were treated with soluble TRAIL (200 ng/ml) in the presence of equal amounts of Fc-fusions or Fc alone. Six hours later, cells were fixed and examined as described in Pan et al., Id. The data (mean \pm SD) shown in FIG. 6B are the percentage of apoptotic nuclei among total nuclei counted (n=4).

[0892] Finally, DR5-Fc had no effect on apoptosis TNF α -induced cell death under conditions where TNFR-1-Fc completely abolished TNF \square killing (FIG. 6C). MCF7 cells were treated with TNF α (40 ng/ml; Genentech, Inc.) in the presence of equal amounts of Fc-fusions or Fc alone. Nuclei were stained and examined 11-15 hours later.

[0893] The new identification of DR5 as a receptor for TRAIL adds further complexity to the biology of TRAIL-initiated signal transduction.

EXAMPLE 7

[0894] Assays to Detect Stimulation or Inhibition of B Cell Proliferation and Differentiation

[0895] Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B-cell responsive-

ness including IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B-cell populations. One of the best-studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays that allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

[0896] Experimental Procedure:

[0897] In Vitro assay—Purified DR5 protein, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of DR5 protein on purified human tonsillar B-cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B-cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and EL-15 synergize with SAC and IgM cross-linking to elicit B-cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B-cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B-cells as assessed by expression of CD45R (B220). Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10⁵ B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 \times 10⁻⁵M β -ME, 100 U/ml penicillin, 10 μ g/ml streptomycin, and 10⁻⁵ dilution of SAC) in a total volume of 150 μ l. Proliferation or inhibition is quantitated by a 20 h pulse (1 μ Ci/well) with ³H-thymidine (6.7 Ci/mM) beginning 72 hours post factor addition. The positive and negative controls are IL-2 and medium respectively.

[0898] In Vivo assay—BALB/c mice are injected (i.p.) twice per day with buffer only, or with 2 mg/Kg of DR5 protein, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal and DR5 protein-treated spleens identify the results of the activity of DR5 protein on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B-cell marker, anti-CD45R (B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

[0899] Flow cytometric analyses of the spleens from DR5 protein-treated mice is used to indicate whether DR5 protein specifically increases the proportion of ThB+, CD45R (B220) dull B-cells over that which is observed in control mice.

[0900] Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and DR5 protein-treated mice.

[0901] The studies described in this example test the activity in DR5 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of DR5 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of DR5.

EXAMPLE 8

[0902] T-Cell Proliferation Assay

[0903] A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ^3H -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 μl /well of mAb to CD3 (HIT3a, Pharminogen) or isotype-matched control mAb (B33.1) overnight at 4° C. (1 $\mu\text{g}/\text{ml}$ in 0.05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells ($5 \times 10^4/\text{well}$) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of DR5 protein (total volume 200 μl). Relevant protein buffer and medium alone are controls. After 48 hours at 37° C., plates are spun for 2 minutes at 1000 rpm and 100 μl of supernatant is removed and stored at -20° C. for measurement of IL-2 (or other cytokines) if an effect on proliferation is observed. Wells are supplemented with 100 μl of medium containing 0.5 μCi of ^3H -thymidine and cultured at 37° C. for 18-24 hr. Wells are harvested and incorporation of ^3H -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control, which enhances proliferation. Control antibody, which does not induce proliferation of T-cells, is used as the negative controls for the effects of DR5 proteins.

[0904] The studies described in this example test the activity in DR5 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of DR5 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of DR5.

EXAMPLE 9

[0905] Effect of DR5 on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells

[0906] Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- α , causes a rapid change in surface phenotype (increased expression of MHC class I and II,

costimulatory and adhesion molecules, downregulation of FC γ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

[0907] FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of DR5 or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4° C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

[0908] Effect on the Production of Cytokines

[0909] Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ($10^6/\text{ml}$) are treated with increasing concentrations of DR5 for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, Minn.)). The standard protocols provided with the kits are used.

[0910] Effect on the Expression of MHC Class II, Costimulatory and Adhesion Molecules

[0911] Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T-cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

[0912] FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of DR5 or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4° C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

[0913] Monocyte Activation and/or Increased Survival

[0914] Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. DR5, agonists, or antagonists of DR5 can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, Md.) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

[0915] Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2×10^6 /ml in PBS containing PI at a final concentration of 5 μ g/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

[0916] Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of 5×10^5 cells/ml with increasing concentrations of DR5 and under the same conditions, but in the absence of DR5. For IL-12 production, the cells are primed overnight with WFN- γ (100 U/ml) in presence of DR5. LPS (10 ng/ml) is then added. Conditioned media are collected after 24 h and kept frozen until use. Measurement of TNF- α , IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g., R & D Systems (Minneapolis, Minn.)) and applying the standard protocols provided with the kit.

[0917] Oxidative burst. Purified monocytes are plated in 96-well plates at $2-1 \times 10^5$ cell/well. Increasing concentrations of DR5 are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640+10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 μ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H₂O₂ produced by the macrophages, a standard curve of a H₂O₂ solution of known molarity is performed for each experiment.

[0918] The studies described in this example test the activity in DR5 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of DR5 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of DR5.

EXAMPLE 10

[0919] The Effect of DR5 on the Growth of Vascular Endothelial Cells

[0920] On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at $2-5 \times 10^4$ cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnique, Inc.). On day 2, the

medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. DR5 protein of SEQ ID NO. 2, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

[0921] An increase in the number of HUVEC cells indicates that DR5 may proliferate vascular endothelial cells.

[0922] The studies described in this example test the activity in DR5 protein. However, one skilled in the art could easily modify the exemplified studies to test the activity of DR5 polynucleotides (e.g., gene therapy), agonists, and/or antagonists of DR5.

EXAMPLE 11

[0923] Production of an Antibody

[0924] Hybridoma Technology

[0925] The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing DR5 are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of DR5 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

[0926] Monoclonal antibodies specific for protein DR5 are prepared using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with DR5 polypeptide or, more preferably, with a secreted DR5 polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56° C.), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 μ g/ml of streptomycin.

[0927] The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP20), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones, which secrete antibodies capable of binding the DR5 polypeptide.

[0928] Alternatively, additional antibodies capable of binding to DR5 polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody, which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an

animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones, which produce an antibody, whose ability to bind to the DR5 protein-specific antibody can be blocked by DR5. Such antibodies comprise anti-idiotypic antibodies to the DR5 protein-specific antibody and are used to immunize an animal to induce formation of further DR5 protein-specific antibodies.

[0929] For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed infra. (See, for review, Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et al., U.S. Pat. No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulian et al., *Nature* 312:643 (1984); Neuberger et al., *Nature* 314:268 (1985).)

[0930] Isolation Of Antibody Fragments Directed Against DR5 From A Library Of scFvs

[0931] Naturally occurring V-genes isolated from human PBLs are constructed into a large library of antibody fragments which contain reactivities against polypeptides of the present invention to which the donor may or may not have been exposed (see, e.g., U.S. Pat. No. 5,885,793 incorporated herein in its entirety by reference).

[0932] Rescue of the Library

[0933] A library of scFvs is constructed from the RNA of human PBLs as described in WO92/01047. To rescue phage displaying antibody fragments, approximately 10^9 *E. coli* harboring the phagemid are used, to inoculate 50 ml of 2 \times TY containing 1% glucose and 100 μ g/ml of ampicillin (2 \times TY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2 \times TY-AMP-GLU, 2 \times 10⁸ TU of delta gene 3 helper phage (M13 gene III, see WO92/01047) are added and the culture incubated at 37° C. for 45 minutes without shaking and then at 37° C. for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 minutes and the pellet resuspended in 2 liters of 2 \times TY containing 100 μ g/ml ampicillin and 50 μ g/ml kanamycin and grown overnight. Phages are prepared as described in WO92/01047.

[0934] M13 gene III is prepared as follows: M13 gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C. without shaking and then for a further hour at 37° C. with shaking. Cells are pelleted (IEC-Centra 8, 4000 revs/min for 10 min), resuspended in 300 ml 2 \times TY broth containing 100 μ g ampicillin/ml and 25 μ g kanamycin/ml (2 \times TY-AMP-KAN) and grown overnight, shaking at 37° C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 μ m filter (Minisart NML; Sartorius) to

give a final concentration of approximately 10¹³ transducing units/ml (ampicillin-resistant clones).

[0935] Panning of the Library

[0936] Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 mg/ml or 10 mg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37° C. and then washed 3 times in PBS. Approximately 10¹³ TU of phage are applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log *E. coli* TG1 by incubating eluted phage with bacteria for 30 minutes at 37° C. The *E. coli* are then plated on TYE plates containing 1% glucose and 100 μ g/ml ampicillin. The resulting bacterial library is then rescued with M13 gene III helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

[0937] Characterization of Binders

[0938] Eluted phages from the 3rd and 4th rounds of selection are used to infect *E. coli* HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtiter plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see e.g., WO92/01047) and then by sequencing.

EXAMPLE 12

[0939] Tissue Distribution of DR5 Gene Expression

[0940] Northern blot analysis was carried out to examine DR5 gene expression in human tissues, using methods described by, among others, Sambrook et al., cited above. A cDNA probe containing the entire nucleotide sequence of the DR5 protein (SEQ ID NO:1) was labeled with ³²P using the rediprime® DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe was purified using a CHROMA SPIN-100® column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe was then used to examine various human tissues for DR5 mRNA.

[0941] Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) were obtained from Clontech (Palo Alto, Calif.) and examined with labeled probe using ExpressHyb® hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots were mounted and exposed to film at -70° C. overnight. The films were developed according to standard procedures. Expression of DR5 was detected in heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, uterus, small intestine, colon, peripheral blood leukocytes (PBLs), lymph node, bone marrow, and fetal liver.

[0942] Expression of DR5 was also assessed by Northern blot in the following cancer cell lines, HL60 (promyelocytic leukemia), HeLa cell S3, K562 (chronic myelogenous leukemia), MOLT4 (lymphoblast leukemia), Raji (Burkitt's lymphoma), SW480 (colorectal adenocarcinoma), A549 (lung carcinoma), and G361 (melanoma), and was detected in all of the cell lines tested.

EXAMPLE 13

[0943] Method of Determining Alterations in the DR5 Gene

[0944] RNA is isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease). cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:1. Suggested PCR conditions consist of 35 cycles at 95° C. for 30 seconds; 60-120 seconds at 52-58° C.; and 60-120 seconds at 70° C., using buffer solutions described in Sidransky, D., et al., *Science* 252:706 (1991). PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTHERM Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons of DR5 are also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations in DR5 are then cloned and sequenced to validate the results of the direct sequencing.

[0945] PCR products of DR5 are cloned into T-tailed vectors as described in Holton, T. A. and Graham, M. W., *Nucleic Acids Research*, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations in DR5 not present in unaffected individuals.

[0946] Genomic rearrangements are also observed as a method of determining alterations in the DR5 gene. Genomic clones isolated using techniques known in the art are nick-translated with digoxigenin-deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson, Cg. et al., *Methods Cell Biol.* 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the DR5 genomic locus.

[0947] Chromosomes are counter-stained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, Vt.) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, Ariz.) and variable excitation wavelength filters. (Johnson, Cv. et al., *Genet. Anal. Tech. Appl.*, 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, N.C.) Chromosome alterations of the genomic region of DR5 (hybridized by the probe) are identified as insertions, deletions, and translocations. These DR5 alterations are used as a diagnostic marker for an associated disease.

EXAMPLE 14

[0948] Method of Detecting Abnormal Levels of DR5 in a Biological Sample

[0949] DR5 polypeptides can be detected in a biological sample, and if an increased or decreased level of DR5 is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

[0950] For example, antibody-sandwich ELISAs are used to detect DR5 in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to DR5, at a final concentration of 0.2 to 10 µg/ml. The antibodies are either monoclonal or polyclonal and are produced using technique known in the art. The wells are blocked so that non-specific binding of DR5 to the well is reduced.

[0951] The coated wells are then incubated for >2 hours at RT with a sample containing DR5. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded DR5.

[0952] Next, 50 µl of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

[0953] 75 µl of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution is then added to each well and incubated 1 hour at room temperature to allow cleavage of the substrate and fluorescence. The fluorescence is measured using a microtiter plate reader. A standard curve is prepared using the experimental results from serial dilutions of a control sample with the sample concentration plotted on the X-axis (log scale) and fluorescence or absorbance on the Y-axis (linear scale). The DR5 polypeptide concentration in a sample is then interpolated using the standard curve based on the measured fluorescence of that sample.

EXAMPLE 15

[0954] Method of Treating Decreased Levels of DR5

[0955] The present invention relates to a method for treating an individual in need of a decreased level of DR5 biological activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of DR5 antagonist. Preferred antagonists for use in the present invention are DR5-specific antibodies.

[0956] Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of DR5 in an individual can be treated by administering DR5, preferably in a soluble and/or secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of DR5 polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of DR5 to increase the biological activity level of DR5 in such an individual.

[0957] For example, a patient with decreased levels of DR5 polypeptide receives a daily dose 0.1-100 g/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in a soluble and/or secreted form.

EXAMPLE 16

[0958] Method of Treating Increased Levels of DR5

[0959] The present invention also relates to a method for treating an individual in need of an increased level of DR5 biological activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of DR5 or an agonist thereof.

[0960] Antisense technology is used to inhibit production of DR5. This technology is one example of a method of decreasing levels of DR5 polypeptide, preferably a soluble and/or secreted form, due to a variety of etiologies, such as cancer.

[0961] For example, a patient diagnosed with abnormally increased levels of DR5 is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the is determined to be well tolerated.

EXAMPLE 17

[0962] Method of Treatment Using Gene Therapy—Ex Vivo

[0963] One method of gene therapy transplants fibroblasts, which are capable of expressing soluble and/or mature DR5 polypeptides, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37° C. for approximately one week.

[0964] At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

[0965] pMV-7 (Kirschmeier, P. T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads. The cDNA encoding DR5 can be amplified using PCR primers that correspond to the 5' and 3' end encoding sequences respectively. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform *E. coli* HB101, which are then

plated onto agar containing kanamycin for the purpose of confirming that the vector contains properly inserted DR5.

[0966] The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the DR5 gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the DR5 gene (the packaging cells are now referred to as producer cells).

[0967] Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a Millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether DR5 protein is produced.

[0968] The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

EXAMPLE 18

[0969] Method of Treatment Using Gene Therapy—In Vivo

[0970] Another aspect of the present invention is using in vivo gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) DR5 sequences into an animal to increase or decrease the expression of the DR5 polypeptide. The DR5 polynucleotide may be operatively linked to a promoter or any other genetic elements necessary for the expression of the DR5 polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Pat. Nos. 5,693,622, 5,705,151, 5,580,859; Tabata H. et al., *Cardiovasc. Res.* 35:470-479 (1997); Chao J. et al., *Pharmacol. Res.* 35:517-522 (1997); Wolff J. A. *Neuromuscul. Disord.* 7:314-318 (1997); Schwartz B. et al., *Gene Ther.* 3:405-411 (1996); Tsurumi Y. et al., *Circulation* 94:3281-3290 (1996) (incorporated herein by reference).

[0971] The DR5 polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The DR5 polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[0972] The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formu-

lations, lipofectin or precipitating agents and the like. However, the DR5 polynucleotides may also be delivered in liposome formulations (such as those taught in Felgner P. L. et al. *Ann. NY Acad. Sci.* 772:126-139 (1995), and Abdallah B. et al. *Biol. Cell* 85:1-7 (1995)) which can be prepared by methods well known to those skilled in the art.

[0973] The DR5 polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[0974] The DR5 polynucleotide construct can be delivered to the interstitial space of tissues within an animal, including muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

[0975] For the naked DR5 polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DR5 polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[0976] The dose response effects of injected DR5 polynucleotide in muscle in vivo are determined as follows.

Suitable DR5 template DNA for production of mRNA coding for DR5 polypeptide is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

[0977] Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The DR5 template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

[0978] After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 μ m cross-section of the individual quadriceps muscles is histochemically stained for DR5 protein expression. A time course for DR5 protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DR5 DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages and other treatment parameters in humans and other animals using DR5 naked DNA.

EXAMPLE 19

[0979] A DR5-Fc Fusion Protein Inhibits B Cell Proliferation in Vitro in a Co-stimulatory Assay

[0980] A DR5-Fc polypeptide was prepared that consists of a soluble form of DR5 (corresponding to amino acids -51 to 133 of SEQ ID NO:2) linked to the Fc portion of a human IgG1 immunoglobulin molecule. The ability of this protein to alter the proliferative response of human B-cells was assessed in a standard co-stimulatory assay. Briefly, human tonsillar B-cells were purified by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population was routinely greater than 95% B-cells as assessed by expression of CD19 and CD20 staining. Various dilutions of rHuNeurotuke-alpha (International Application Publication No. WO 98/18921) or the control protein rHuIL2 were placed into individual wells of a 96-well plate to which was added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M 2ME, 100U/ml penicillin, 10 μ g/ml streptomycin, and 10^{-5} dilution of formalin-fixed *Staphylococcus aureus* Cowan I (SAC) also known as Pansorbin (Pan)) in a total volume of 150 μ l. DR5-Fc was then added at various concentrations. Plates were then placed in the incubator (37° C. 5% CO₂, 95% humidity) for three days. Proliferation was quantitated by a 20-hour pulse (1 μ Ci/well) of ³H-thymidine (6.7 Ci/mM) beginning 72 hours post factor addition. The positive and negative controls are IL-2 and medium, respectively.

[0981] The results of this experiment confirmed that DR5-Fc inhibited B-cell proliferation in the co-stimulatory assay using *Staphylococcus Aureus* Cowan 1 (SAC) as priming

agent and Neutrokin-alpha as a second signal (data not shown). It is important to note that other Tumor Necrosis Factor Receptors (TNFR) fusion proteins (e.g., DR4-Fc (International Application Publication No. WO 98/32856), TR6-Fc (International Application Publication No. WO 98/31799), and TR9-Fc (International Application Publication No. WO 98/56892)) did not inhibit proliferation.

EXAMPLE 20

[0982] Sensitive and Specific Immunohistochemical Assays for the Detection of TRAIL Receptors DR4 and DR5.

[0983] Specific and sensitive assays for the detection of DR4 and DR5 receptors in formalin-fixed, paraffin-embedded human tumor tissues were developed. Affinity purified rabbit polyclonal antibodies, specific for DR4 and DR5, were optimized using a two step assay utilizing the EnVision®+visualization system. The optimal antigen retrieval was determined to be heat induced epitope retrieval (HIER). The optimal antibody concentrations were established while employing an incubation time of 30 minutes for the DR4 assay and 60 minutes for the DR5 assay. The reactions were visualized with 3'3-diaminobenzidine (DAB+).

[0984] Sections from formalin-fixed, paraffin-embedded pellets from cell lines previously characterized by flow cytometry for surface expression of DR4 and DR5 demonstrated optimal IHC staining using both assays. Similarly, tumor-enriched xenograft models showed IHC staining with minimal background staining of the surrounding non-reactive mouse tissue. Optimal staining for both DR4 and DR5 was observed on a wide range of human tumor specimens, including colon, lung and breast carcinoma. Staining of the tumor cells was favorable with minimal background staining in the stroma.

[0985] Materials

[0986] Recombinant DR4 and DR5 Antigens:

[0987] DR4 and DR5 were expressed in NSO cells. The proteins were secreted in culture media. DR4 and DR5 were purified by a three-step procedure combining HQ (PerSeptive Biosystems) anion exchange chromatography at pH 8.5 using a salt gradient of 0-1 M NaCl) Hydrophobic Interaction Chromatography on a Phenyl (high sub) Sepharose column (Amersham Bioscience) and a Hydroxyapatite column for final purification and removing high salt. Purified protein is endotoxin free and 99% pure in N-terminal sequencing and HPLC-RPC.

[0988] Primary antibody/recombinant antigen preparations in optimal concentrations were mixed for 30 minutes at room temperature prior to application to the specimen.

[0989] Antibodies:

[0990] Affinity purified rabbit polyclonal antibodies specific for DR4 and DR5 were produced by HGS. Optimal antibody titers were established using control specimens and diluted in Antibody Diluent with background reducing components (DakoCytomation code S3022).

[0991] Target Retrieval:

[0992] Heat-induced epitope retrieval (HIER) was performed using target retrieval solution, TRS (DakoCytoma-

tion code S1700) in a pressure cooker (Farberware Programmable Pressure Cooker #FPC400) for 30 minutes at high pressure.

[0993] Detection Reagents:

[0994] Immunostaining was performed using the EnVision+visualization system (DakoCytomation code K4011). This detection system utilizes an HRP enzyme labelled polymer backbone conjugated to mouse secondary antibodies. The reactions were visualized with 3'3-diaminobenzidine (DAB+; DakoCytomation code K3468).

[0995] Cell Pellet Models:

[0996] Approximately one million cells were suspended in 100 L FACS buffer (PBS with 0.1% sodium azide and 0.1% BSA) and incubated with PE-conjugated antibodies directed against DR4 (eBiosciences #12-6644-73), DR5 (eBiosciences #12-9908-73) or isotype matched control (eBiosciences # 12-4719-71). Cells were incubated with antibodies for 10 to 20 minutes at room temperature, washed once in FACS buffer and pelleted by centrifugation. The cells were resuspended in 0.5 µg/ml propidium iodide for live/dead discrimination and analyzed on a FACScan using CellQuest software (BD Immunocytometry Systems).

[0997] Control cell lines used were as follows: MDA-MB-231 (breast carcinoma), Colo-205 (colon carcinoma), HT1080 (colon carcinoma), H460 (lung carcinoma), H2122 (lung carcinoma) and ST486 (lymphoma). These control cell lines were chosen based upon supportive flow cytometric, mRNA analytical, in vitro cytotoxic response to agonist DR4 and DR5 antibodies and/or protein chemical data to characterize expression of both DR4 and DR5. Four-micron sections of formalin-fixed, paraffin-embedded cell pellet blocks were mounted on silanized slides, deparaffinized with Histo-Clear (National Diagnostics) and rehydrated in graded alcohol.

[0998] Xenograft Models:

[0999] Colo205 Colon: Female Swiss athymic mice (7-8 weeks of age, 20 g average body weight) were used for xenograft models. On day 0, 1x10⁷ Colo205 cells (ATCC CCL-222) were implanted subcutaneously in the lower right flank of the mice. Once tumors had grown to approximately 100 mm³ (day 9) tumors were harvested.

[1000] MDA-MB-231 Breast: Female Swiss nude mice (7-10 weeks of age) were used for xenograft models. On day 0, 10⁶ MDA-MB-231 cells (HGS stock) were implanted subcutaneously SC lower right mammary area (inguinal area) of the mice. Once tumors had grown to a approximately 25 mm³ (day 6) tumors were harvested. Tumors were excised and placed in 10% neutral buffered formalin (NBF) for 24 hours. Tumors were then trimmed, processed, and embedded in paraffin using routine histologic techniques. Four-micron sections of formalin-fixed, paraffin-embedded xenograft tissue blocks were mounted on silanized slides, deparaffinized with Histo-Clear (National Diagnostics) and rehydrated in graded alcohol.

[1001] Human Tissue Specimens: Four-micron sections of formalin-fixed, paraffin-embedded normal and neoplastic human tissue were mounted on silanized slides. The tissue sections were deparaffinized in Histo-Clear (National Diagnostics) and rehydrated in graded alcohols. Some specimens used for the development of these assays used tissue samples

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provided by the Cooperative Human Tissue Network which is funded by the National Cancer Institute.

[1002] In conclusion, two highly specific IHC assays have been developed for the detection of DR4 and DR5 expression in formalin-fixed, paraffin-embedded human tissues. Xenograft and cell pellet controls were used to establish assay specificity. Optimal IHC staining for both DR4 and DR5 was observed on a wide variety of formalin-fixed, paraffin-embedded human tumor tissues, including lung, colon and breast carcinoma. DR5 tumor expression was stronger and more widespread than DR4. Adjacent normal tissues have significantly weaker expression. The observed staining pattern was generally heterogeneous and either membrane or cytoplasmic in appearance. These assays allow for sensitive and specific detection of DR4 and DR5 expression in human tissue samples.

[1003] It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[1004] The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in

the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.

[1005] Further, the Sequence Listing submitted herewith, in paper form, is hereby incorporated by reference in its entirety.

[1006] Additionally, the disclosures and teachings contained in the specifications and sequence listings of U.S. Provisional Application Ser. No. 60/608,429, filed Sep. 10, 2004, U.S. Provisional Application Ser. No. 60/551,811, filed Mar. 11, 2004, U.S. patent application Ser. No. 10/774,622 filed Feb. 10, 2004, U.S. patent application Ser. No. 10/648,825 filed Aug. 27, 2003, U.S. Provisional Application Ser. No. 60/413,747, filed on Sep. 27, 2002, U.S. Provisional Application Ser. No. 60/406,307, filed on Aug. 28, 2002, U.S. patent application Ser. No. 10/005,842 filed Dec. 7, 2001, U.S. patent application Ser. No. 09/874,138 filed Jun. 6, 2001, U.S. application Ser. No. 09/565,009, filed May 4, 2000, U.S. Provisional Application Ser. No. 60/148,939, filed Aug. 13, 1999, U.S. Provisional Application Ser. No. 60/133,238, filed May 7, 1999, U.S. Provisional Application Ser. No. 60/132,498, filed May 4, 1999, U.S. application Ser. No. 09/042,583, filed Mar. 17, 1998, U.S. Provisional Application Ser. No. 60/054,021, filed Jul. 29, 1997, and U.S. Provisional Application Ser. No. 60/040,846, filed Mar. 17, 1997, are each herein incorporated by reference in their entireties.

SEQUENCE LISTING

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<160> NUMBER OF SEQ ID NOS: 14

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cctaccgcc atg gaa caa cgg gga cag aac gcc ccg gcc gct tcg ggg gcc      171
    Met Glu Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala
    -50           -45           -40

cgg aaa agg cac ggc cca gga ccc agg gag ggc cggtt ggc agg cct      219
Arg Lys Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro
    -35           -30           -25

ggg ccc cgg gtc ccc aag acc ctt gtg ctc gtt gtc gcc gcg gtc ctg      267
Gly Pro Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu
    -20           -15           -10

ctg ttg gtc tca gct gag tct gct ctg atc acc caa caa gac cta gct      315
Leu Leu Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala
    -5            -1   1           5           10

ccc cag cag aga gcg cca caa aag agg tcc agc ccc tca gag      363

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Pro Gln Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu			
15	20	25	
gga ttg tgt cca cct gga cac cat atc tca gaa gac ggt aga gat tgc			411
Gly Leu Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys			
30	35	40	
atc tcc tgc aaa tat gga cag gac tat agc act cac tgg aat gac ctc			459
Ile Ser Cys Lys Tyr Gly Gln Asp Tyr Ser Thr His Trp Asn Asp Leu			
45	50	55	
ctt ttc tgc ttg cgc tgc acc agg tgt gat tca ggt gaa gtg gag cta			507
Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu			
60	65	70	75
agt ccc tgc acc acg acc aga aac aca gtg tgt cag tgc gaa gaa ggc			555
Ser Pro Cys Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly			
80	85	90	
acc ttc cgg gaa gaa gat tct cct gag atg tgc cgg aag tgc cgc aca			603
Thr Phe Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr			
95	100	105	
ggg tgt ccc aga ggg atg gtc aag gtc ggt gat tgt aca ccc tgg agt			651
Gly Cys Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser			
110	115	120	
gac atc gaa tgt gtc cac aaa gaa tca ggc atc atc ata gga gtc aca			699
Asp Ile Glu Cys Val His Lys Glu Ser Gly Ile Ile Gly Val Thr			
125	130	135	
gtt gca gcc gta gtc ttg att gtg gct gtt gtt tgc aag tct tta			747
Val Ala Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu			
140	145	150	155
ctg tgg aag aaa gtc ctt cct tac ctg aaa ggc atc tgc tca ggt ggt			795
Leu Trp Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly			
160	165	170	
ggt ggg gac cct gag cgt gtg gac aga agc tca caa cga cct ggg gct			843
Gly Gly Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala			
175	180	185	
gag gac aat gtc ctc aat gag atc gtg agt atc ttg cag ccc acc cag			891
Glu Asp Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln			
190	195	200	
gtc cct gag cag gaa atg gaa gtc cag gag cca gca gag cca aca aca ggt			939
Val Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly			
205	210	215	
gtc aac atg ttg tcc ccc ggg gag tca gag cat ctg ctg gaa ccg gca			987
Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala			
220	225	230	235
gaa gct gaa agg tct cag agg agg ctg ctg gtt cca gca aat gaa			1035
Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu			
240	245	250	
ggt gat ccc act gag act ctg aga cag tgc ttc gat gac ttt gca gac			1083
Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp			
255	260	265	
tgg gtg ccc ttt gac tcc tgg gag ccg ctc atg agg aag ttg ggc ctc			1131
Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu			
270	275	280	
atg gac aat gag ata aag gtg gct aaa gct gag gca gcg ggc cac agg			1179
Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg			
285	290	295	
gac acc ttg tac acg atg ctg ata aag tgg gtc aac aaa acc ggg cga			1227
Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg			
300	305	310	315
gat gcc tct gtc cac acc ctg ctg gat gcc ttg gag acg ctg gga gag			1275

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Asp Ala Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu			
320	325	330	
aga ctt gcc aag cag aag att gag gac cac ttg ttg agc tct gga aag	1323		
Arg Leu Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys			
335	340	345	
tcc atg tat cta gaa ggt aat gca gac tct gcc atg tcc taagtgtat	1372		
Phe Met Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser			
350	355	360	
tctcttcagg aagttagacc ttccctgggt tacctttt ctggaaaaag cccaaactgga	1432		
cgtccagtcag taggaaatgt ccacaattgt cacatgaccg gtactgaaag aaactctccc	1492		
atccaaacatc acccagtggta tggaacatcc tgtaactttt cactgcactt ggcattattt	1552		
ttataagctg aatgtgataa taaggacact atggaaaaaa aaaaaaaaa	1600		
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Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro			
-35	-30	-25	-20
Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu			
-15	-10	-5	
Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln			
-1	1	5	10
Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu			
15	20	25	
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser			
30	35	40	45
Cys Lys Tyr Gly Gln Asp Tyr Ser Thr His Trp Asn Asp Leu Leu Phe			
50	55	60	
Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro			
65	70	75	
Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe			
80	85	90	
Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys			
95	100	105	
Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile			
110	115	120	125
Glu Cys Val His Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala			
130	135	140	
Ala Val Val Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp			
145	150	155	
Lys Lys Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly			
160	165	170	
Asp Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp			
175	180	185	
Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro			
190	195	200	205
Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn			

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210	215	220
Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala		
225	230	235
Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp		
240	245	250
Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val		
255	260	265
Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp		
270	275	285
Asn Glu Ile Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr		
290	295	300
Leu Tyr Thr Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala		
305	310	315
Ser Val His Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu		
320	325	330
Ala Lys Gln Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met		
335	340	345
Tyr Leu Glu Gly Asn Ala Asp Ser Ala Met Ser		
350	355	360

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<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

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Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro			
20	25	30	
His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys			
35	40	45	
Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys			
50	55	60	
Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp			
65	70	75	80
Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu			
85	90	95	
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val			
100	105	110	
Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg			
115	120	125	
Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe			
130	135	140	
Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu			
145	150	155	160
Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu			
165	170	175	
Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr			
180	185	190	
Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser			
195	200	205	

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Gly Thr Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu
210 215 220

Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys
225 230 235 240

Ser Lys Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu
245 250 255

Gly Glu Leu Glu Gly Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
260 265 270

Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val
275 280 285

Pro Ser Ser Thr Phe Thr Ser Ser Thr Tyr Thr Pro Gly Asp Cys
290 295 300

Pro Asn Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly
305 310 315 320

Ala Asp Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn
325 330 335

Pro Leu Gln Lys Trp Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp
340 345 350

Thr Asp Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro
355 360 365

Leu Arg Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu
370 375 380

Ile Asp Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln
385 390 395 400

Tyr Ser Met Leu Ala Thr Trp Arg Arg Arg Thr Pro Arg Arg Glu Ala
405 410 415

Thr Leu Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly
420 425 430

Cys Leu Glu Asp Ile Glu Glu Ala Leu Cys Gly Pro Ala Ala Leu Pro
435 440 445

Pro Ala Pro Ser Leu Leu Arg
450 455

<210> SEQ ID NO 4

<211> LENGTH: 335

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser
20 25 30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Val Glu Thr Gln Asn
35 40 45

Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro
50 55 60

Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65 70 75 80

Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
85 90 95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
100 105 110

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Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
 115 120 125
 Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp
 130 135 140
 Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
 145 150 155 160
 Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
 165 170 175
 Leu Cys Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
 180 185 190
 Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
 195 200 205
 Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu
 210 215 220
 Ser Asp Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met
 225 230 235 240
 Thr Leu Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu
 245 250 255
 Ala Lys Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu
 260 265 270
 Gln Lys Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys
 275 280 285
 Glu Ala Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys
 290 295 300
 Thr Leu Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser
 305 310 315 320
 Asp Ser Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val
 325 330 335

<210> SEQ ID NO 5
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 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
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 Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg
 20 25 30
 Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys
 35 40 45
 Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
 50 55 60
 Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
 65 70 75 80
 Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
 85 90 95
 Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
 100 105 110
 Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
 115 120 125
 Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys

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130	135	140
Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr		
145	150	155
160		
Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys		
165	170	175
Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala		
180	185	190
Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala		
195	200	205
Gly Leu Val Val Pro Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr		
210	215	220
Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly		
225	230	235
240		
Met Glu Ala Leu Thr Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp		
245	250	255
Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys		
260	265	270
Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr		
275	280	285
Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro		
290	295	300
Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser		
305	310	315
320		
Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr		
325	330	335
Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg		
340	345	350
Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile		
355	360	365
Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln		
370	375	380
Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met		
385	390	395
400		
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acttggcatt attttgtna agctgaatgt gataataagg gcactgatgg aaatgtctgg	240
atcattccgg ttgtgcgtac tttgagattt gngttgggg atgtncattg tgtttgacag	300
cactttttttn atccctaattg tnaaatgcnt nattgattt tgantgggg gttaacattg	360
gttaaaggntn cccntntgac acagtagntg gtncccgact tanaatngnn gaanangatg	420
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<212> TYPE: DNA

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cggaatgatc cagacatttc cataggtcct tattatcaca ttcagcttat aaaataatgc	180
caagtgcagt gaaaagttac aggatgttcc atccactggg tggatt	226

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What is claimed is:

1. An isolated polypeptide selected from the group consisting of:
 - (a) the polypeptide of SEQ ID NO:2; and
 - (b) a fragment of the polypeptide of (a).
2. The polypeptide of claim 1, wherein said fragment binds TRAIL.
3. The polypeptide of claim 1, wherein said fragment inhibits apoptosis.
4. The polypeptide of claim 1, wherein said fragment induces apoptosis.
5. The polypeptide of claim 1, wherein said polypeptide comprises amino acids n¹ to 360 of SEQ ID NO:2, wherein n¹ represents an integer from -50 to 355.
6. The polypeptide of claim 2, wherein said polypeptide comprises amino acids n¹ to 360 of SEQ ID NO:2, wherein n¹ represents an integer from -50 to 355.
7. The polypeptide of claim 3, wherein said polypeptide comprises amino acids n¹ to 360 of SEQ ID NO:2, wherein n¹ represents an integer from -50 to 355.
8. The polypeptide of claim 4, wherein said polypeptide comprises amino acids n¹ to 360 of SEQ ID NO:2, wherein n¹ represents an integer from -50 to 355.
9. The polypeptide of claim 1, wherein said fragment is soluble.
10. The polypeptide of claim 2, wherein said fragment is soluble.
11. The polypeptide of claim 3, wherein said fragment is soluble.
12. An isolated polypeptide comprising an amino acid sequence at least 80% identical to the amino acid sequence of SEQ ID NO:2.
13. The polypeptide of claim 12, wherein said polypeptide binds TRAIL.
14. The polypeptide of claim 12, wherein said polypeptide inhibits apoptosis.
15. The polypeptide of claim 12, wherein said polypeptide induces apoptosis.
16. The polypeptide of claim 12, wherein said polypeptide comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:2.
17. The polypeptide of claim 16, wherein said polypeptide comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:2.
18. The polypeptide of claim 13, wherein said polypeptide comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:2.
19. The polypeptide of claim 18, wherein said polypeptide comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:2.
20. The polypeptide of claim 14, wherein said polypeptide comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:2.
21. The polypeptide of claim 20, wherein said polypeptide comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:2.
22. The polypeptide of claim 15, wherein said polypeptide comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO:2.
23. The polypeptide of claim 22, wherein said polypeptide comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:2.
24. A dimer, trimer, or tetramer of the polypeptide of claim 12.
25. A dimer, trimer, or tetramer of the polypeptide of claim 13.
26. A dimer, trimer, or tetramer of the polypeptide of claim 14.
27. A dimer, trimer, or tetramer of the polypeptide of claim 15.
28. A composition comprising the polypeptide of claim 1, and a pharmaceutically acceptable carrier.
29. A composition comprising the polypeptide of claim 2, and a pharmaceutically acceptable carrier.
30. A composition comprising the polypeptide of claim 3, and a pharmaceutically acceptable carrier.
31. A composition comprising the polypeptide of claim 4, and a pharmaceutically acceptable carrier.
32. A composition comprising the polypeptide of claim 9, and a pharmaceutically acceptable carrier.
33. A composition comprising the polypeptide of claim 10, and a pharmaceutically acceptable carrier.
34. A composition comprising the polypeptide of claim 11, and a pharmaceutically acceptable carrier.
35. An isolated polypeptide encoded by a DNA comprising the nucleotide sequence of SEQ ID NO:1.
36. An isolated polypeptide comprising amino acids n² to 133 of SEQ ID NO:2, wherein n² represents an integer from -51 to 128, and wherein said polypeptide binds TRAIL.
37. The polypeptide of claim 36, wherein said polypeptide comprises amino acids 1 to 133 of SEQ ID NO:2.
38. The polypeptide of claim 36, wherein said polypeptide is soluble.
39. The polypeptide of claim 1, wherein said fragment comprises the cysteine-rich domains of SEQ ID NO:2.

- 40.** The polypeptide of claim 39, wherein said fragment comprises amino acids 33 to 128 of SEQ ID NO:2.
- 41.** The polypeptide of claim 39, wherein said polypeptide binds TRAIL.
- 42.** The polypeptide of claim 39, wherein said polypeptide inhibits apoptosis.
- 43.** The polypeptide of claim 39, wherein said polypeptide induces apoptosis.
- 44.** An isolated soluble polypeptide comprising an amino acid sequence at least 80% identical to the sequence of amino acids 1 to 133 of SEQ ID NO:2.
- 45.** The polypeptide of claim 44, wherein said polypeptide binds TRAIL.
- 46.** The polypeptide of claim 44, wherein said polypeptide inhibits apoptosis.
- 47.** The polypeptide of claim 44, wherein the polypeptide comprises an amino acid sequence at least 90% identical to the sequence of amino acids 1 to 133 of SEQ ID NO:2.
- 48.** The polypeptide of claim 45, wherein the polypeptide comprises an amino acid sequence at least 90% identical to the sequence of amino acids 1 to 133 of SEQ ID NO:2.
- 49.** The polypeptide of claim 46, wherein the polypeptide comprises an amino acid sequence at least 90% identical to the sequence of amino acids 1 to 133 of SEQ ID NO:2.
- 50.** A multimer comprising at least two polypeptides of claim 36.
- 51.** A multimer comprising at least two polypeptides of claim 37.
- 52.** A multimer comprising at least two polypeptides of claim 38.
- 53.** A multimer comprising at least two polypeptides of claim 39.
- 54.** A multimer comprising at least two polypeptides of claim 40.
- 55.** A multimer comprising at least two polypeptides of claim 41.
- 56.** A multimer comprising at least two polypeptides of claim 42.
- 57.** A multimer comprising at least two polypeptides of claim 43.
- 58.** A multimer comprising at least two polypeptides of claim 44.
- 59.** A multimer comprising at least two polypeptides of claim 45.
- 60.** A multimer comprising at least two polypeptides of claim 46.
- 61.** A multimer comprising at least two polypeptides of claim 47.
- 62.** A multimer comprising at least two polypeptides of claim 48.
- 63.** A multimer comprising at least two polypeptides of claim 49.
- 64.** The multimer of claim 50, wherein the multimer is a dimer or trimer.
- 65.** The multimer of claim 51, wherein the multimer is a dimer or trimer.
- 66.** The multimer of claim 52, wherein the multimer is a dimer or trimer.
- 67.** The multimer of claim 55, wherein the multimer is a dimer or trimer.
- 68.** The multimer of claim 56, wherein the multimer is a dimer or trimer.
- 69.** The multimer of claim 57, wherein the multimer is a dimer or trimer.
- 70.** The multimer of claim 64, wherein the multimer is a dimer.
- 71.** A composition comprising the polypeptide of claim 36, and a pharmaceutically acceptable carrier.
- 72.** A composition comprising the polypeptide of claim 41, and a pharmaceutically acceptable carrier.
- 73.** A composition comprising the polypeptide of claim 42, and a pharmaceutically acceptable carrier.
- 74.** A composition comprising the polypeptide of claim 43, and a pharmaceutically acceptable carrier.
- 75.** A composition comprising the dimer, trimer, or tetramer of claim 24, and a pharmaceutically acceptable carrier.
- 76.** A composition comprising the dimer, trimer, or tetramer of claim 25, and a pharmaceutically acceptable carrier.
- 77.** A composition comprising the dimer, trimer, or tetramer of claim 26, and a pharmaceutically acceptable carrier.
- 78.** A composition comprising the dimer, trimer, or tetramer of claim 27, and a pharmaceutically acceptable carrier.
- 79.** A composition comprising the multimer of claim 50, and a pharmaceutically acceptable carrier.
- 80.** A composition comprising the multimer of claim 55, and a pharmaceutically acceptable carrier.
- 81.** A composition comprising the multimer of claim 56, and a pharmaceutically acceptable carrier.
- 82.** A composition comprising the multimer of claim 57, and a pharmaceutically acceptable carrier.
- 83.** A composition comprising the multimer of claim 64, and a pharmaceutically acceptable carrier.
- 84.** An isolated polynucleotide encoding the polypeptide of claim 1.
- 85.** An isolated antibody that specifically binds the polypeptide of claim 1.

* * * * *

Tanshinone IIA Facilitates TRAIL Sensitization by Up-regulating DR5 through the ROS-JNK-CHOP Signaling Axis in Human Ovarian Carcinoma Cell Lines

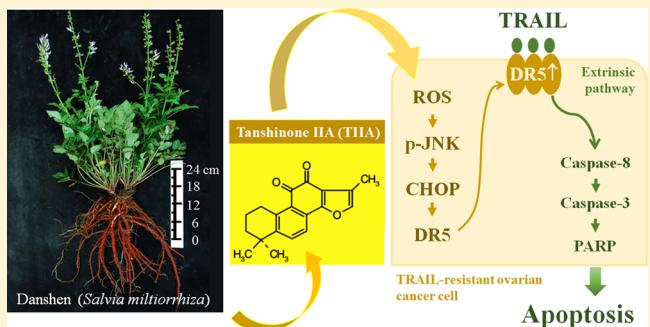
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[‡]Institute of Biomedical Sciences, [§]Agricultural Biotechnology Center, ^{||}Ph.D. Program in Translational Medicine, and [⊥]Rong Hsing Research Center for Translational Medicine, National Chung Hsing University, Taichung, Taiwan

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ABSTRACT: Tanshinone IIA (TIIA) extracted from *Salvia miltiorrhiza* has been shown to possess antitumor and TRAIL-sensitizing activity. The involvement of DR5 in the mechanism whereby TIIA exerts its effects is unknown. This study aimed to explore the mechanism underlying TIIA augmentation of TRAIL-induced cell death in ovarian carcinoma cells. Cell viability was determined by MTS assay. Real-time RT-PCR and Western blotting were used to assess the mRNA and protein expression of relating signaling proteins. Transcriptional activation was explored by a dual-luciferase reporter assay. We found that TIIA sensitized human ovarian carcinoma cells to TRAIL-induced extrinsic apoptosis. Combined treatment with subtoxic concentrations of TIIA and TRAIL was more effective than single treatments with respect to cytotoxicity, clonogenic inhibition, and the induction of caspase-8 and PARP activity in ovarian carcinoma cell lines TOV-21G and SKOV3. TIIA induced DR5 protein and mRNA expression in a concentration-dependent manner. DR5/Fc treatment markedly suppressed the TRAIL cytotoxicity enhanced by TIIA. These results indicate that DR5 plays an essential role in TIIA-induced TRAIL sensitization and that induction of DR5 by TIIA is mediated through the up-regulation of CCAAT/enhancer-binding protein homologous protein (CHOP). Knockdown of CHOP gene expression by shRNA attenuated DR5 up-regulation and rescued cell viability under the treatment of TIIA-TRAIL combination. TIIA promoted JNK-mediated signaling to up-regulated CHOP and thereby inducing DR5 expression as shown by the ability of a JNK inhibitor to potently suppress the TIIA-mediated activation of CHOP and DR5. In addition, the quenching of ROS using NAC prevented the induction of JNK phosphorylation and CHOP induction. Furthermore, inhibition of ROS by NAC significantly attenuated TRAIL sensitization by TIIA. Taken together, these data suggest that TIIA enhances TRAIL-induced apoptosis by upregulating DR5 receptors through the ROS-JNK-CHOP signaling axis in human ovarian carcinoma cells.



INTRODUCTION

Epithelial ovarian carcinoma (EOC) is the leading cause of death among gynecological malignancies in women worldwide. Most patients are diagnosed at an advanced stage and have poor prognoses. Currently, the standard treatment for advanced ovarian cancer is surgical tumor resection followed by chemotherapy.¹ However, most patients relapse with tumors that acquire resistance to chemotherapy. Therefore, novel therapeutic agents and strategies are needed for treating this devastating disease. We identified a novel antiovary cancer agent, tanshinone IIA (TIIA), a natural product derived from the roots of *Salvia miltiorrhiza*.² We revealed that TIIA induces proapoptotic and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-sensitizing effects, implicating this active compound as a potential therapeutic agent or TRAIL-based therapy for cancer chemoprevention or chemotherapy.

TIIA is reported to possess anti-inflammatory, antioxidative, and antitumor activities.^{3–8} The ability of TIIA to induce apoptosis in cancer cells has been established, and several molecular targets have been identified, including activation of JNK,⁹ p38 MAPK¹⁰ and p53^{11,12} and induction of reactive oxygen species (ROS),¹³ as well as inhibition of PI₃K/AKT,¹⁴ STAT3,¹⁵ CHOP,¹⁶ HIF-1 α , and VEGF.⁷ Whereas apoptosis is generally initiated by either the intrinsic or extrinsic stimuli, the intrinsic pathway is triggered by cellular damage and leads to the cleavage and consequent activation of caspase-9, which in turn sets off the caspase cascade that stimulates apoptosis. The extrinsic pathway is triggered by the binding of tumor necrosis factor- α (TNF- α), TNF-related apoptosis-inducing ligand

Received: April 15, 2015

Published: July 23, 2015

(TRAIL), or Fas ligands to their receptors and leads to the cleavage and activation of caspase-8, which is recruited to the “death-inducing signal complex” (DISC) to initiate apoptosis. Cellular FLICE-inhibitory protein (c-FLIP) is structurally homologous to caspase-8; therefore, it competes with caspase-8 for binding to the DISC complex but is devoid of caspase activity, thus precluding caspase-8 activation in a dominant-negative manner. However, the mechanism whereby TIIA activates the extrinsic pathway has not been fully elucidated.

TRAIL is a potential candidate for anticancer therapy based on its selective suppression of tumor growth *in vivo* and *in vitro* with little or no effect on normal cells.^{17,18} TRAIL belongs to the TNF family which includes cytokines such as TNF- α and Fas ligands. The soluble TRAIL is a homotrimer which initiates extrinsic apoptosis through binding to death receptors (DRs) 4 and 5 expressed on the cell surface in a variety of tumor cells.^{19,20} Although TRAIL effectively induces the apoptosis of cancer cells, repeated application leads to resistance through multiple mechanisms.^{21,22} Suggested mechanisms include dysregulation of death receptors and the defective production of DISC.^{23–25} Thus, agents that can up-regulate death receptors have the potential to sensitize the apoptotic effects of TRAIL.^{26,27}

In this study, we explored the role of DR5 in TRAIL sensitization by TIIA in EOC cells. Recently, numerous signaling molecules have become known to trigger DR5 induction, including the activation of mitogen-activated protein kinases (MAPKs) and the binding of CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) transcription factor to the DR5 promoter.²⁸ The signaling event involved in survival, growth arrest, or programmed cell death includes the activation of the MAPK pathway. The MAPK pathways consist of JNK, p38 MAPK, and ERK. The JNK/p38-MAPK pathway plays a central role in apoptosis,²⁹ especially chemical-induced apoptosis, whereas ERK is involved in cell survival, proliferation, differentiation, and migration. In this study, we present evidence supporting the fact that TIIA up-regulates DR5 expression through activating the ROS-JNK-CHOP signaling axis, leading to sensitization of EOC cells to TRAIL-induced apoptosis.

MATERIALS AND METHODS

Reagents. Tanshinone IIA (TIIA, Lot number 00020043-010, purity >96.1%) was purchased from ChromaDex Inc. (Irvine, CA, USA). Purified TIIA was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and was stored in the dark at –20 °C until use. The final concentration of DMSO used in all experiments was 0.1% (v/v). Recombinant human TRAIL (Gibco; Grand Island, NY, USA) was prepared as a 100 µg/mL stock solution and stored in aliquots at –20 °C before use. 2',7'-Dichlorodihydrofluorescein diacetate (H₂DCFDA) was purchased from Sigma. Pyromycin-2HCl, NAC (N-acetylcysteine), SB203580 (p38 MAPK-specific inhibitor), and SP600125 (JNK-specific inhibitor) were purchased from TOCRIS (Ellisville, Missouri, USA). Thapsigargin (Calbiochem; Darmstadt, Germany) was prepared as a 1 mM stock solution in DMSO. Human recombinant DR5/Fc chimeric protein was purchased from R&D Systems (Minneapolis, MN, USA).

Cell Culture. Three human ovarian cancer cell lines CaOV3 (ATCC HTB-75), TOV-21G (BCRC 60407), and SKOV3 (ATCC HTB-77) were used in this study. CaOV3 cells were grown in DMEM medium with 10% heat-inactivated fetal bovine serum (FBS) (Gibco; Grand Island, NY, USA). TOV-21G cells were grown in a 1:1 mixture of MCDB 105 and medium 199 with 15% FBS. SKOV3 cells were grown in McCoy's 5α medium with 10% FBS supplemented with 100

U/mL penicillin and 100 mg/mL streptomycin. The media and supplements were purchased from Invitrogen (Carlsbad, CA, USA). All cell lines were cultured at 37 °C in a 5% CO₂ atmosphere.

Cell Viability Assays. The effect of TRAIL or TIIA on cell viability was determined by the MTS assay as previously described.³⁰ In brief, cultures were established in 96-well flat bottomed microtiter plates in growth medium containing 10% FBS. Cell suspensions (100 µL; 5–15 × 10³ cells) were added to each well and allowed to attach overnight. The medium was then changed, and the cells were maintained in media alone or in the presence of the indicated amount of drug in a final volume of 150 µL of 2%-FBS culture medium. After 48 h of incubation, the medium in each well was replaced with 100 µL of fresh phenol-red-free culture medium, followed by the addition of 20 µL of One Solution Reagent MTS (Promega; Madison, WI, USA). The plate was then incubated for 2 h at 37 °C in a humidified, 5% CO₂ environment before measuring the absorbance (A) at 490 nm using a Sunrise absorbance reader (Tecan; Grodig, Austria). Cell viability was calculated as $(A_{\text{sample}} - A_{\text{blank}})/(A_{\text{control}} - A_{\text{blank}}) \times 100\%$, while cytotoxicity = 100% – cell viability (%). All experiments were repeated at least three times with triplicate samples in each experiment.

Determination of Combination Index Value (CI). The effect of drug combination was evaluated with Calcsyn software v2.1 (Biosoft) using the Chou-Talalay method.³¹ In this model, CI values <1.0 indicate synergy of drug combination, whereas CI values >1.0 indicate an antagonistic effect. Synergistic effects can be further graded based on the calculated CI value: < 0.1, very strong; 0.1–0.3, strong synergism; 0.3–0.7, synergism; 0.7–0.85, moderate synergism; 0.85–0.9, slight synergism; and 0.9–1.1, additive effect.

Clonogenic Assay. The effect of TRAIL or TIIA on colony formation was determined by a clonogenic assay as previously described.³⁰ In brief, cells were plated onto 60 mm dishes at a density of 5 × 10³ cells/dish, incubated overnight, and treated with the indicated doses of drugs for 24 h. At the end of drug treatments, cells were washed twice with phosphate-buffered saline (PBS) and then trypsinized to determine cell numbers. Drug-treated cells were then seeded at a density of 200 cells per 60 mm-dish in triplicate for each treatment. TOV-21G cells were then allowed to form colonies by incubation in drug-free medium for 10 days. To count the numbers of colonies, the cell monolayer was rinsed twice with PBS, followed by staining with 1% crystal violet solution in 30% ethanol. Colonies comprising 50 or more cells were counted under a microscope. The plating efficiency was calculated as the ratio of the number of colonies counted to the number of cells seeded. A plating efficiency of 50–60% was routinely achieved. The same procedure was repeated at least 3 times.

Western Blot Analysis. Immunoblotting was performed as previously described.³² Briefly, whole cell lysates were boiled in sample buffer (100 mM Tris-HCl, 4% SDS, 0.2% bromophenol blue, 20% glycerol, and 10% dithiothreitol). Equal amounts of protein (20–50 µg) were separated by SDS-PAGE using 10–15% polyacrylamide gel. After electrophoresis, the proteins were transferred onto a FluoroTrans W membrane (Pall Life Sciences, USA), blocked with 5% skim milk, and then probed with antibodies. Antibodies against caspase-8 (NEB 9746), DR5 (NEB 3696), cleaved PARP (NEB 9546), JNK (NEB 9258), p-JNK (NEB 9251), p38 (NEB 9212), and p-p38 (NEB 9211) were purchased from Cell Signal (Beverly, MA, USA). The antibody against CHOP/GADD153 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The β-tubulin antibody was purchased from Sigma. The signals were detected by using enhanced superSignal West Pico chemiluminescence (Pierce/Life Technologies; South San Francisco, CA, USA).

Analysis of Cell Surface Expression of DR5. To analyze the cell surface expression of DR5, cells were treated with 0, 1, 3, 10 µM TIIA or 100 ng/mL TRAIL for 24 h, stained with PE mouse IgG1, κ isotype control, or PE mouse antihuman DR5 antibody (BD Pharmingen) for 30 min at 4 °C according to the manufacturer's instructions, resuspended in phosphate-buffered saline, and finally analyzed by flow cytometry.

Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction. The levels of *DR5* and *CHOP* mRNA were

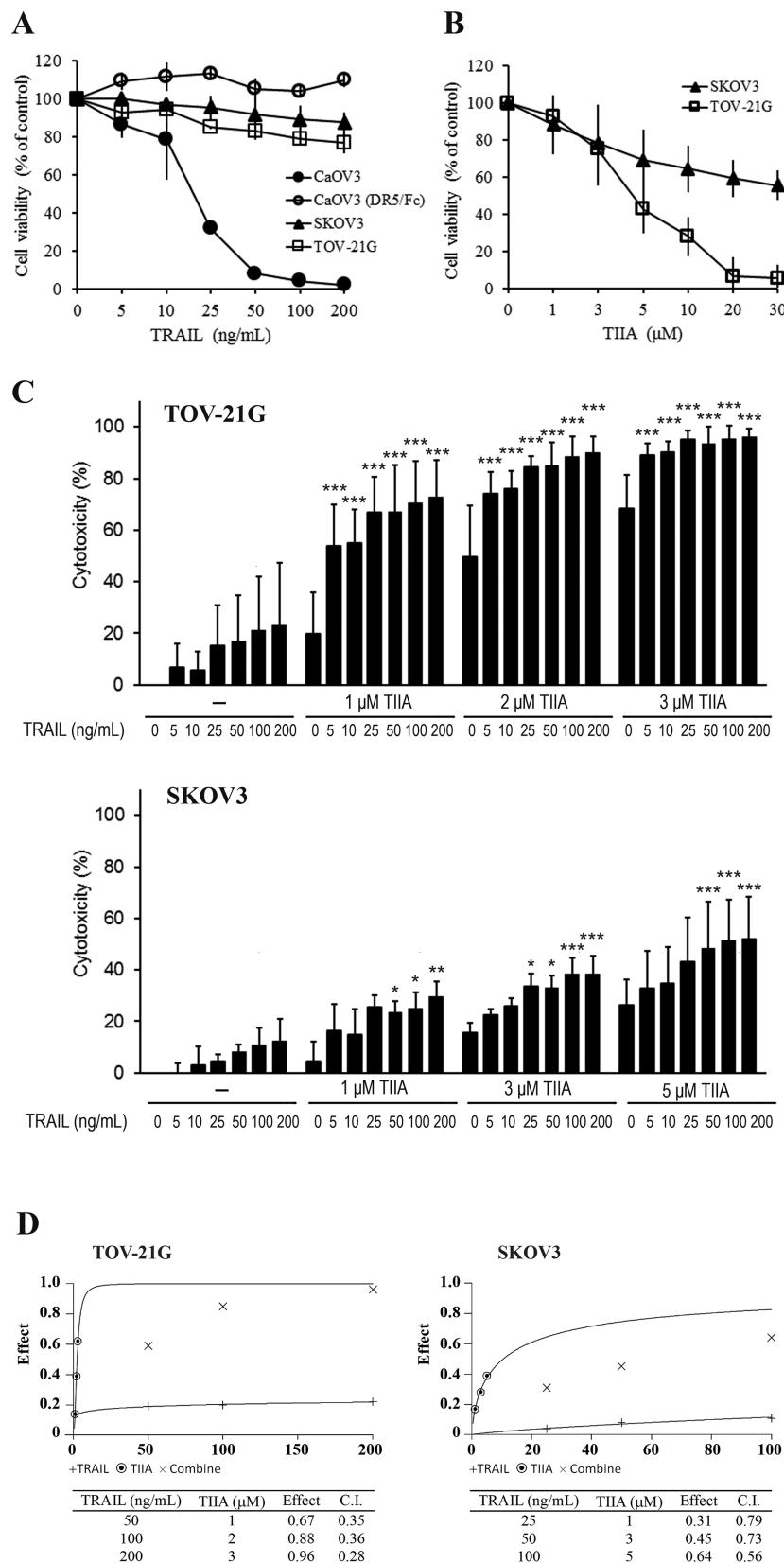


Figure 1. continued

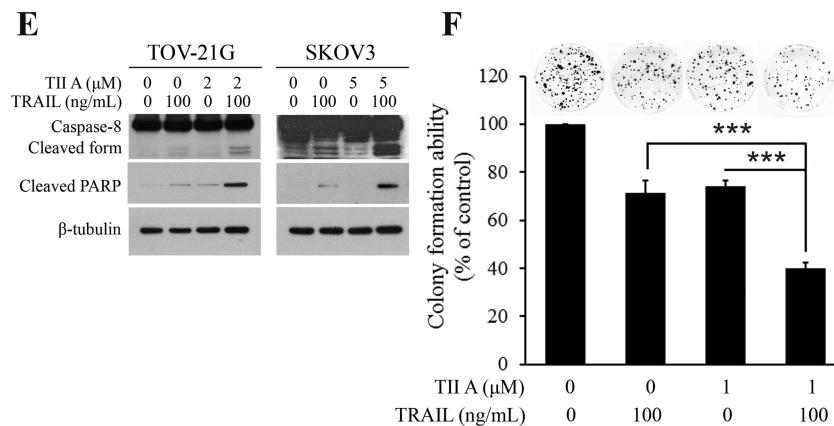


Figure 1. TIIA enhances the TRAIL-induced extrinsic apoptotic pathway in human EOC cell lines. (A) Effect of TRAIL on the viability in EOC cells. Cell viability was determined by MTS assay. Cells were exposed to TRAIL (0–200 ng/mL) for 48 h. CaOV3 cells were preincubated with or without the DRS/Fc chimeric protein blocking DRS receptor (1 μg/mL) for 1 h and stimulated for 48 h by TRAIL. (B) Effect of TIIA on the viability in SKOV3 and TOV-21G cells. Cells were treated without (DMSO control) or with increasing doses of TIIA (1 μM–30 μM) for 48 h, followed by the determination of cell viability using the MTS assay. (C) Cells were treated for 1 h with TIIA (0, 1, 2, or 3 μM for TOV-21G; 0, 1, 3, or 5 μM for SKOV3), followed by the indicated doses of TRAIL for a total of 48 h, and cell viability was determined. The values are expressed as the means ± SEM; **p* < 0.05, ***p* < 0.01, and ****p* < 0.005, significant difference compared to the TIIA only treatment. (D) The dose–effect curves were generated by CalcuSyn software to fit the experimental points. × symbol designates the combination effect. Cells were exposed to the combination in a nonconstant ratio. CI analysis to determine synergy was carried out using CalcuSyn software as described in Materials and Methods. (E) Western blotting of extracts from TOV-21G and SKOV3 cells treated with vehicle control, TRAIL (100 ng/mL) alone, TIIA (2 μM for TOV-21G; 5 μM for SKOV3) alone, or a combination of both drugs were assayed for caspase-8, cleaved PARP, and β-tubulin. (F) Clonogenic assay was performed to determine the survival effect of TIIA–TRAIL cotreatment in TOV-21G cells. ****p* < 0.005.

determined by quantitative real-time reverse transcription–polymerase chain reaction (qRT-PCR). Total RNA extracted from mock- or drug-treated cells was isolated using the guanidinium thiocyanate method (trizol; Invitrogen) following the manufacturer's protocol. Total RNA (10 μg) was reverse-transcribed into the first strand of cDNA using ImProm-II Reverse Transcription reagents (Promega; Madison, WI, USA) using random hexamers as the primer. PCR reactions were set up in a 20 μL reaction volume using SYBR Green PCR Master Mix (Applied Biosystems, USA) with the following primer pairs: DRS forward, 5'-AgTCAgAgCATCTgCTggAAC-3'; DRS reverse, 5'-AgCACTgTCTCAgAgTCTCAg-3'; CHOP forward, 5'-ACAgAgCCAAATCAgAgCTg-3'; and CHOP reverse, 5'-AAgCACATCTgCTTTCAggTg-3'. Gene expression levels were normalized to that of TATA-binding protein (TBP). Final results were expressed as the ratio of copy numbers of CHOP mRNA to TBP mRNA and are presented as the mean ± SEM of 3 independent experiments.

Dual-Luciferase Reporter Assays. TOV-21G cells were transiently transfected with the CHOP promoter luciferase reporter plasmid (pCHOP-Luc) in combination with plasmid-expressing β-galactosidase using the jet PEI transfection reagent (Polyplus, New York, NY, USA) and allowed to grow for an additional 24 h. Subsequently, cells were treated with various doses of TIIA (0, 10, and 20 μM) for 24 h, and the drug-treated cell lysates were prepared and subjected to the dual luciferase assay according to the manufacturer's protocol. The internal control plasmid pGL4.18 was included in all transfections. To normalize transfection efficiency, we used the Tropix dual-light reporter gene assay system according to the manufacturer's instructions (Applied Biosystems/Life Technologies, South San Francisco, CA, USA). Luciferase activity was normalized to β-galactosidase activity, and final data were presented as the fold-change in luciferase activity between treated and untreated cells.

Evaluation of Intracellular ROS Levels. The levels of ROS in TOV-21G cells were examined and determined by flow cytometry (BD FACSCalibur). 2',7'-Dichlorofluorescein diacetate (H₂DCFDA) is a cell-permeable nonfluorescent probe. 2',7'-Dichlorofluorescein diacetate is de-esterified intracellularly and turns to highly fluorescent 2',7'-dichlorofluorescein upon oxidation.³³ Cells were treated with or without TIIA for 0.5 h to determine the influence of ROS. Cells were harvested and resuspended in 500 μL of H₂DCFDA solution (10 μM),

incubated at 37 °C for 30 min, washed twice, and analyzed by flow cytometry.

shRNA Interference for the Knockdown of the CHOP on TOV-21G Cell Line. For retroviral infection, 293T cells were plated on 10 cm dishes at a density of 1×10^6 cells/dish. After 24 h, the cells were transfected with the retroviral constructs pMKO.1-puro or pMKO.1-puro-CHOP-shRNA,³⁴ and viral particles were harvested at 24 and 48 h post-transfection. TOV-21G cells ($0.5\text{--}1 \times 10^5$ /well) were seeded in 6 cm-well plates to yield 50–60% confluence. After 24 h, cells were transfected using jetPEI transfection reagent (Polyplus) with 2 μg/mL puromycin and either the empty pMKO.1 vector or the CHOP expression plasmid (pMKO.1-shCHOP). At 48 h after transfection, cells from each well were passed to 96-well plates and selected in medium supplemented with puromycin (2 μg/mL) for 14 days. Downregulated CHOP expression in these transient transfectants was verified by immunoblotting.

Statistical Analysis. All assays were repeated in at least three independent experiments, and the results are expressed as the mean ± standard error of mean (SEM). Differences between groups were examined for statistical significance using the unpaired two-tailed Student's *t*-test. Statistical significance was regarded at *p* < 0.05.

RESULTS

TIIA Sensitizes Human Ovarian Cancer Cells to TRAIL-Induced Extrinsic Apoptosis. To explore the effect of TIIA on TRAIL-induced cell death, TRAIL sensitivity was first examined in an array of human ovarian carcinoma cell lines. To this end, the viability of CaOV3, SKOV3, and TOV-21G cells after 48 h-treatment with TRAIL (0–200 ng/mL) was determined. We found that CaOV3 cells were highly sensitive to TRAIL-induced cytotoxicity, with an IC₅₀ of 19.22 ng/mL (Figure 1A). In contrast, both SKOV3 and TOV-21G cells were resistant to the cytotoxic effects of TRAIL. Notably, functional blockade of TRAIL binding to DRS using the recombinant human DRS/Fc chimeric protein was found to completely rescue the viability of TRAIL-treated CaOV3 cells (Figure 1A). This result illustrates that DRS-mediated apoptotic signaling is required for TRAIL-induced killing of

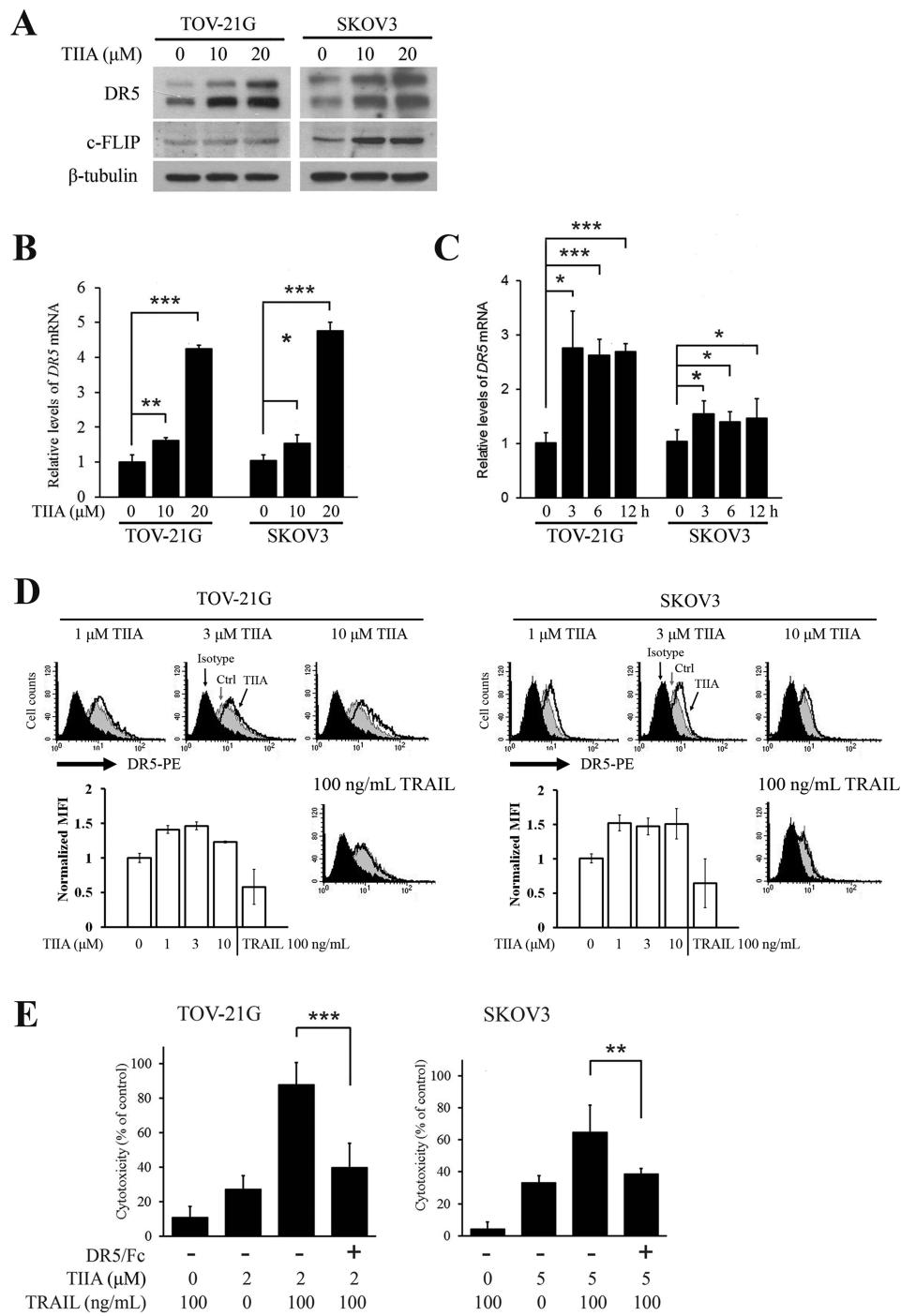


Figure 2. TIIA increases the expression of DR5 protein that regulates the sensitivity to TRAIL. (A) TOV-21G and SKOV3 cells were treated with 0, 10, or 20 μM TIIA for 24 h, and cell extracts were prepared for Western blotting of DR5 and c-FLIP. (B) The expression of *DR5* mRNA was determined by real-time RT-PCR. TOV-21G and SKOV3 cells were treated with 0, 10, or 20 μM TIIA for 12 h (TOV-21G) or 3 h (SKOV3). (C) Cells were treated with 10 μM TIIA for 0, 3, 6, or 12 h; *DR5* mRNA expression was then measured. (D) Effect of TIIA or TRAIL on the surface expression of DR5 in TOV-21G and SKOV3 cells with flow cytometry. Black histograms, isotype control; gray histograms, solvent control; black line, treated cells with TIIA or TRAIL. (E) After treatment with TIIA for 2 h (2 μM for TOV-21G; 5 μM for SKOV3), cells were preincubated without or with 1 $\mu\text{g}/\text{mL}$ DR5/Fc for 2 h. Cells were then treated with 100 ng/mL TRAIL for 24 h and the cytotoxicity determined by MTS assay. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

CaOV3 cells. Given their resistance to TRAIL-induced cytotoxicity, SKOV3 and TOV-21G cells were used to investigate TIIA-induced TRAIL sensitization. SKOV3 and TOV-21G cells were also treated for 48 h with increased dosage (0–30 μM) of TIIA for follow-up evaluation of cell viability (Figure 1B). We found that TIIA suppressed the viability in a

concentration-dependent manner, with TOV-21G being more sensitive to TIIA treatment (IC_{50} : $4.9 \pm 0.6 \mu\text{M}$) than SKOV3 ($\text{IC}_{50} > 30 \mu\text{M}$). Importantly, subtoxic doses of TIIA (1–3 μM) effectively sensitized TOV-21G cells to TRAIL-induced killing (Figure 1C). Likewise, TIIA increased TRAIL sensitivity of SKOV3 cells, though requiring higher drug doses. As shown

in Figure 1C, 5 ng/mL of TRAIL started to significantly reduce viability in TOV-21G cells treated with TIIA alone at doses of 1 μ M, 2 μ M, and 3 μ M. In SKOV3 cells, 50 ng/mL of TRAIL began to induce significant viability reduction when cells were treated with TIIA alone at doses of 1 μ M, 2 μ M, and 3 μ M. Furthermore, isobologram analyses were performed to characterize the effect of TIIA-TRAIL combination. It is clear to note that treatment with indicated doses of TRAIL in combination with various concentrations of TIIA all yield combination indexes lower than 1.0, thus confirming that TIIA synergistically enhances TRAIL-induced cell death (Figure 1D). Particularly, a marked synergism was observed in TIIA (2 μ M)-TRAIL (100 ng/mL) in TOV-21G cells and in TIIA (5 μ M)-TRAIL (100 ng/mL) in SKOV3 cells, which yield combination index (CI) values of 0.36 and 0.56, respectively. At the biochemical level, cotreatment with TIIA markedly enhanced the levels of cleavage and thus the activation of caspase-8 and PARP elicited by TRAIL alone in both cell lines tested (Figure 1E), confirming the capability of TIIA to up-regulate TRAIL-induced extrinsic death receptor apoptosis. Lastly, the effect of TIIA on the clonogenic assay of TRAIL-treated TOV-21G cells was examined. As shown in Figure 1F, treatment with either drug alone led to a mild decrease in colony formation ($71.3 \pm 5.1\%$ and $74.2 \pm 2.4\%$ of solvent control after treatment with TRAIL or TIIA alone, respectively). By contrast, TRAIL combined with TIIA showed a marked decrease in the number of colony formation ($40.1 \pm 2.3\%$ of untreated cells; $p < 0.005$).

TIIA Up-regulates DR5 to Facilitate TRAIL Sensitization. Accumulating evidence has highlighted the increased expression level of DR5 as one of the critical mechanisms responsible for overcoming TRAIL resistance.^{22,23,35} Thus, we asked whether TIIA induces TRAIL sensitization by up-regulating DR5 expression. Our results show that TIIA concentration-dependently increases the levels of DR5 protein in TOV-21G and SKOV3 cells (Figure 2A). It is known that the death receptor-induced pathway leads to the recruitment of caspase-8 or -10 to the DISC for activation. DISC signaling can be inhibited by the expression of c-FLIP, a physiologically dominant-negative mutant of caspase-8 that leads to the formation of a signaling inactive DISC.³⁶ Intriguingly, the level of c-FLIP remained relatively constant in TOV-21G cells and was even elevated in SKOV3 cells on TIIA stimulation (Figure 2A). Additionally, we found that TIIA (0, 10, and 20 μ M) also elicited a concentration-dependent increase in DR5 mRNA levels (Figure 2B), with the increase in DR5 mRNA expression reaching its maximal level as early as 3 h after TIIA treatment (Figure 2C). We further performed flow cytometry analysis to validate that the increase in DR5 levels induced by TIIA corresponds to the increased DR5 expression at the cell surface. As shown in Figure 2D, TIIA significantly elevated DR5 levels at the cell surface as low as 1 μ M, whereas stimulation of TRAIL (100 ng/mL) failed to induce cell-surface DR5 expression. Lastly, we elucidated the role of DR5 up-regulation in TIIA-induced TRAIL sensitization. The proapoptotic action of DR5 in TOV-21G and SKOV3 cells was functionally blocked using the DR5/Fc chimeric protein, followed by TRAIL-TIIA cotreatment and subsequent evaluation of cell viability. In both TOV-21G and SKOV3 cells, DR5/Fc treatment markedly suppressed the level of TRAIL-induced cytotoxicity enhanced by TIIA (Figure 2E). In particular, the cytotoxicity induced by TRAIL-TIIA cotreatment decreased from $80.2 \pm 9.4\%$ to $43.9 \pm 13.0\%$ ($p < 0.005$) and from $70.4 \pm 19.2\%$ to $38.3 \pm 15.0\%$ ($p < 0.01$) in TOV-21G and SKOV3 cells, respectively.

TIIA Transcriptionally Induces CHOP for DR5 Up-regulation. We next sought to elucidate the role of CHOP in TIIA-induced DR5 up-regulation. TIIA treatment led to a time-dependent increase in CHOP and DR5 protein expression (Figure 3A). Real-time RT-PCR analysis revealed that the level

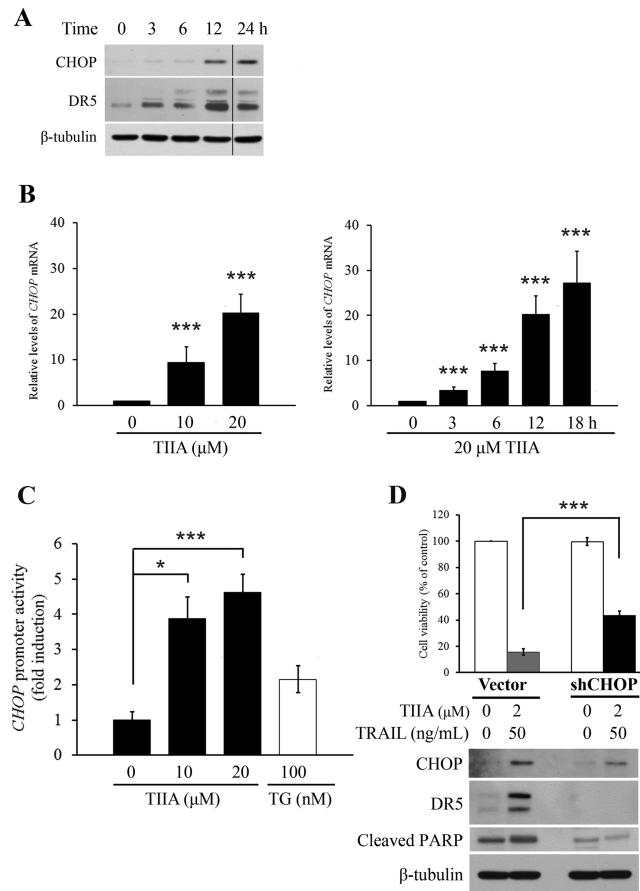


Figure 3. TIIA induces upregulation of CHOP to increase DR5 expression in TOV-21G cells. (A) Cells were treated with 10 μ M TIIA for 0, 3, 6, 12, or 24 h followed by Western blot analysis of CHOP and DR5. (B) TIIA increases the levels of CHOP mRNA concentration- and time-dependently. Cells were treated with 0, 10, or 20 μ M TIIA for 12 h (left) and with 20 μ M TIIA for 0, 3, 6, 12, or 18 h (right); the expression of CHOP mRNA was then measured. (C) Effect of TIIA on CHOP promoter activity. TOV-21G cells were treated with TIIA or thapsigargin (TG) for 24 h after transfection with a reporter comprising the CHOP promoter (pGL4-CHOP-Luc) in a β -galactosidase expression vector. TG, an ER stress inducer. (D) TOV-21G cells were transfected with shRNA directed against CHOP mRNA. Transfected cells were treated with or without 2 μ M TIIA plus 50 ng/mL TRAIL for 24 h, and cell viability was assessed using the MTS assay. Western blotting was used to confirm CHOP knockdown and examine its effect on TIIA-induced DR5 up-regulation. * $p < 0.05$; *** $p < 0.005$.

of CHOP mRNA was increased by TIIA treatment in both a concentration- and time-dependent manner (Figure 3B). To further explore whether TIIA up-regulates CHOP at the transcriptional level, we generated a luciferase reporter construct for probing the activity of the CHOP promoter. TOV-21G cells were treated for 24 h with various doses of TIIA (0, 10, 20 μ M) or 100 nM of thapsigargin (TG), a well-known ER stress inducer, followed by a dual luciferase reporter assay to monitor the CHOP promoter activity. As shown in

Figure 3C, TIIA treatment induced dose-dependent activation of the CHOP promoter. To determine the role of TIIA-mediated CHOP promoter induction in DR5 up-regulation and TRAIL sensitization, we examined the effect of TIIA in TOV-21G cells expressing CHOP shRNA. TIIA-TRAIL cotreatment failed to up-regulate DR5 and elicit PARP cleavage in shCHOP cells (Figure 3D). Thus, TIIA mediates the up-regulation of DR5 for TRAIL sensitization through the CHOP promoter.

TIIA-Induced Up-regulation of CHOP and DR5 Requires JNK. Given the involvement of JNK and p38 MAPK in apoptosis induction and the role of JNK in the regulation of CHOP and DR5 expression, we tested the effect of TIIA on JNK and p38 MAPK phosphorylation. As shown in Figure 4A, TIIA treatment led to a concentration-dependent

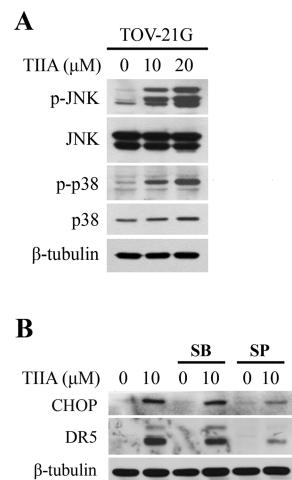


Figure 4. JNK-mediated signaling up-regulates CHOP, leading to DR5 induction. (A) Immunoblot represents the phosphorylation of JNK and p38 upon TIIA treatment in TOV-21G cells. The sample blots were stripped and reported with antibodies for nonphosphorylated proteins to verify equal protein loading. (B) TOV-21G cells were pretreated with the p38 MAPK inhibitor (SB203580) or JNK inhibitor (SP600125) for 1 h and then treated with 10 μ M TIIA for 24 h. Western blotting was used to analyze the extracts for CHOP and DR5 expression.

increase in the levels of phosphorylated JNK (Thr183/Tyr185) and phosphorylated p38 MAPK (Thr180/Tyr182), indicating that TIIA induced the activation of both JNK and p38 MAPK. The addition of a JNK inhibitor potently suppressed the TIIA-mediated induction of CHOP and DR5, whereas inhibition of p38 MAPK exerted no effect (Figure 4B). Thus, TIIA likely promotes JNK-mediated signaling to up-regulate CHOP, thereby inducing DR5 expression.

ROS Induced by TIIA Is Required for JNK Activation and CHOP Induction. We observed a clear increase in ROS production in TOV-21G cells following TIIA treatment (Figure 5A). Cells pretreated with the ROS scavenger NAC potently suppressed the TIIA-evoked JNK phosphorylation and CHOP induction, thereby abrogating PARP to cleavage (Figure 5B). Inhibition of ROS by NAC significantly attenuated TRAIL-induced cell death potentiated by TIIA, as the viability of TOV-21G cells cotreated with TIIA and TRAIL was rescued by NAC from $19.3 \pm 1\%$ to $31.6 \pm 4.9\%$ of drug-untreated cells ($p < 0.005$) (Figure 5C).

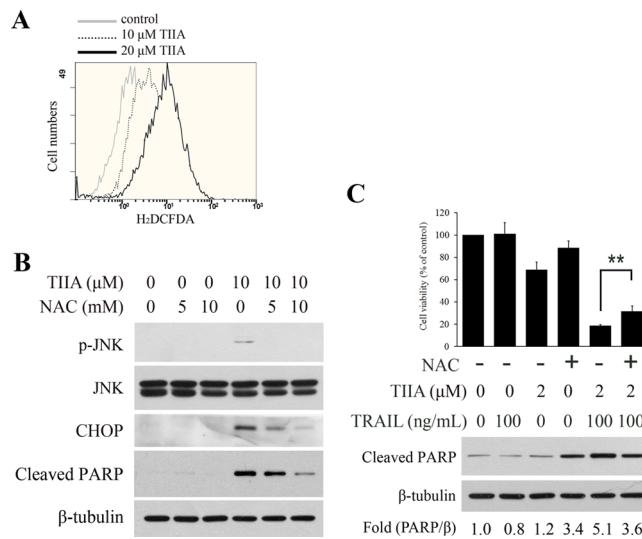


Figure 5. JNK phosphorylation and CHOP induction by TIIA are ROS-dependent. (A) TOV-21G cells were treated with TIIA (gray line, vehicle control; dotted line, 10 μ M; and solid line, 20 μ M), and labeled with H₂DCFDA to examine ROS production. (B) Inhibition of p-JNK, decrease in CHOP and PARP cleavage by NAC in TOV-21G cells. Cells were pretreated with NAC for 1 h and then treated with 10 μ M TIIA for 24 h; whole-cell lysates were analyzed by immunoblot. (C) Pretreatment with antioxidants blocks the cell death induced by TIIA plus TRAIL. TOV-21G cells were pretreated with 10 mM NAC for 1 h and further treated with 2 μ M TIIA plus 100 ng/mL TRAIL for 24 h. Cell viability was assessed using the MTS assay (** $p < 0.01$).

DISCUSSION

This study investigated the mechanisms whereby TIIA induces TRAIL sensitization. We observed that TIIA treatment markedly sensitized the TRAIL-resistant human EOC cell lines TOV-21G and SKOV3 to TRAIL-induced cell death and increased caspase-8 and PARP processing (Figure 1). Furthermore, we provide evidence that DR5 up-regulation underlies TIIA-mediated TRAIL sensitization (Figure 2). Additionally, CHOP was found to be transcriptionally induced by TIIA and was proved to be essential for TIIA-elicited DR5 up-regulation (Figure 3). We confirmed that TIIA-mediated up-regulation of CHOP and DR5 requires JNK, whose activation is induced by ROS evoked by TIIA (Figure 4). To the best of our knowledge, this study is the first to report that TIIA engages the ROS-JNK-CHOP signaling axis to up-regulate DR5 for sensitizing cells to TRAIL-induced extrinsic apoptosis (Figure 5).

Effective cancer therapeutics must induce cell death selectively, affecting malignant tumor cells but not normal cells. TRAIL is considered a promising candidate for medical applications because it triggers apoptosis in a variety of tumor cells but shows little toxicity in normal cells.³⁷ One mechanism that may contribute to the cancer-specific cytotoxicity of TRAIL is the differential expression of its receptors. To date, 5 TRAIL-specific receptors have been identified. DR5 and DR4 are proapoptotic TRAIL receptors, while decoy receptors (DcR1 and DcR2) and osteoprotegerin (OPG) contain mutations in the death domain and are unable to transmit apoptotic signals.^{38,39} DR4 and/or DR5 are expressed in most cancer cell lines, whereas DcR expression is less frequent and does not appear to correlate with resistance to TRAIL.⁴⁰ According to the numerous clinical trials of TRAIL, one major obstacle facing the use of TRAIL for treatment is the resistance

exhibited by many cancer cells.³⁸ Such resistance occurs in cells with low levels of DR4 and DR5 expression on the cell surface. To our knowledge, this is the first report demonstrating that DR5 receptors play an essential role in the sensitization effect of TIIA on TRAIL's proapoptotic action in TRAIL-resistant human ovarian cancer cells. Several chemicals induce resensitization to TRAIL-mediated apoptosis through the upregulation of DR5, a TRAIL receptor.^{27,41–46} Our results show that TIIA strongly affects the expression of DR5, and blockade of DR5 expression leads to the inhibition of TIIA-mediated TRAIL sensitization (Figure 2). An alternative way to circumvent TRAIL resistance is through the down-regulation of c-FLIP, an endogenous inhibitor of caspase-8 activation.^{22,23,47} Resistance to TRAIL has also been correlated with high levels of c-FLIP which shows that it is structurally similar to caspase-8 but has no protease activity. Along this line, we noticed an elevated expression of c-FLIP in SKOV3 cells after TIIA treatment, whereas the levels of c-FLIP remained relatively constant in TIIA-treated TOV-21G cells (Figure 2A). These results therefore exclude the possible involvement of c-FLIP in TIIA-induced TRAIL sensitization and also might be a reason why TIIA failed to cause profound cell death in TRAIL-treated SKOV3 cells, the same as that in TOV-21G. Thus, we propose that TIIA might serve as an effective adjunctive reagent in resensitizing TRAIL-resistant cells to TRAIL-induced apoptosis through up-regulation of DR5. These findings provide a foundation for developing TIIA as a TRAIL sensitizer that can overcome TRAIL resistance.

In light of numerous reports that induction of DR5 is transcriptionally regulated by CHOP,^{28,48} we examined the role of CHOP in DR5 induction. We found that CHOP plays a critical role in the expression of DR5 induced by TIIA. Results of Western blot analysis, real time RT-PCR or luciferase assay, and gene-silencing suggest that TIIA-mediated DR5 induction in TOV-21G cells occurs at the transcriptional level via increased CHOP gene transcription. We observed that TIIA treatment led to a time-dependent increase in the level of CHOP protein, along with the induction of DR5 expression (Figure 3A). In addition, TIIA induced up-regulation of CHOP mRNA (Figure 3B) and activation of the CHOP promoter (Figure 3C). Furthermore, silencing of CHOP abolished the effect of TIIA-mediated TRAIL sensitization on induction of DR5 and apoptosis (Figure 3D). Taken together, this evidence indicates that CHOP plays an essential role in DR5 regulation and the sensitization of TRAIL on TIIA treatment.

We examined the involvement of the signaling molecules JNK and p38 MAPK in this process. Figure 4 shows that JNK and p38 were activated by TIIA; pretreatment with a JNK inhibitor, but not a p38 inhibitor, decreased TIIA-induced upregulation of CHOP and DR5, suggesting that JNK activation may be responsible for TIIA-induced upregulation of CHOP and DR5. This finding is consistent with previous studies showing that JNK-dependent CHOP induction regulates apoptosis.⁴⁹ ROS triggers a variety of signal transduction pathways that lead to cell growth, differentiation, or death. Important downstream mediators of ROS-induced signaling include MAPKs,⁵⁰ such as JNK and p38 MAPK. We next investigated whether scavenging of ROS could abolish the TIIA-induced sensitization of cells to TRAIL. Figure 5 shows that cell treatment with TIIA alone induced the upregulation of CHOP, the phosphorylation of JNK, and PARP cleavage; these activities were all dramatically inhibited by the addition of the ROS scavenger NAC. We also found that induction of ROS is

critical for the sensitization of cells to TRAIL by TIIA. Quenching of ROS also abrogated the effect of TIIA on TRAIL-induced apoptosis. These results suggest that ROS generated by TIIA play an essential role in CHOP-dependent DR5 up-regulation and in the increase in apoptosis induced by TIIA plus TRAIL. It is worth noting that NAC treatment or CHOP silencing induces significant but not complete rescue of cell viability under the treatment of TRAIL-TIIA combination. This finding suggested that the NAC-JNK-CHOP-DR5 signaling axis identified in this study represents one of the mechanisms underlying TIIA-mediated TRAIL sensitization. In fact, previous studies have demonstrated a number of mechanisms involved in the sensitization of cancer cell lines to TRAIL-induced apoptosis. These include up-regulation of death receptors, increased DISC formation, upregulation of proapoptotic proteins, and suppression of antiapoptotic proteins. Our recent study identified that down-regulation of antiapoptotic survivin also plays an important role in TIIA-induced TRAIL sensitization.⁵¹ In conclusion, as summarized in Figure 6, the present study provides the first mechanistic

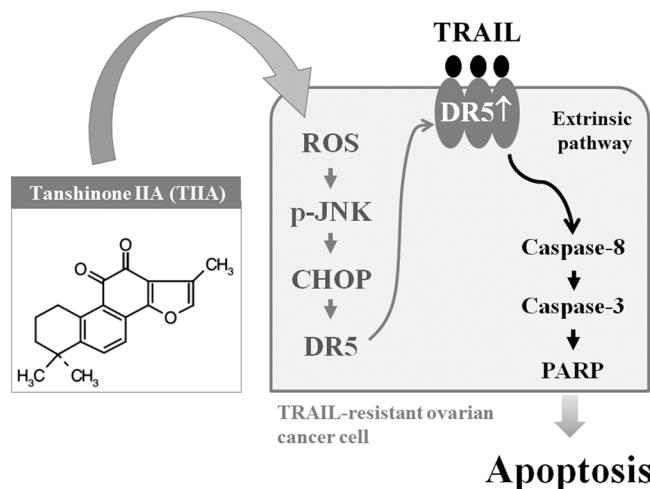


Figure 6. Diagram showing the mechanism by which tanshinone IIA (TIIA) sensitizes TRAIL-resistant epithelial ovarian carcinoma (EOC) to TRAIL. TIIA enhances TRAIL-induced apoptosis by up-regulating the expression of DR5 via the ROS-JNK-CHOP signal cascade.

evidence that pretreatment of cells with TIIA effectively recovers or enhances the sensitivity of TRAIL in human EOC cells. TIIA markedly sensitized TRAIL-resistant human EOC cell lines TOV-21G and SKOV3 to TRAIL-induced cell death, likely due to the increased level of processing/activation of caspase-8 and PARP. We identified DR5 up-regulation as a key mechanism leading to TIIA-mediated TRAIL sensitization. Additionally, CHOP was found to be transcriptionally induced by TIIA and was later proved responsible for TIIA-induced DR5 up-regulation. Moreover, we confirmed that TIIA-elicited up-regulation of CHOP and DR5 requires p-JNK, whose activation is evoked by ROS initiated by TIIA. Collectively, these lines of evidence are the first to support the notion that TIIA engages the ROS-JNK-CHOP signaling axis to up-regulate DR5, thereby sensitizing cells to TRAIL-induced extrinsic apoptosis.

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Author Contributions

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Funding

This work was supported by grants from the following donors: Central Taiwan University of Science and Technology, Taichung, Taiwan (CTU102-P-17 and CTU103-P-18); National Chung Hsing University and Agricultural Research Institute, Council of Agriculture, Executive of Yuan, R.O.C. (NCHU-TARI 9904 and NCHU-TARI 10104); Taichung Veterans General Hospital and National Chung Hsing University, Taichung, Taiwan (TCVGH-NCHU997606); and The Ministry of Education, Taiwan, R.O.C. under the ATU plan.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

TIIA, tanshinone IIA; DRS, death receptor 5; c-FLIP, Ccellular FLICE (FADD-like IL-1 β -converting enzyme)-inhibitory protein; FADD, Fas-associated death domain; ROS, reactive oxygen species; CHOP, C/EBP homologous protein; JNK, c-Jun N-terminal kinase; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; z-VAD.fmk, N-benzyloxycarbonyl Val-Ala-Asp (O -methyl)-fluoromethylketone; PARP, poly-(ADP-ribose)polymerase; NAC, (*N*-acetylcysteine); EOC, epithelial ovarian carcinoma

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Epigenetic and expression analysis of *TRAIL-R2* and *BCL2*: on the TRAIL to knowledge of apoptosis in ovarian tumors

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Received: 27 March 2013 / Accepted: 15 October 2013 / Published online: 5 November 2013
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Abstract

Objective This study assesses *TRAIL-R2* (TNF-related apoptosis-inducing ligand receptor 2) and *BCL2* (B cell CLL/lymphoma 2) expression as well as CpG island methylation within the *TRAIL-R2* promoter in ovarian serous tumors and primary and metastatic serous EOC (epithelial ovarian cancer).

Methods RNA and DNA were obtained from women with normal ovarian tissues ($n = 18$), ovarian serous cystadenoma tumors ($n = 11$) and serous EOC ($n = 16$) using Trizol®. Quantitative PCR was performed to quantify the relative levels of *TRAIL-R2* and *BCL2*. The methylation frequency of the *TRAIL-R3* promoter was assessed using a methylation-specific PCR assay after DNA bisulfite conversion. Differences between the groups were evaluated using the χ^2 , Mann–Whitney *U* or Kruskal–Wallis tests, as indicated.

Results We identified *TRAIL-R2* and *BCL2* mRNA expressed in all ovarian tumor groups, and there were significant differences between the groups. Both genes had

low expression levels in ovarian serous cystadenoma and primary EOC tumors when compared with metastatic EOC. Methylation of the *TRAIL-R2* promoter was frequently observed in all groups; however, there were no statistically significant associations.

Conclusions Primary EOC is associated with lower *TRAIL-R2* and *BCL2* expression levels, while metastatic EOC is associated with higher expression of these genes. Promoter DNA methylation was not related to this finding, suggesting there are other mechanisms involved in transcriptional control.

Keywords Ovarian tumors · *TRAIL-R2* · *BCL2* · Gene expression · DNA methylation

Introduction

Apoptotic cell death can be triggered by two alternative, convergent pathways: the extrinsic pathway, which is mediated by the activation of membrane-bound death receptors (DRs), and the intrinsic pathway, which is mediated by mitochondria [5, 10, 40]. The extrinsic pathway operates through the DRs, which are activated by binding their cognate ligands, such as TRAIL, a TNF-related apoptosis-inducing ligand that is a type II transmembrane protein [5, 13, 19, 45].

Four homologous human receptors for TRAIL have been identified. Two DRs, DR4/TRAIL-R1/TNFRSF10A and DR5/TRAIL-R2/TNFRSF10B [39, 41, 48], contain an extracellular cysteine-rich domain that recruits adaptor proteins, such as the Fas-associated death domain (FADD), and initiator CASPASES (PROCASPASES-8/10), thereby transducing the apoptotic signal [33, 34]. In contrast, neither the decoy receptor (TRAIL-R3/TRID/DcR1/

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TNFRSF10C), which lacks a cytoplasmic domain [8, 39, 41], nor the TRAIL receptor 4 (TRAIL-R4/DcR2/TNFRSF10D), which contains a truncated cytoplasmic death domain, transduces apoptotic signals directly [7]. In this report, the TRAIL receptors are called TRAIL-R1, TRAIL-R2, TRAIL-R3 and TRAIL-R4.

The apoptotic signal transduced by TRAIL receptors can cause alterations in mitochondrial membrane integrity resulting in cytochrome c efflux and formation of the apoptosome [26]. In this context, *BCL2* (B-cell CLL/lymphoma 2) family members play a central role in regulating the intrinsic pathway. A total of 25 genes have been identified in the *BCL2* family, which encode for a mix of proapoptotic and antiapoptotic proteins [16] that are classified by sequence homology in four α -helical segments from BH1 to BH4. The highly conserved antiapoptotic genes (e.g., *BCL2*, *BCL-X*, *BCL-XL*, *BCL-XS* and *BCL-WL*) contain all four BH domains, of which the BH1–BH3 domains structurally form a pocket capable of binding the BH3 domains of the other family proteins [45]. The antiapoptotic proteins *BCL2* and *BCL-XL* bind and sequester the proapoptotic BH3-only proteins, thereby preventing BAX and BAK activation, or bind the activated conformers of BAX and BAK as a mechanism of cell survival [10]. A cell's susceptibility to apoptosis is influenced by the equilibrium of several components of both the proapoptotic and antiapoptotic proteins [1]. Because the TRAIL preference to kill tumor cells, it is a promising anti-cancer agent and the sensitivity to TRAIL-induced apoptosis is an important factor influencing the therapeutic response to TRAIL. However, the basis for the resistance and sensitivity of tumor cells to TRAIL-induced apoptosis is not completely understood [25]. TRAIL resistance could be mediated by a sort of defects in signaling pathway including inactivating mutations in *TRAIL-R1* and *TRAIL-R2*, loss of the initiator CASPASE-8 and BAX, and overexpression of *BCL2* [36].

Furthermore, detachment from the extracellular matrix induces programmed cell death and this cell detachment-induced apoptosis has been a phenomenon called anoikis [25, 37, 44]. This special form of cell death is an important mechanism terminating the physiological cell life cycle by neglecting and is triggered by inadequate or inappropriate cell–matrix contacts [37] and it could be the second hypothesis to justify the resistance of tumor cells to TRAIL-induced apoptosis. Several authors postulate the involvement of the death receptors, like FADD and TRAIL protein, in anoikis with subsequent activation of CASPASE-8 led to cell death, generally through the intrinsic pathway that *BCL2* family members play a central role in regulating [15]. *BCL2* can interact with BMF (*BCL-2-modifying factor*) to act as sensitizer that binds pro-survival *BCL-2* protein to displace activator proapoptotic

BH3-only proteins (i.e., BID or *BCL2*-interacting mediator of cell death (BIM)) [18]. After the cell has detached from the extracellular matrix, BIM and BMF are released from cytoskeleton, interact with protein *BCL2* and neutralize its antiapoptotic action [18, 23]. Anoikis resistance may be explained in turn impacts metastatic aggressiveness by allowing cells to survive following detachment from the origin tissue matrix [23].

Epithelial ovarian cancer (EOC) is a silent disease that is usually diagnosed at an advanced stage and, currently, represents the most fatal gynecologic malignancy [2]. At the cellular and molecular levels, ovarian cancers are remarkably heterogeneous, a factor that makes EOC a difficult disease to treat effectively [2, 20, 27]. One of the major disappointments in the field of ovarian cancer research is the failure of the currently established therapies to cure tumors. More recent developments in apoptosis research have made it possible to devise novel therapeutic approaches that exploit this process to treat cancer. The observation that certain types of cancer express cell surface death receptors has triggered interest in exploring the potential of receptor ligation as a novel anti-cancer modality [32, 34]. However, for these agents to succeed, alone or in combination with other drugs, a deep understanding of their roles in specific signaling pathways and of how molecules in the tumor may interfere with the actions of these drugs is required. Otherwise, a promising strategy is to become ineffective due to the lack of tumor biology knowledge.

In this study, we aimed to investigate the expression of two apoptotic genes, *TRAIL-R2* and *BCL2*, to gain further insight into the apoptosis profile of EOC. Our postulate was that *TRAIL-R2* and *BCL2* expression in EOC could be considered to play an important role in resistance to apoptosis and/or anoikis which represents an unfavorable prognostic indicator for this type of human malignancy. Additionally, we investigated whether methylation at single CpGs regulates *TRAIL-R2* transcription. This strategy is used to identify the targets of epigenetic silencing in various tumor types, and DNA methylation is a frequent epigenetic event for the tumor suppressor genes that are active in many human cancers [29, 35, 43, 50].

Materials and methods

Patients and histology

Forty-five female patients were prospectively evaluated in the following three groups: serous EOC ($n = 16/45$), ovarian serous cystadenoma ($n = 11/45$) and normal ovary ($n = 18/45$). The study was performed in accordance with the Ethical Committee for Research in Human Beings

guidelines of the Institution. Informed consent was obtained from all patients involved in the study. Eligible cases were women with adnexal masses suggestive of malignancy who were scheduled to undergo cytoreductive surgery and pathologically confirmed diagnosis of serous epithelial ovarian cancer as soon as women with a uterine myoma who were scheduled to undergo total hysterectomy as clinically indicated. Tumor staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO) recommendations [14]. The EOC patient samples were collected from the primary tumor ($n = 16/45$), and an additional sample of metastatic tumor was collected in the same patients when extra-pelvic disease larger than 1 cm was observed ($n = 5/16$). Normal ovarian epithelial tissue samples were taken from post-menopausal women who required a bilateral oophorectomy. After excision, the samples were immediately frozen in liquid nitrogen and stored at -80°C for later use.

The isolation of RNA and genomic DNA (gDNA)

For RNA extraction, the preserved tissue samples (50–100 mg) were homogenized in 1 mL of TRIzol[®] (Life Technologies, Carlsbad, California, USA), and RNA from the tissues was isolated according to the manufacturer's instructions. The recovered RNA was then treated with an RNase-free DNase Set (Qiagen, Hilden, Germany). gDNA was isolated from the inter-organic phase of the TRIzol[®]-chloroform mixture and then combined with 0.7 mL of back extraction buffer (4 M guanidine isothiocyanate, 50 mM sodium citrate and 1 M Tris pH 8.0). After vigorous mixing by inversion, phase separation was achieved by centrifugation at 14,000 $\times g$ for 15 min at 4°C . The gDNA was then precipitated from the upper aqueous phase using 0.7 mL of isopropanol. The isolated gDNA from each fraction was dissolved in 20 μL of Milli-Q water and stored at -20°C until use. The concentrations of the RNA or gDNA as well as the 260/280 absorbance ratio were measured using a NanoVuePathlength Calibration Fluid Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

cDNA synthesis

cDNA was generated from 2 mg of total RNA using Illustra Ready-to-Go RT-PCR Beads (GE Healthcare, Little Chalfont, Buckinghamshire, UK) in a total volume of 50 μL in accordance with the manufacturer's instructions.

Quantitative PCR analysis

The qRT-PCR was performed using an Agilent MX 3005P detection system (Stratagene, La Jolla, CA, USA). There

were duplicate 10 μL reactions containing 1 X Brilliant II SYBR[®] Green QPCR Master Mix (Agilent Technologies, La Jolla, CA, USA); 0.2 μL of Rox (1:500); 0.25 μM of forward primer/0.20 μM of reverse primer for *TRAIL-R2* or *BCL2* and 0.2 μM of each primer for TATA binding protein (*TBP*), and 40 ng/ μL cDNA (RNA equivalent) for each experiment.

The *TBP* was used as a reference locus for normalization. The sequences of the specific primers used in this study for *TBP* (GenBank ID: NM_003194) [30], *TRAIL-R2* (GenBank ID: NM_147187.2) [28] and *BCL2* (GenBank ID:666) (PrimerBank ID 6456033a2 [49]) have been previously reported.

The PCR cycling conditions were performed as follows: 95 °C for 10 min and 40 cycles of 95 °C for 30 s, annealing at 60 °C for 60 s and extension at 72 °C for 30 s.

The optimization of the qRT-PCR was performed according to the manufacturer's instructions. A sample without a template (no cDNA in the PCR) was included as a control in each assay. A melting curve was constructed for each primer pair to confirm product specificity. The 2[−Delta Delta C(T)] method was used to calculate the relative quantitation values from the data of an individual sample. The data for each sample were normalized with the normalizer gene for comparison with normal tissue, showing fold differences in *TRAIL-R2* and *BCL2* expression [31].

Bisulfite conversion

The gDNA was modified with sodium bisulfite using a Bisulfite Conversion Kit (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions.

Methylation-specific polymerase chain reaction (MSP)

The MSP was performed as described previously [21]. *TRAIL-R2* Primer sets to amplify bisulfite-modified DNA were designed to produce a 102-base-pair PCR fragment. The primer sequences were unmethylated-specific 5' TTA-TAGTTCGGGCGTAGTC 3' (sense) and 5' AAATCT CGTCAACGCAATC 3' (antisense) and methylated-specific 5' AAATTATAGTTGGGGTAGTT 3' (sense) and 5' AAAATCTCATCACACAATCCT 3' (antisense).

The MSP was performed in a total volume of 15 μL containing 10 ng of bisulfite-treated DNA, 1 X Epitech Master Mix (Qiagen, Hilden, Germany) and 0.3 μM of each primer. Reactions containing unmethylated DNA, methylated DNA or unmethylated bisulfite-converted DNA (Epitech Control DNA Set—Qiagen, Hilden, Germany) were performed as controls. The amplification was performed with the following conditions: 95 °C for 10 min; 35 cycles of 95 °C for 1 min, 60 °C for 1 min, and 72 °C

Table 1 Clinicopathological characteristics of the patients who provided the ovarian samples

	Normal ovary (n = 18)	Cystadenoma (n = 11)	EOC (n = 16)	p value
Age (years)	50.61 ± 2.13	54.3 ± 5.1	59.1 ± 2.7	0.128
Parity (births)	2.83 ± 0.44	2.3 ± 0.8	1.9 ± 0.3	0.356
CA-125 (U/mL)				
CA <330 (U/mL)		11 (100 %)	8 (50 %)	
CA >330 (U/mL)		0	8 (50 %)	
Tumor differentiation degree				
G2			6 (37.5 %)	
G3			10 (62.5 %)	
Cytoreduction				
Optimal (<1 cm)			7 (43.8 %)	
Suboptimal (>1 cm)			9 (56.3 %)	
Stage				
Stage I and II			5 (31.3 %)	
Stage III			8 (50 %)	
Stage IV			3 (18.7 %)	
Ascites				
Yes			10 (64.3 %)	
No			6 (35.7 %)	
Recurrence				
Yes			6 (37.5 %)	
No			10 (62.5 %)	
Mortality				
Yes			6 (37.5 %)	
No			10 (62.5 %)	
Menarche		12.8 ± 0.4	12.8 ± 3.4	0.991
Menopause				
Yes	18 (100 %)	6 (54.5 %)	13 (81.3 %)	0.008
No	0	5 (45.5 %)	3 (18.8 %)	

The values represent the mean ± standard error or n (percentage). The comparison between groups was performed using a χ^2 square test or an ANOVA as indicated. The median serum level of CA-125 was considered to determine the cut-off point. The tumor differentiation degree was classified as moderately differentiated (G2) or poorly differentiated (G3). Cytoreduction was considered optimal for a residual tumor <1 cm after tumor resection surgery. The tumor was staged according to the 2009 recommendations of FIGO (International Federation of Gynecology and Obstetrics). A p value ≤0.05 was considered to be statistically significant

EOC Epithelial ovarian carcinoma

for 30 s; and a final extension step for 10 min at 72 °C. The amplified MSP products (20 µL) were separated by electrophoresis onto 7.5 % non-denaturing polyacrylamide gels and visualized by silver nitrate staining.

Statistics

All statistical analyses were performed with the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA). The clinical data were expressed as a percentage and the mean ± standard error. The gene expression levels were expressed as the median with the interquartile range. The differences between the groups were evaluated using the χ^2 , ANOVA, Mann–Whitney U and Kruskal–Wallis tests

as indicated. A p value <0.05 was considered statistically significant.

Results

Patients

The patients included in this study were between 32 and 81 years old; the mean age was 54 ± 5 years (p = 0.128). The mean parity was 2.3 ± 0.3 births, with a range between 0 and 8 deliveries (p = 0.356). The only statistically significant difference between the groups was in the frequency of menopause (p = 0.008). The stage (FIGO)

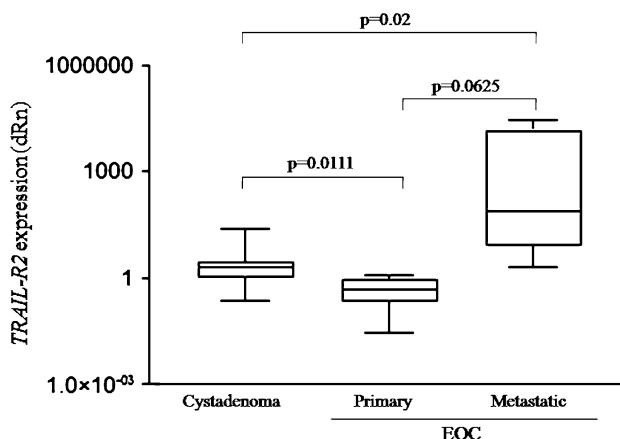


Fig. 1 The association of *TRAIL-R2* expression in ovarian tumors. The values shown represent the *TRAIL-R2* expression levels. The horizontal line indicates the median expression ratio, and the box plots demonstrate the interquartile range (25–75 %). The 10th–90th percentile ranges are also shown. The differences between groups were evaluated using a Mann–Whitney *U* test and Kruskal–Wallis test. *EOC* epithelial ovarian carcinoma

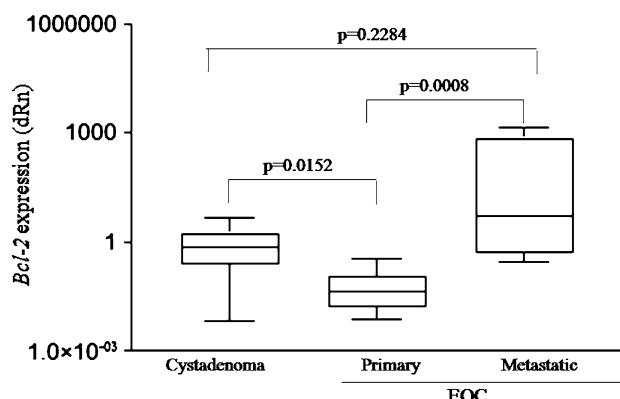


Fig. 2 The association of *BCL2* expression in ovarian tumors. The values shown represent the *BCL2* expression levels. The horizontal line indicates the median expression ratio, and the box plots demonstrate the interquartile range (25–75 %). The 10th–90th percentile ranges are also shown. The differences between groups were evaluated using a Mann–Whitney *U* test and Kruskal–Wallis test. *EOC* epithelial ovarian carcinoma

was I/II in 5 patients (31.3 %), III in 8 patients (50 %) and IV in 3 patients (18.7 %) in the EOC group.

The tumor was moderately differentiated (G2) in 6 (37.5 %) cases and poorly differentiated in 10 (62.5 %). The general sample characteristics are listed in Table 1.

The gene expression data

We identified *TRAIL-R2* and *BCL2* mRNA using qRT-PCR in all normal and ovarian tumor tissues, suggesting that the *TRAIL-R2* and *BCL2* genes are expressed in all cases (Figs. 1, 2).

The median *TRAIL-2* expression ratio in ovarian serous cystadenoma was 2.11 (1.04–2.71), and that in the primary EOC was 0.48 (0.21–0.09); the metastatic EOC expression ratio was 76.33 (7.66–14,502.47). Both the ovarian serous cystadenoma tumor and primary EOC groups had lower *TRAIL-R2* expression than the metastatic EOC samples (≥ 159.02 -fold higher expression than the primary EOC group). Significant differences were observed in the *TRAIL-R2* expression levels when all the ovarian tumor groups were compared ($p = 0.0008$) as well as between the ovarian serous cystadenoma tumors and primary EOC and ovarian serous cystadenoma tumors and metastatic EOC group ($p = 0.01$ and $p = 0.02$, respectively). No statistical significance was observed when comparing primary EOC and metastatic EOC ($p = 0.06$) (Fig. 1).

For the *BCL2* expression levels, significant differences were observed when all the ovarian tumor groups were compared ($p = 0.03$). The median *BCL2* expression ratio in the ovarian serous cystadenoma was 0.8 (0.2–1.9), while in the primary EOC, the expression ratio was 0.4 (0.02–0.1). A significant difference in the *BCL2* expression level was observed between these groups ($p = 0.01$). In metastatic EOC, overexpression of *BCL2* was also detected with a median expression ratio of 5.4 (0.6–365.8). This level of *BCL2* expression was also significantly higher than the level found in primary EOC ($p = 0.0008$), although no statistical significance was seen when ovarian serous cystadenoma tumors were compared with metastatic EOC ($p = 0.2$) (Fig. 2).

The DNA methylation status of *TRAIL-R2*

With the use of primers specific for methylated or unmethylated *TRAIL-R2*, a product of 102 bp was detectable in both the positive controls from an MSP that used bisulfite-treated genomic DNA as the template. The results from the MSP products that were run onto 7.5 % non-denaturing polyacrylamide gels are shown in Fig. 3.

The methylation of at least one allele (hemimethylation) of *TRAIL-R2* was found in 40 % of the normal tissues, 40 % of the ovarian serous cystadenoma, 60 % of the primary EOC and 60 % of the metastatic EOC samples. However, no statistically significant associations were found between the methylation statuses of the *TRAIL-R2* promoter in these groups ($p = 0.87$) (Fig. 4).

Discussion

EOC is the deadliest gynecologic malignancy, and 80 % of patients who respond initially to treatment have recurrent disease and develop treatment resistance. Different latencies indicate the need for a better understanding of the

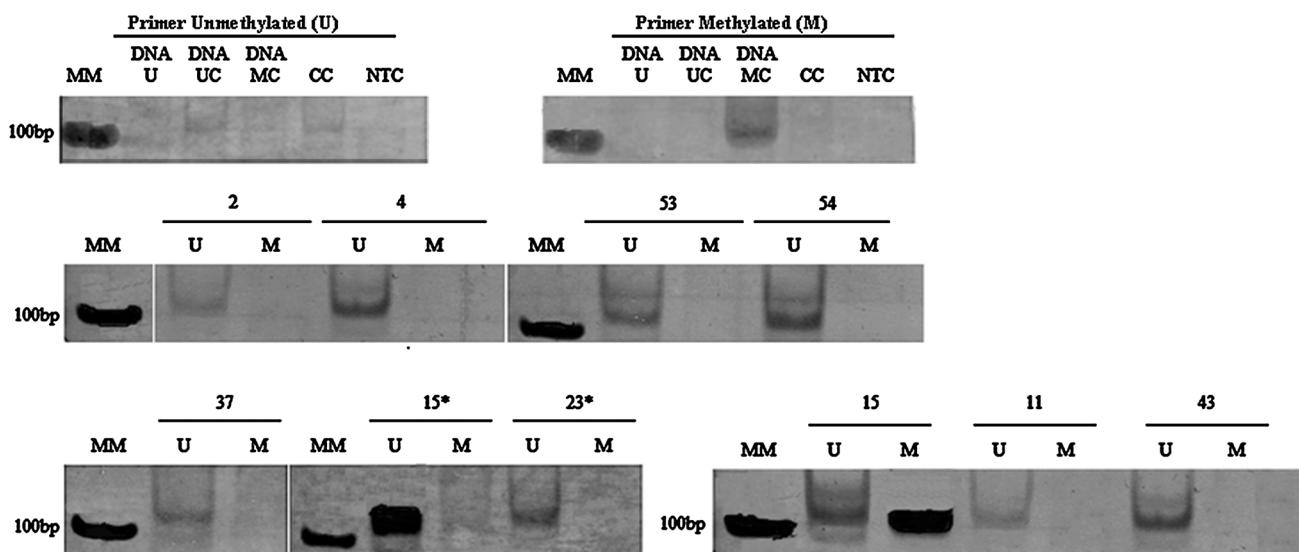


Fig. 3 An analysis of the CpG island promoter methylation status in *TRAIL-R2* by the methylation-specific polymerase chain reaction (MSP) assay. The 102-base-pair *TRAIL-R2* product was analyzed onto 7.5 % non-denaturing polyacrylamide gels. *U* represents the amplified products from primers specific to an unmethylated DNA sequence in the *TRAIL-R2* promoter, and *M* represents the amplified products from primers specific to a methylated sequence in the *TRAIL-R2* promoter. *MM* DNA molecular marker, *DNA U* unmethylated control DNA, *DNA UC* unmethylated control DNA after bisulfite conversion, *MC* bisulfite converted methylated control DNA (Qiagen, Hilden,

Germany), which serves as a control for the methylated and unmethylated sequences; *CC* bisulfite converted control DNA that serves as a methylSEQR™ bisulfite conversion control; case numbers 2 and 4: ovarian serous cystadenoma; 11, 15, 37 and 43: primary EOC; 15* and 23*: metastatic EOC; 53 and 54 normal ovarian tissue. The sample was considered to be unmethylated when the product was detected with the primer *U*, and the sample was considered to be hemimethylated when the product was detected with the primers *U* and *M*

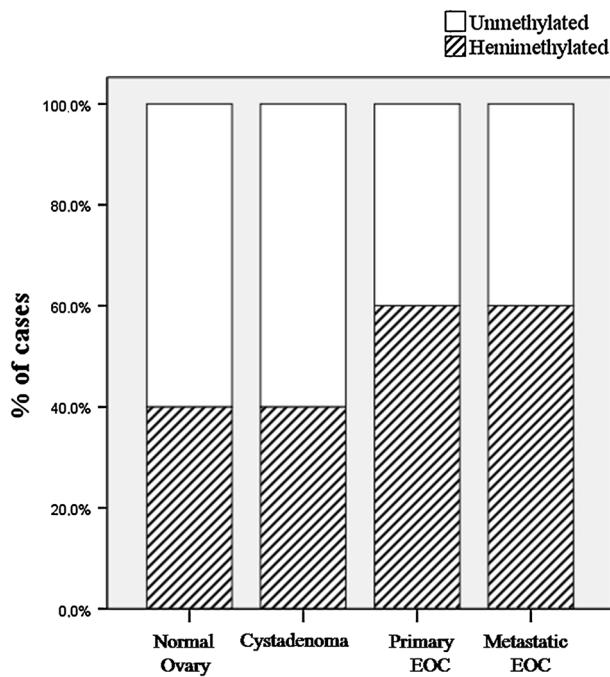


Fig. 4 The frequency of aberrant CpG island methylation in the *TRAIL-R2* promoter. Hemimethylation was detected in 40 % of the normal tissues, 40 % of the ovarian serous cystadenoma, 60 % of the primary EOC and 60 % of the metastatic EOC samples. No statistically significant associations between these groups were found by the Kruskal-Wallis test ($p = 0.87$)

disease and identification of new therapeutic targets or treatment modalities [2, 51].

Some factors have been identified in the study of drug resistance, such as the expression of multidrug resistance proteins, hypoxia in the tumor microenvironment, and resistance to apoptosis by the overexpression of survival factors and down-regulation of death signaling proteins [3]. In EOC, TRAIL signaling not only may influence the growth of cancer cells, but may also determine immunologic responses and could reflect the patient sensitivity or resistance to chemotherapy. Thus, it is the interplay of all of these factors that influences the disease endpoints and, ultimately, patient prognosis [12, 27, 40, 52]. To better understand the involvement of *TRAIL-R2* and *BCL2* on TRAIL signaling and apoptosis resistance in EOC, we evaluated the genetic expression and epigenetic variation of *TRAIL-R2* and the genetic expression of *BCL2* in ovarian serous cystadenoma, primary and metastatic EOC samples and normal ovarian tissues.

We demonstrated a higher *TRAIL-R2* expression level in metastatic EOC compared with ovarian serous cystadenoma and primary EOC. This result was calculated as a quantity relative to that in normal ovarian tissue, wherein the normal tissue sample was assigned an arbitrary quantity of "1" and all tumor samples were expressed in terms of their fold difference relative to the normal sample [31]. Our

results are consistent with the investigations of head and neck squamous cell carcinoma in which there was a significant down-regulation of *TRAIL-R2* expression in primary tumors [11].

Our study did not show any association between higher expression of *TRAIL-R2* observed in metastatic EOC and a survival advantage for patients. In agreement with our result, Elrod et al. [11] showed no survival advantage for metastatic head and neck squamous cell carcinoma that had high expression of *TRAIL-R2*. Indeed, in the cell lines from different cancers, including EOC, sensitivity to TRAIL was associated with high expression levels of the death receptors *TRAIL-R1* and *TRAIL-R2* and reduced expression levels of decoy receptors, such as *TRAIL-R3* [27, 41]. These reports indicate that TRAIL signaling is more complex than originally anticipated and it can be accomplished in various ways, including a reduction of the death receptor, ligand, and effector protein levels or the up-regulation of antiapoptotic proteins [38].

Additionally, we examined the expression of *BCL2* family members that might contribute to a greater pro-survival effect and apoptosis resistance in EOC. We observed overexpression of *BCL2* in metastatic EOC, and this expression level was also significantly higher than in primary EOC. These results are consistent with those in the literature showing that *BCL2* is overexpressed in several tumors (Burkitt's lymphoma, pancreatic carcinoma, melanoma, and neuroblastoma), and this increased expression is associated with increased proliferation and resistance to chemotherapy in many types of cancers, including EOC [6, 33]. Considering that antiapoptotic proteins can form heterodimers with multidomain proapoptotic proteins, such as *BAX* and *BAK* and that this interaction may prevent mitochondrial outer membrane permeabilization, which otherwise neutralizes the proapoptotic function [27]. The lower *BCL2* mRNA expression found in the primary EOC tissue samples could be made a biomarker favorable for the role of TRAIL in EOC, because it may increase apoptosis by TRAIL as well as ovarian cells can detach from their substrate and to involve in the subsequent cell death. In metastatic EOC, a relative resistance to death by apoptosis and the escape of tumors cells from anoikis could be explained by overexpression of *BCL2* which inhibits apoptotic signal transduced by *TRAIL-R2* and other *BCL2* family members that mediate anoikis.

The underexpression of *TRAIL-R2* in primary EOC inspired us to analyze the mechanisms involved in its down-regulation. We looked for promoter hypermethylation, which can selectively down-regulate gene expression. In agreement with previous studies [42, 47], we found high frequencies of *TRAIL-R2* methylation in at least one allele of the gene in both normal and ovarian tumor tissues, but it is not in concordance with the loss of gene expression

observed in ovarian serous cystadenoma and primary EOC. The *TRAIL-R2* hemimethylated alleles in metastatic EOC did not result in the gene expression silencing as observed in others human tumors [9, 22, 42]. There were no significant differences between the methylation profile and ovarian histological subtypes detected in our cohort, which was probably due to the limited number of patients and controls.

This study provides a useful basis for further analysis of epigenetic alterations in EOC and their controversial influence on gene expression, which is due to many reasons. First, the occasional reduction of *TRAIL-R2* expression that is observed with different DNA methylation levels could be explained by the lack of upstream transcription signals or by possible gene mutations [17, 24]. Second, the epigenetic silencing of gene transcription does not occur by promoter methylation changes alone but is mediated by a complex series of molecular events that cause remodeling of the chromatin configuration [17] as in some other tumors, such as small cell lung cancer [42]. Third, it is known that the methylation initiates at one or more chromosomal CpG sites in a promoter region and spreads to adjacent sites along the DNA strand until it meets a counteracting force in the form of open, active chromatin [17, 46]. Based on our previous findings of *TRAIL-R3* underexpression in primary EOC showing hemimethylation of the *TRAIL-R3* promoter frequently found in the neoplasia samples [4] and the fact that four receptors are mapped in a tandem fashion to the human chromosome 8p22-21, we can hypothesis that methylation (and resultant silencing) of the antiapoptotic *TRAIL-R3* genes may result in the silencing of the adjacent proapoptotic *TRAIL-R2* gene.

The absence of an association between the methylation data with the histological groups should be interpreted with caution. The most important limitation of this study is the relatively small number of patients and controls due to the low prevalence of the disease and ethical criteria control selected. Further studies with larger numbers of patients and longer follow-up are necessary to assess the accurate diagnostic and prognostic impact of *TRAIL-R2* and *BCL2* expression in EOC women.

In conclusion, the *TRAIL-R2* and *BCL2* genes exhibited differential expression between the tumor groups, and promoter DNA methylation appears an insufficient mechanism for *TRAIL-R2* gene silencing in primary EOC. We have highlighted the advantage of looking at two genes implicated in the same signaling pathway and the importance of a holistic approach when dealing with a specific pathway because the somatory effect of the deregulation of each member is what generates the downstream effects of the pathway. It remains to be determined in a larger series of patients whether these gene expressions can be used

clinically as markers of disease progression, either by themselves or in combination with other pathways.

Acknowledgments This work was supported by the Fundação de Amparo à Pesquisa do estado de Minas Gerais, FAPEMIG #PPM-CDS-00246-09, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Capes and Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq. The authors would like to thank Francisco de Oliveira Vieira for artwork help and the Pro-Rector of Research of the Universidade Federal de Minas Gerais for additional financial support.

Conflict of interest The authors have no conflicts of interest to declare.

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The extrinsic apoptosis pathway and its prognostic impact in ovarian cancer

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ARTICLE INFO

Article history:

Received 16 June 2009

Available online 3 December 2009

Keywords:

Apoptosis

Ovarian cancer

TRAIL

Prognosis

ABSTRACT

Objective. Death ligand FasL, its agonistic receptor Fas, tumor necrosis factor related apoptosis inducing ligand (TRAIL) and its agonistic death receptors DR4 and DR5 are implied in carcinogenesis, tumor immune surveillance and response to chemotherapy. TRAIL receptor agonists are evaluated as anti-cancer agents. This study aimed to relate expression of death ligands/receptors and downstream initiator caspase 8 and its anti-apoptotic homologue FLICE like inhibitory protein (c-FLIP) in ovarian cancers to chemotherapy response and survival.

Methods. Fas, FasL, TRAIL, DR4, DR5, caspase 8 and c-FLIP were determined immunohistochemically on a tissue microarray containing 382 ovarian cancers. Protein expression profiles were correlated with clinicopathologic variables, chemotherapy response and survival.

Results. Most tumors expressed DR4, DR5, caspase 8 and c-FLIP. High c-FLIP expression was associated with expression of caspase 8 and both TRAIL receptors. TRAIL and Fas were associated with low tumor grade and better progression-free survival (HR 0.63, $p = .018$ and HR 0.54, $p = .012$), respectively, and Fas with disease-specific survival (HR 0.49, $p = 0.009$) in univariate analysis.

Conclusions. Fas and TRAIL loss is associated with dedifferentiation and worse prognosis. Expression of DR4, DR5, caspase 8 and c-FLIP by most ovarian cancers does not correlate with survival. High c-FLIP expression should be taken into account for death receptor targeted therapies.

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Introduction

Ovarian cancer is the fifth most common cause of cancer deaths in women [1]. Late stage disease at diagnosis and acquired resistance to chemotherapy are characteristic for the course of most ovarian cancers. These characteristics exemplify the complexity of ovarian carcinogenesis, of which a defined sequence of progression has not yet been established [2]. The resulting heterogeneity among ovarian cancers and consequently in factors underlying clinical response complicates the definition of prognostic and predictive factors for individualized treatment. Distinctive for all cancers is deregulation of the apoptotic machinery [3]. Apoptosis can be induced through two pathways. In the intrinsic pathway, diverse cellular stressors cause sensors within the cell to promote cytochrome c release from the mitochondria, resulting in the formation of the apoptosome and activation of caspase 3, which sets the final execution phase of apoptosis in motion. The extrinsic apoptotic pathway is activated upon binding of death ligands from the tumor necrosis factor (TNF) family to their cognate receptors at the cell surface. The death ligands TNF

related apoptosis inducing ligand (TRAIL) and Fas ligand (FasL/CD95L) are members of the TNF family [4]. TRAIL can bind five receptors of which death receptor 4 (DR4) and death receptor 5 (DR5) transmit an apoptotic signal [5]. FasL binds to one agonistic receptor, Fas (CD95), and one soluble antagonistic receptor, DcR3 [6]. Trimerization of the receptors upon ligand binding causes formation of a death inducing signaling complex (DISC) in which the initiator caspase 8 is activated. Active caspase 8 cleaves various designated cellular proteins including pro-caspase 3, resulting in apoptosis [4]. An important regulator of caspase 8 activation is its anti-apoptotic homologue c-FLIP, which is up-regulated in many tumor types and involved in resistance to chemotherapy and death receptor induced apoptosis [7].

Sensitivity of cancer cells to death ligand induced apoptosis has resulted in development of death receptor targeted drugs as anti-cancer agents. Because systemic administration of Fas targeted agents caused severe hepatotoxicity in mice [8,9], only therapies directed at local administration are now investigated [10]. The recombinant human (rh) form of TRAIL and agonistic antibodies targeting DR4 and DR5 show efficacy in numerous tumor cell lines, including ovarian cancer cell lines and in various xenograft tumor models in mice, without side effects [11]. These results have led to clinical studies which showed that these agents are well tolerated [12–14].

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Considering the development of targeted therapies for TRAIL receptor and Fas activation, assessing expression of key proteins of the extrinsic pathway in ovarian cancer is of interest. Alterations in the expression of FasL, TRAIL, its receptors [15,16], caspase 8 [15,17] and c-FLIP [7] have been implied in carcinogenesis and may hamper future therapies directed at death receptors. Furthermore, because response to chemotherapeutic drugs can be mediated through death ligand dependent and independent activation of caspase 8, these alterations may cause resistance to chemotherapy [18–20]. Robust co-expression data of proteins involved in the extrinsic pathway may define occurrence of these alterations in tumors and their impact on prognosis. Furthermore, they may assist in patient selection for future therapies targeting the extrinsic pathway.

Therefore, the aim of this study was to evaluate protein expression of Fas, FasL, TRAIL, DR4, DR5, caspase 8 and c-FLIP on a tissue microarray (TMA) containing tumor tissue of 382 ovarian cancer patients and to correlate these expression profiles with clinicopathological characteristics and disease outcome.

Materials and methods

Patients

From ovarian cancer patients treated since 1985 at the University Medical Center Groningen or affiliated hospitals all clinical, pathological and follow-up data have prospectively been stored in a database. Tumor samples from 382 patients were collected on a TMA. Patients with borderline or non-epithelial tumors were excluded. Primary treatment for all patients consisted of surgery and 90% of the patients eligible for systemic treatment received platin-based regimens as described previously [21]. Primary tumor samples obtained at surgery before any systemic treatment was administered were available for 359 patients. When residual tumor mass was present, response to chemotherapy was determined after three or six cycles based on World Health Organization criteria. Intervention surgery was performed after three chemotherapy cycles and second look surgery after six when indicated. Follow-up lasted up to 10 years. All relevant data were filed in a separate anonymous database in which patients were given unique codes to protect patient identity. Database management was restricted to two people with access to the larger database containing all patients' characteristics. Due to these procedures no additional patient or institutional review board approval was required according to Dutch Law.

TMA construction

TMAs were constructed as described previously [21]. Representative tumor tissue samples were selected from hematoxylin and eosin stained slides. Four 0.6-mm cores were punched from each donor block and put into 12 recipient paraffin TMA blocks. Each array contained 240 tissue cores, representing 55 tumor samples in quadruplicate and 10 internal controls in duplicate (composed of five tumor, one benign and four non-tumor samples). From each block 4-μm sections were cut and mounted on aminopropyltriethoxysilane-treated slides.

Antibodies

The TMAs were stained with polyclonal goat anti-TRAIL (1:100, clone K-18, Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal goat anti-DR4 (1:100, clone C20, Santa Cruz Biotechnology), polyclonal rabbit anti-DR5 (1:100, clone PC392, Calbiochem, San Diego, CA), monoclonal mouse anti-Fas (1:50, clone CH-11, Upstate Biotechnology, Temecula, CA), polyclonal rabbit anti-FasL (1:100, clone N-20, Santa Cruz Biotechnology), monoclonal mouse anti-caspase 8 (1:100, clone 1C12, Cell Signaling Technology, Danvers,

MA) and monoclonal mouse anti-c-FLIP, detecting both FLIP_L and FLIP_S (1:10, clone NF6, Alexis, Lausanne, Switzerland).

Immunohistochemistry

Staining procedures for all antibodies were performed as described previously [22–25]. After deparaffinization in xylene and re-hydration in ethanol, antigen retrieval was performed by incubation in citrate buffer at 96 °C for DR5 and FasL or high pressure cooking for c-FLIP and caspase 8. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in all slides except those for Fas detection. Avidin/biotin blocking solutions (Vector Laboratories, Burlingame, UK) were applied for DR4, DR5 and TRAIL. Prior to primary antibody incubation, slides were pre-incubated with 1% human AB serum (DR4, DR5 and FasL) or normal rabbit serum (TRAIL). Primary antibody incubation for c-FLIP was overnight; other antibodies were applied for 1 h. c-FLIP staining was detected by incubation with EnVision (DAKO, Glostrup, Denmark), caspase 8 staining with rabbit anti-mouse peroxidase antibody (DAKO), followed by goat anti-rabbit peroxidase antibody (DAKO) and for all other stainings with appropriate biotinylated secondary antibodies and peroxidase-labeled streptavidin (DAKO). Peroxidase activity was visualized with diaminobenzidine. Slides were counterstained with hematoxylin.

Normal tissue (kidney for FasL, liver for Fas) and tumor sections found positive on previous occasions served as positive control for Fas, FasL, DR4, DR5 and TRAIL staining. Negative controls were obtained by omission of the primary antibody, and by incubation with normal isotype controls. For caspase 8 and c-FLIP controls were used as described previously [24].

All sections were simultaneously reviewed by two observers (E.W.D. and W.B.-v.E.), without knowledge of the clinical data. Independent scoring was performed prior to simultaneous evaluation with agreement of >90% for all stainings. Discordant cases and final scoring were reviewed with a gynecological pathologist (H.H.) and assigned on consensus of opinion. Cores containing <10% tumor tissue and all cases with <2 cores were excluded from final analysis. Staining intensity was estimated and scored semi-quantitatively in four classes for DR4, DR5 and caspase 8 as negative (0), moderate (1), positive (2) and strong positive (3). Staining for Fas, FasL, TRAIL and c-FLIP was scored in three classes as negative (0), moderate (1) and positive (2). If heterogeneous staining intensity occurred between four cores of the same tumor, the highest staining intensity was chosen for final scoring if the core with highest staining contained >50% tumor tissue. For statistical analysis all classes were initially studied separately and then dichotomized. For DR4, DR5 and caspase 8, categories 2 and 3 were considered positive and 1 and 0 as negative. For TRAIL, FasL and Fas, 2 was considered positive and 1 and 0 as negative. For c-FLIP staining 2 and 1 were considered positive and 1 as negative [24].

Statistical analysis

Statistical analysis was performed with SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL). Comparisons between categorical variables were made with χ^2 tests or Fisher exact tests where appropriate. Comparisons between unpaired tumor samples obtained before and after chemotherapy were made using Mann–Whitney U tests. To exclude the possibility of a type I error in these multiple comparisons, p values <0.01 were considered statistically significant. Response to chemotherapy was analyzed using logistic regression analysis for patients with a residual tumor mass ≥ 2 cm receiving platinum-based chemotherapy ($n = 141$). Differences in progression-free survival and disease-specific survival were analyzed with two-sided log-rank testing and Cox proportional hazards analysis. p values <0.05 were considered significant. Progression-free and disease-specific survival were defined, respectively, as time from primary surgery until date of progression or relapse and as time from primary surgery until death

Table 1
Clinicopathological characteristics of the patients ($n = 382$).

	n	%
FIGO stage		
Stage I	73	19.1%
Stage II	36	9.4%
Stage III	223	58.4%
Stage IV	47	12.3%
Missing	3	0.8%
Tumor type		
Serous	226	59.2%
Mucinous	46	12.0%
Clear cell	48	12.6%
Endometrioid	20	5.2%
Adenocarcinoma NOS	19	5.0%
Other	23	6.0%
Tumor grade		
Grade I	68	17.8%
Grade II	90	23.6%
Grade III	159	41.6%
Undifferentiated	14	3.7%
Missing	51	13.4%
Residual disease		
<2 cm	183	47.9%
≥2 cm	173	45.3%
Missing	26	6.8%
Type of chemotherapy		
No chemotherapy	55	14.4%
Platinum monotherapy	164	42.9%
Platinum/taxane containing	119	31.2%
Other regimen	40	10.5%
Missing	4	1.0%
Age at diagnosis (years)		
Median	58.4	
Range (years)	21.8–89.8	
Follow-up (months)		
Median	29.3	
Range (months)	0–213	

due to ovarian cancer. For multivariate analysis, age at diagnosis (<58 years (median), ≥58 years), FIGO stage (I/II (early), III/IV (late)), tumor type (serous, non-serous), tumor grade (grade I/II, grade III/undifferentiated) and residual tumor size after primary surgery (<2 cm, ≥2 cm) were used as covariates.

Results

Patient characteristics

Clinicopathological data are summarized in Table 1. Median follow-up time was 29.3 months (range 0–213); one patient was lost to follow-up. Three hundred seventy-six patients (98.4%) received primary surgery, whereas 6 patients (1.6%) received chemotherapy prior to debulking surgery. Debulking surgery with a residual tumor mass of <2 cm was achieved in 75 (30.5%) late stage patients. Three hundred twenty-three (84.6%) patients received first line chemotherapy, of whom 173 (49.0%) received it as adjuvant therapy with no evidence of residual tumor. In 55 patients no chemotherapy was administered because of stage Ia disease (34 patients, 9%), ineligibility or patient refusal. At the time of data analysis, 22 (20.4%) early and 184 (68.1%) late stage patients had died of ovarian cancer, 3 (2.8%) early and 38 (14.1%) late stage patients were alive with disease and the other patients were alive without evidence of disease. Median progression-free survival was 49.6 (range 0–207) months for early and 11.5 (range 0–149) months for late stage patients. Median disease-specific survival was 57.6 (range 0–207) months and 19.7 (range 0–213) months for early and late stage patients, respectively.

Associations of proteins with clinicopathological characteristics

Staining results were obtained in 92.8–94.7% of primary tumors available ($n = 359$) (Table 2). Staining for all proteins was cytoplasmic, with no apparent membranous staining (Fig. 1).

Most tumors expressed DR5, DR4, caspase 8 and c-FLIP (Table 2). Combining data of 322 tumors with expression results on both DRs showed that 70.8% of tumors expressed both death receptors, 26.7% expressed one receptor and only 2.5% expressed neither DR4 nor DR5. Positive c-FLIP expression was associated with higher differentiation grade ($p = 0.049$). TRAIL, Fas and FasL were less frequently expressed. TRAIL expression was more frequent in tumors of low grade ($p = 0.006$) and reduced in late stage tumors ($p = 0.01$). FasL and Fas expression occurred more often in low grade tumors ($p = 0.03$ and $p < 0.001$, respectively) and were associated with a smaller residual tumor mass after primary surgery ($p = 0.01$ and $p = 0.02$,

Table 2
Staining results and clinicopathological characteristics.

	DR4 (%)	p	DR5 (%)	p	Caspase 8 (%)	p	c-FLIP (%)	p	TRAIL (%)	p	FasL (%)	p	Fas (%)	p
Age														
<58 years	129/169 (76.33)	0.27	159/169 (94.1)	0.62	68/87 (78.2)	0.60	141/166 (84.9)	0.88	33/166 (19.9)	0.25	53/167 (31.7)	0.09	19/166 (11.5)	0.86
>58 years	116/164 (70.7)		161/168 (95.8)		91/112 (81.3)		146/174 (83.9)		26/174 (14.9)		71/174 (40.8)		18/169 (10.7)	
Stage														
Early stage	70/98 (71.4)	0.58	88/93 (94.6)	0.78	73/93 (78.5)	0.70	81/97 (83.5)	0.74	25/97 (25.8)	0.01	40/97 (41.2)	0.26	16/96 (16.7)	0.05
Late stage	174/232 (75)		230/241 (95.4)		198/239 (82.8)		204/240 (85.0)		33/240 (13.8)		82/241 (34.0)		21/236 (8.9)	
Histology														
Serous	148/192 (77.1)	0.10	191/199 (96.0)	0.32	113/144 (78.5)	0.44	168/197 (85.3)	0.65	33/198 (16.7)	0.77	65/197 (33.0)	0.14	20/196 (10.2)	0.60
Non-serous	97/141 (68.8)		129/138 (93.5)		46/55 (83.7)		119/143 (83.2)		26/142 (18.3)		59/144 (41.0)		17/139 (12.2)	
Grade														
Grade I/II	97/137 (70.8)	0.36	131/137 (95.6)	0.61	42/55 (76.4)	0.55	113/139 (81.3)	0.049	34/140 (24.3)	0.006	60/140 (42.9)	0.03	26/139 (18.7)	<0.001
Grade III/ undiff	119/157 (75.8)		150/160 (93.8)		99/122 (81.1)		143/160 (89.4)		19/158 (12.0)		48/149 (30.2)		5/158 (3.2)	
Residual tumor														
<2 cm	113/164 (68.9)	0.10	154/162 (95.1)	1.00	46/58 (79.3)	1.00	135/165 (81.8)	0.38	36/165 (21.8)	0.08	69/165 (41.8)	0.01	22/163 (13.5)	0.02
≥2 cm	114/147 (77.6)		145/153 (94.8)		103/130 (79.2)		131/153 (85.6)		21/153 (13.7)		44/154 (28.6)		8/150 (5.3)	

p values are derived from χ^2 analysis.

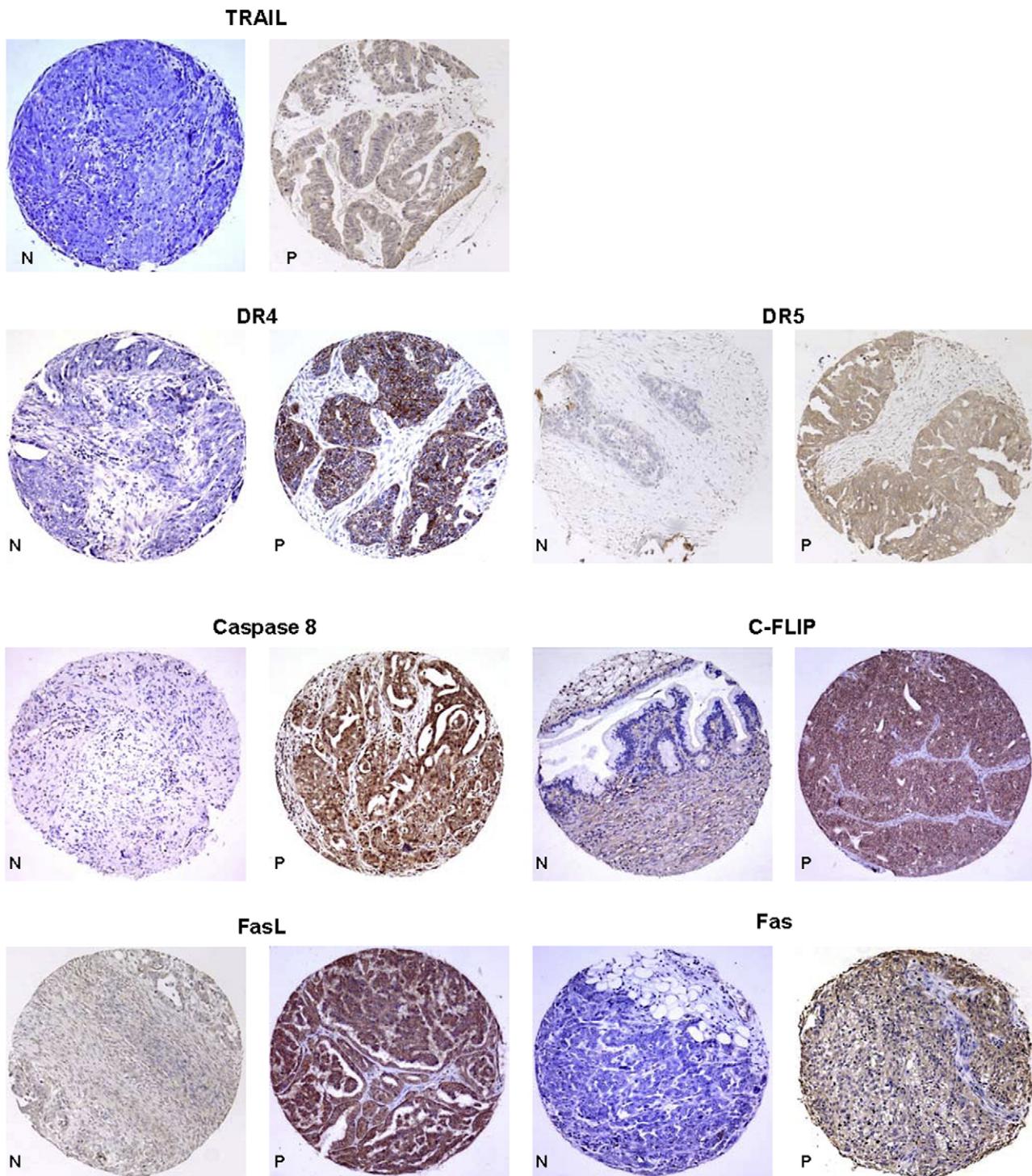


Fig. 1. Results of immunostaining for TRAIL, DR4, DR5, caspase 8, c-FLIP, FasL and Fas. N stands for negative and P for positive staining (magnification $\times 10$).

respectively). When early and late stage tumors were analyzed separately, TRAIL was not associated with grade, while late stage tumors of low grade expressed FasL and Fas more frequently ($p=0.02$ and $p=0.001$, respectively). After adjustment for multiple testing, only the associations of TRAIL and Fas with low grade tumors sustained.

Associations between proteins

Comparison of protein expression profiles of biological relevance revealed several associated profiles (Supplementary Table 1A and B).

In early stage tumors, DR5 was positively associated with caspase 8 staining ($p=0.008$). In late stage tumors, DR4 staining correlated with positive FLIP staining ($p=0.001$) and negative TRAIL staining ($p=0.007$). Strikingly, 75.7% of the tumors expressing both DRs also expressed c-FLIP, while tumors with reduced expression of at least one DR were more often associated with negative c-FLIP expression (60.5%) ($p<0.0001$) (Fig. 2). These associations were also observed for early ($p=0.004$) and late stage tumors ($p=0.001$) separately. Caspase 8 staining was positively correlated with c-FLIP ($p=0.008$) in late stage tumors. Finally, in late stage tumors negative Fas expression was associated with negative FasL staining ($p=0.008$). For

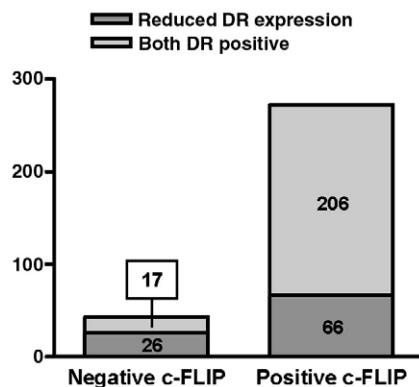


Fig. 2. Death receptor expression in relation to c-FLIP expression in early and late stage ovarian tumors.

188 patients data on p53 staining were available [21]. No relationships were identified between the protein expression of the proteins under study and p53.

Protein expression in pre- and post-chemotherapy tumor samples

Comparison of staining patterns in paired tumor samples ($n = 43$) revealed no alterations in protein expression profiles after chemotherapy. When primary tumor samples were compared with all post-chemotherapy samples available, Fas expression was reduced in post-chemotherapy samples ($p = 0.048$).

Response to chemotherapy and survival in relation to protein staining

To assess the presence of a correlation between expression of the proteins under study and response to chemotherapy, univariate regression analysis was performed in 141 patients with a residual tumor mass ≥ 2 cm after initial surgery, who received platinum-based chemotherapy. Expression profiles were not correlated with response to chemotherapy.

Positive TRAIL expression was associated with a better progression-free survival in log-rank tests and univariate Cox proportional hazard analysis (HR 0.63, 95% CI 0.42–0.92, $p = 0.018$) (Supplementary Table 2). However, this association was not found when the data were analyzed separately in early and late stages (Fig. 3). Positive Fas staining was associated with better progression-free and disease-specific survival ($p = 0.012$ and $p = 0.008$, respectively) (Figs. 4A and B), which was also observed in Cox proportional hazard analyses (HR 0.54, 95% CI 0.33–0.88, $p = 0.012$ and HR 0.49, 95% CI 0.28–0.84 $p = 0.009$, respectively). In subgroup analysis Fas was not associated with survival in early and late stage tumors.

In multivariate analysis only advanced stage and a residual tumor ≥ 2 cm after primary surgery were independent predictors of poor progression-free survival (HR 3.92, 95% CI 2.17–7.084, $p < 0.0001$ and HR 1.94, 95% CI 1.33–2.83, $p = 0.001$, respectively) and disease-specific survival (HR 3.3, 95% CI 1.73–6.29, $p < 0.0001$ and HR 2.11, 95% CI 1.41–3.16, $p < 0.0001$, respectively).

Discussion

In the largest study to date analyzing the protein expression of the death ligands TRAIL, FasL, their cognate agonistic receptors, caspase 8 and c-FLIP in ovarian cancers, we showed that the majority of cancers expressed at least one death receptor, as well as caspase 8 and its anti-apoptotic homologue c-FLIP. Moreover, these data show that derangement of the Fas/FasL system, which is implied in malignant transformation of the ovaries [26] is indeed the case in human ovarian cancer. In addition, ovarian tumors that have retained Fas expression are better differentiated and have a better progression-free and disease-specific survival, which support data showing that loss of Fas expression is implied in dedifferentiation and acquisition of a higher malignant potential in several cancers [27–29]. Previous studies examining protein expression of Fas, FasL or both in ovarian cancers showed substantial variation which can be explained by small sample sizes, inclusion of tumors classified as benign, borderline and malignant and different use of antibodies and scoring systems [23,30–32]. Moreover, in agreement with a previous study [33] we used cut-off values for definition of positive or negative staining based on dichotomization of staining classes according to their association with prognosis.

FasL expression is commonly reported to increase with malignant progression and tumor grade, which was not observed in our study [29,32]. In addition, FasL expression was not associated with a worse prognosis and is therefore not supportive for the tumor counterattack hypothesis [34] in ovarian cancers.

TRAIL expression was associated with lower tumor grade and better progression-free survival when all tumors were analyzed. In previous studies in ovarian cancers TRAIL was also associated with low tumor grade [35] and early stage [22,35], but not with prognosis [22,35,36]. Among colon adenomas and carcinomas loss of TRAIL expression occurred in a subset of colon carcinomas [37] and in samples spanning oral cancer progression it was an early event in carcinogenesis [38]. These results suggest that loss of TRAIL expression represents a survival advantage for tumor cells, possibly because they evade apoptosis induction by para- or autocrine released TRAIL. This is supported by a study which showed that in response to interferon-gamma Ewing tumor cells produce and secrete functional TRAIL that induces apoptosis in unstimulated Ewing tumor cells [39].

The majority of tumors in our study expressed DR4, DR5, caspase 8 and c-FLIP. A striking finding was the association of c-FLIP

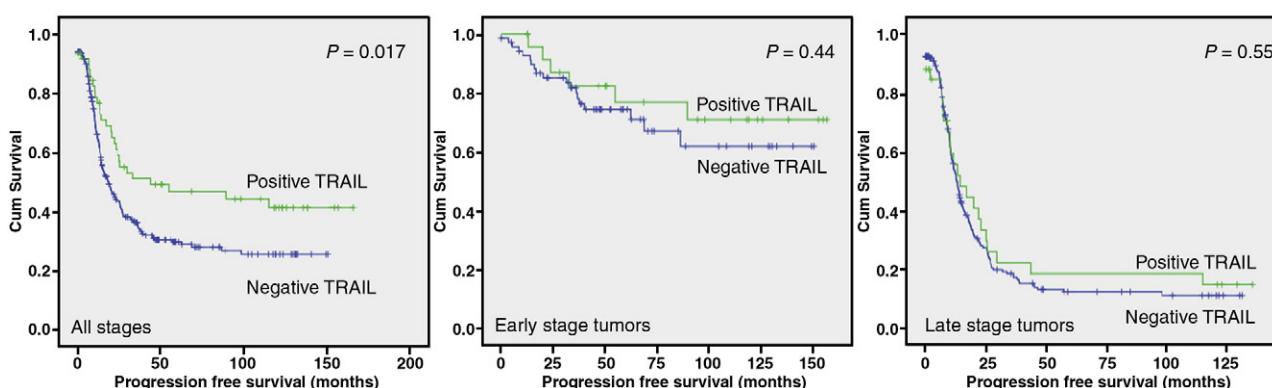


Fig. 3. Progression-free survival according to TRAIL expression in all patients and early and late stage tumors separately.

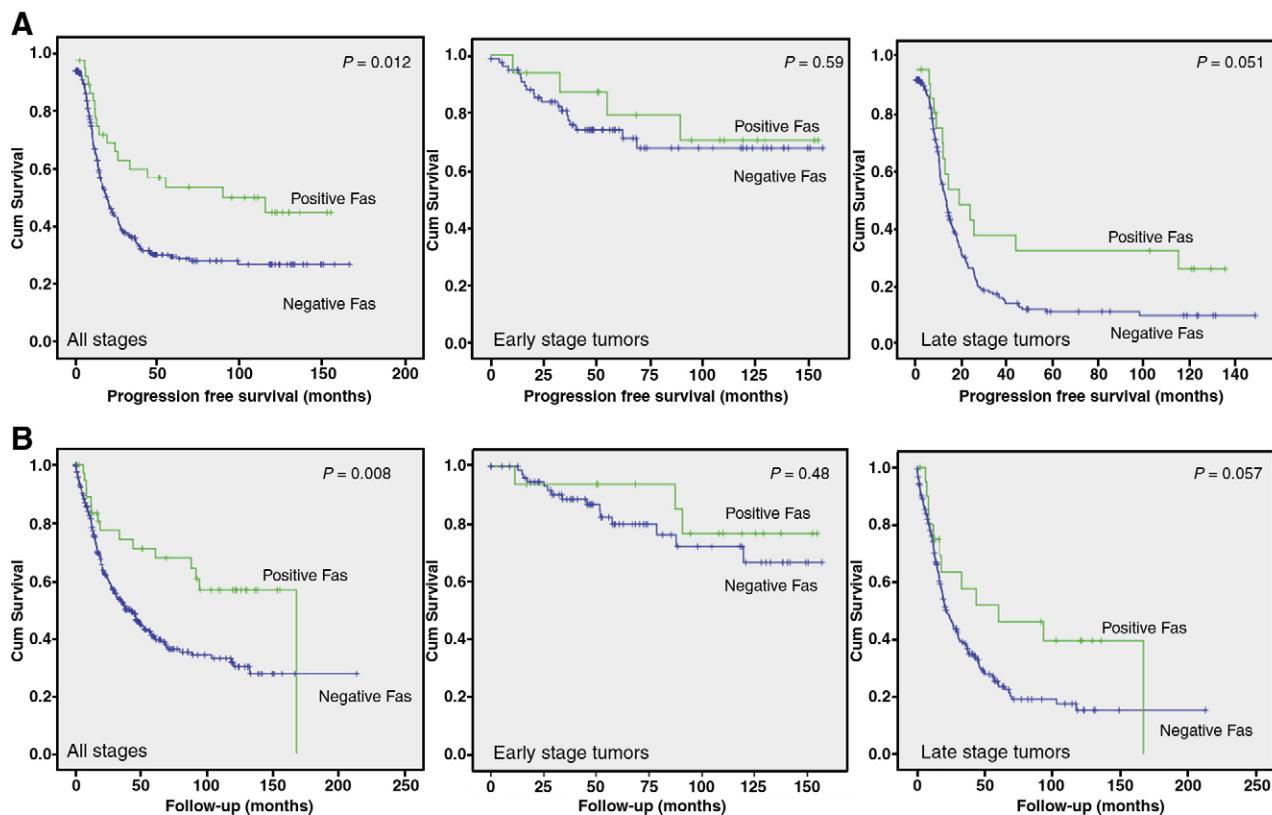


Fig. 4. (A) Progression-free survival according to Fas expression in all patients and early and late stage tumors separately. (B) Disease-specific survival according to Fas expression in all patients and early and late stage tumors separately.

expression with expression of both death receptors and with caspase 8, which suggests that apoptotic death receptor signaling is counteracted in ovarian cancers. These associations were, however, not correlated with prognosis, nor were the individual proteins. Up-regulation of the anti-apoptotic caspase 8 homologue c-FLIP was reported in several tumor types [7] and was associated with a poor clinical outcome in Burkitt lymphomas [40] and bladder urothelial carcinomas [41]. In vitro, c-FLIP induces resistance to Fas and TRAIL receptor targeted drugs in vitro [42–45] and is therefore a target for modulating death receptor induced apoptosis. In colon cancer patients, high DR4 expression was an independent prognostic factor for worse disease-free and overall survival [46]. High DR5 expression was associated with decreased survival in univariate analysis in ovarian cancers [35] and was independently associated with decreased survival in breast and small lung cancers [47,48]. These different results underline the complexity of death receptor signaling, which is not only dependent on expression of its constituents, but also on external factors and the intracellular apoptotic machinery and might therefore be tissue specific. Moreover, it becomes increasingly evident that single prognostic factors, e.g., HER2 and hormone receptors in breast and c-kit in GIST tumors, are rather an exception than the rule. Considering the redundancy of signaling pathways, it is not surprising that in most tumors numerous factors are likely to influence prognosis [49]. Furthermore, although alterations in the death receptor pathway are involved in chemoresistance [19,50,51], the main tumorcidal mechanism of most conventional drugs is not likely to act through the extrinsic pathway. Therefore, our results show that in ovarian cancers loss of TRAIL and Fas expression represents an important aspect in dedifferentiation and escape from tumor immune surveillance. In addition, deregulation of the extrinsic pathway by c-FLIP expression occurs, but these changes are not of critical significance for disease outcome. They may, however, be of significance for future therapies targeting the extrinsic pathway. Clinical studies with rhTRAIL,

agonistic antibodies directed at DR4 or DR5 and Fas are ongoing. Membranous DR expression on tumors is a pre-requisite for these drugs to be effective as anti-cancer agents, but functionality of the downstream signaling pathway is of equal importance. Therefore, it needs to be established whether these protein expression profiles correlate with functionality of the death receptor pathway in ovarian cancers, which can be achieved by relating clinical responses to TRAIL receptor agonists with tumor characteristics.

Resistance to death receptor targeted agents and to conventional chemotherapies can be overcome by combining these drugs. Many different mechanisms were described to be involved in this synergy, including down-regulation of c-FLIP [52]. Consequently, combinations of conventional therapeutics and death receptor drugs warrant further development as novel strategies for cancer treatment.

In conclusion, loss of Fas and TRAIL is associated with dedifferentiation and a worse prognosis in ovarian cancers. Expression of anti-apoptotic c-FLIP is associated with caspase 8 and death receptor expression, which should be considered for future death receptor targeted therapies in ovarian cancer.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygyno.2009.09.014.

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Analysis of Death Receptor 5 and Caspase-8 Expression in Primary and Metastatic Head and Neck Squamous Cell Carcinoma and Their Prognostic Impact

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Abstract

Death receptor 5 (DR5) and caspase-8 are major components in the extrinsic apoptotic pathway. The alterations of the expression of these proteins during the metastasis of head and neck squamous cell carcinoma (HNSCC) and their prognostic impact have not been reported. The present study analyzes the expression of DR5 and caspase-8 by immunohistochemistry (IHC) in primary and metastatic HNSCCs and their impact on patient survival. Tumor samples in this study included 100 primary HNSCC with no evidence of metastasis, 100 primary HNSCC with lymph node metastasis (LNM) and 100 matching LNM. IHC analysis revealed a significant loss or downregulation of DR5 expression in primary tumors with metastasis and their matching LNM compared to primary tumors with no evidence of metastasis. A similar trend was observed in caspase-8 expression although it was not statistically significant. Downregulation of caspase-8 and DR5 expression was significantly correlated with poorly differentiated tumors compared to moderately and well differentiated tumors. Univariate analysis indicates that, in HNSCC with no metastasis, higher expression of caspase-8 significantly correlated with better disease-free survival and overall survival. However, in HNSCC with LNM, higher caspase-8 expression significantly correlated with poorer disease-free survival and overall survival. Similar results were also generated when we combined both DR5 and caspase-8. Taken together, we suggest that both DR5 and caspase-8 are involved in regulation of HNSCC metastasis. Our findings warrant further investigation on the dual role of caspase-8 in cancer development.

Citation: Elrod HA, Fan S, Muller S, Chen GZ, Pan L, et al. (2010) Analysis of Death Receptor 5 and Caspase-8 Expression in Primary and Metastatic Head and Neck Squamous Cell Carcinoma and Their Prognostic Impact. PLoS ONE 5(8): e12178. doi:10.1371/journal.pone.0012178

Editor: Torbjörn Ramqvist, Karolinska Institutet, Sweden

Received May 4, 2010; **Accepted** July 14, 2010; **Published** August 16, 2010

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Funding: This work was supported by the Georgia Cancer Coalition Distinguished Cancer Scholar award (SYS) and National Institutes of Health SPORE P50 grant CA128613 (SYS and FRK for Project 2). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

More than 35,000 people in the United States and more than 500,000 worldwide are estimated to be diagnosed with head and neck squamous cell carcinoma (HNSCC) annually [1,2]. The presence of metastasis in patients with head and neck cancer is common and the 5-year survival rate for patients with lymph node metastasis is approximately 25–50% [3]. Better treatments for metastatic HNSCC are urgently needed. However, our understanding of the factors that regulate metastasis in this disease is limited.

Death receptor 5 (DR5) is one of the cell surface receptors that when activated by its ligand, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), induces the activation of the extrinsic apoptotic pathway in humans [4]. DR5 has been shown to be overexpressed in several types of cancer including colon, lung and cervical cancer [5–9]. Increased DR5 expression was also associated with reduced survival in non-small cell lung cancer [7,9]. A recent mouse study has shown that deficiency of TRAIL receptor in mice (only one receptor for TRAIL in mouse) enhances lymph node metastasis (LNM) without affecting primary tumor

development [10], suggesting that TRAIL receptor or TRAIL-TRAIL receptor interaction may be critical for regulation of tumor metastasis. Agonistic antibodies targeting DR5 are currently in clinical trials for treatment of various types of cancer [11]. Currently, the role of DR5 in metastasis is unknown and the expression of DR5 in primary and metastatic HNSCC has not been examined.

Caspase-8 is the first caspase activated during death receptor-initiated apoptosis [12]. There is evidence of increased expression of caspase-8 in several types of cancer including colorectal and rectal, gastric, pancreatic, and breast cancers [13,14–17]. Besides, it has been also shown that caspase-8 expression is lost or inactivated in certain types of cancer such as small cell lung cancer, neuroblastoma, gastric carcinoma and hepatocellular carcinoma [18–25]. Loss of caspase-8 has been associated with metastasis in neuroblastoma [26]. However, it has also been recently shown that caspase-8 is associated with cell migration and can promote metastasis in apoptotic resistant cells [27,28]. Moreover, a loss of caspase-8 was reported to be associated with unfavorable survival in childhood medulloblastoma [29]. Caspase-8 expression in HNSCC, particularly in metastatic HNSCC, has not been documented.

Thus, this study was particularly interested in comparing the expression patterns of DR5 and caspase-8 between primary HNSCC without LNM and HNSCC with LNM. To this end, we performed immunohistochemistry (IHC) to detect DR5 and caspase-8 on three groups of tumor samples from patients with either primary tumors with no evidence of LNM, primary tumors with LNM and the matching LNM.

Materials and Methods

Tissue Specimens

This study was approved by the Institutional Review Board at Emory University. Tissues were obtained from surgical specimens of patients who had HNSCC diagnosed at Emory University Hospital and whose initial treatment was surgery without receiving prior treatment with radiation and/or chemotherapy. The selection criteria of the available formalin-fixed and paraffin-embedded tissue blocks included 2 patient groups: primary HNSCC with LNM (Tu^{+met}), their paired LNM, and primary HNSCC with negative LNM (Tu^{-met}). In the Tu^{-met} group, if any patient developed metastases within 2 years of the initial procedure, they were excluded from the study. Each category has 100 samples. The clinical information on the samples was obtained from the surgical pathology files in the Department of Pathology at Emory University according to the regulations of the Health Insurance Portability and Accountability Act (HIPAA). This was a retrospective study which used tissue samples from surgical specimens dated prior to April 14, 2003 and therefore was exempt for consent requirement from HIPPA regulations. The clinicopathologic parameters for the 2 study groups, including age, gender, smoking history, tumor location, and histologic grade are listed in Table 1.

IHC

Formalin-fixed, paraffin-embedded tissue sections were used for IHC. Tissues were deparaffinized, hydrated through graded ethanol, and microwaved in 100 mmol/L sodium citrate for 5 minutes at high power and 10 minutes at low power for antigen retrieval. Detection of caspase-8 and DR5 was performed following the DAKO Visualization System instructions using 3,3-diaminobenzidine tetrahydrochloride substrate to visualize the proteins (DAKO, Carpinteria, CA). The slides were incubated with caspase-8 polyclonal antibody (1:100 dilution) (NeoMarkers, Fremont, CA) or DR5 polyclonal antibody (1:250 dilution) (ProSci, Inc., Poway, CA) overnight at 4°C.

Both percentage of positive staining in tumor cells and intensity of staining were scored. The intensity of IHC staining was measured by using a numerical scale (0 = no expression, 1 = weak expression, 2 = moderate expression, 3 = strong expression). The staining data were finally quantified as the weighted index (WI) (WI = % positive stain in tumor × intensity score) as previously described [30,31]. The WI was determined by 2 individuals, and the final values were the average of the two readings.

Statistical Analysis

Median differences of the WIs for Caspase-8 and DR5 among different groups were assessed with Mann-Whitney-Wilcoxon rank test. Median differences between paired samples Tu^{+met} and LNM were analyzed with Wilcoxon signed rank test. Correlations of the WIs and the clinical characteristics for caspase-8 and DR5 were performed in all the patients and within each group and in the combined sample (Tu^{-met} and Tu^{+met}) after adjusting with patients' metastatic status. Logistic regression model was applied to assess association between the WIs and binary variables (gender

Table 1. Clinic-pathologic features of the non-metastatic and metastatic patient groups.

Clinical Parameters	Non-metastatic Group	Metastatic Group
Average age (years)	62.5	60.4
Gender		
Men	62	68
Women	41	33
Smokers	81*	91**
Tumor location		
Oral cavity	62	40
Oropharynx	7	33
Larynx	34	28
Tumor classification		
T1	42	24
T2	31	38
T3	14	17
T4	15	22
Lymph node status		
N1	-	19
N2	-	74
N3	-	8
Histologic grade		
WD	30	3
MD	60	75
PD	11	23

*Six patients with unknown smoking status.

**Four patients with unknown smoking status.

doi:10.1371/journal.pone.0012178.t001

and smoking status). Kruskal-Wallis tests were performed for categorical variables with more than two categories (tumor site, tumor size, node status and differentiation status). The Cox proportional hazards model was used for univariate and multivariable survival analysis for continuous Caspase-8 and DR5. The proportional hazards assumption was assessed using Schoenfeld residuals. Caspase-8 and DR5 were also dichotomized as low and high based on the observed mean value. The log-rank test was used to test whether Kaplan-Meier survival estimators with different Caspase-8 or DR5 levels are statistically different. Multivariable analyses were performed with those clinical variables shown to be statistically significant in the univariate analyses. All data processing and statistical analyses were conducted using SAS version 9 (SAS Institute, Cary, NC).

Results

Detection of DR5 Expression in HNSCC

IHC analysis of DR5 was performed on 100 samples in each group. A total of 94 samples in Tu^{-met} , 92 samples in Tu^{+met} and 85 samples in LNM group had acceptable tumor tissues for evaluation. All samples in Tu^{-met} and Tu^{+met} were positive for DR5 staining, whereas LNM tissues were 96% (82/85) positive for DR5 staining. Figure 1 shows examples of DR5 staining in different groups. DR5 was expressed primarily in the cytoplasm of tumor cells. Some tumor stromal cells including fibroblasts and immune cells were also positive for DR5. DR5 expression was often decreased or lost in Tu^{+met} and corresponding LNM

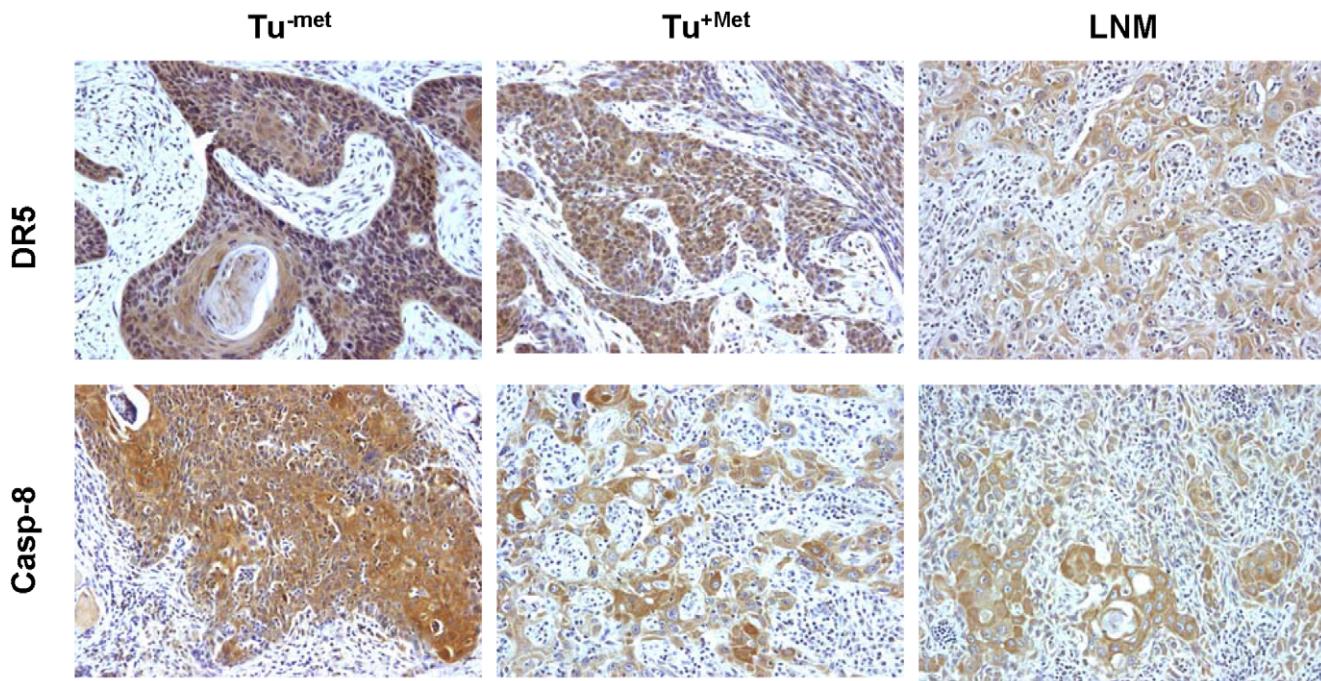


Figure 1. Representative IHC staining of DR5 and caspase-8 in different groups of HNSCC (200×).
doi:10.1371/journal.pone.0012178.g001

samples. The analysis of the WI for DR5 showed that there was a statistically significant difference between DR5 expression in Tu^{-met} and in Tu^{+met} and their matching LNM samples (Figure 2A). Specifically, primary tumors without LNM (Tu^{-met}) had significantly higher DR5 expression compared to both the primary tumors with LNM (Tu^{+met}) and to LNM.

Detection of Caspase-8 Expression in HNSCC

IHC analysis of caspase-8 was also conducted in 100 samples in each group. Among these samples, 96 Tu^{-met}, 91 Tu^{+met} and 86 LNM samples could be evaluated. Some examples of caspase-8 staining were presented in Fig. 1. Similar to DR5 staining, caspase-8 staining was also primarily cytoplasmic. Some tumor stromal cells including fibroblasts and immune cells were positive for caspase-8. Tumors in Tu^{-met} group exhibited a trend towards a higher WI of caspase-8 than those in Tu^{+met} and LNM groups; however, this result was not statistically significant (Fig. 2B).

DR5 Expression and its Correlation with Clinical Parameters

We further analyzed the correlation between DR5 expression and multiple clinical variables including gender, age at diagnosis, smoking status, tumor site, tumor size, tumor stage, histologic grade, node status, overall survival and disease free survival.

- 1) DR5 and tumor site. Univariate analysis showed that there was a significant difference in the location of the site of the tumor, characterized as oropharynx, larynx and oral cavity and the WI of DR5. Specifically, tumors in Tu^{+met} group arising in the oral cavity had significantly higher DR5 expression than tumors from this group that arose in the oropharynx or larynx ($P=0.0196$).
- 2) DR5 and histologic grade. The histologic grade of the tumor samples were characterized as well differentiated (WD), moderately differentiated (MD) and poorly differentiated

(PD). By univariate analysis, there was a significant correlation between histologic grade and DR5 expression. Primary tumors in Tu^{+met} and their matching LNM characterized as PD showed a significantly lower WI compared to MD and WD tumors in these groups (Figure 3A). Furthermore, multivariable analysis showed that in Tu^{+met} group, the PD tumors showed a significantly lower WI compared to tumors that were characterized as MD or WD ($P=0.0207$). When combining all the patient tumor samples, univariate and multivariable analysis showed that histologic tumor grade was significantly correlated with DR5 expression. Specifically those tumors identified as PD have a lower DR5 WI compared to tumors identified as MD and WD. WD tumors have a higher WI compared to MD tumors. This result is consistent with analysis of the DR5 WI and histologic grade when comparing each group of tumors (Tu^{+met}, LNM, and Tu^{-met}) where we saw a decrease in DR5 expression as the tumors became less differentiated.

- 3) DR5 and smoking status. Smoking status and DR5 expression was found to be significantly correlated when combining all tumor samples. By univariate and multivariable analysis, a lower DR5 WI was significantly associated with smokers compared to non-smokers when both groups of tumor samples were combined (i.e., Tu^{-Met} and Tu^{+Met}) ($P<0.05$).
- 4) DR5 and patient survival. We analyzed whether DR5 expression has any effect on patient survival. Neither univariate nor multivariable Cox proportional hazards model revealed significant association between DR5 expression and patient survivals in either Tu^{-met} or Tu^{+met} group ($P>0.05$). However, when DR5 expression was dichotomized into low or high in terms of the observed mean value, higher DR5 expression was significantly associated with poorer disease-free survival ($P=0.0458$) in Tu^{+met} group (Fig. 4B).

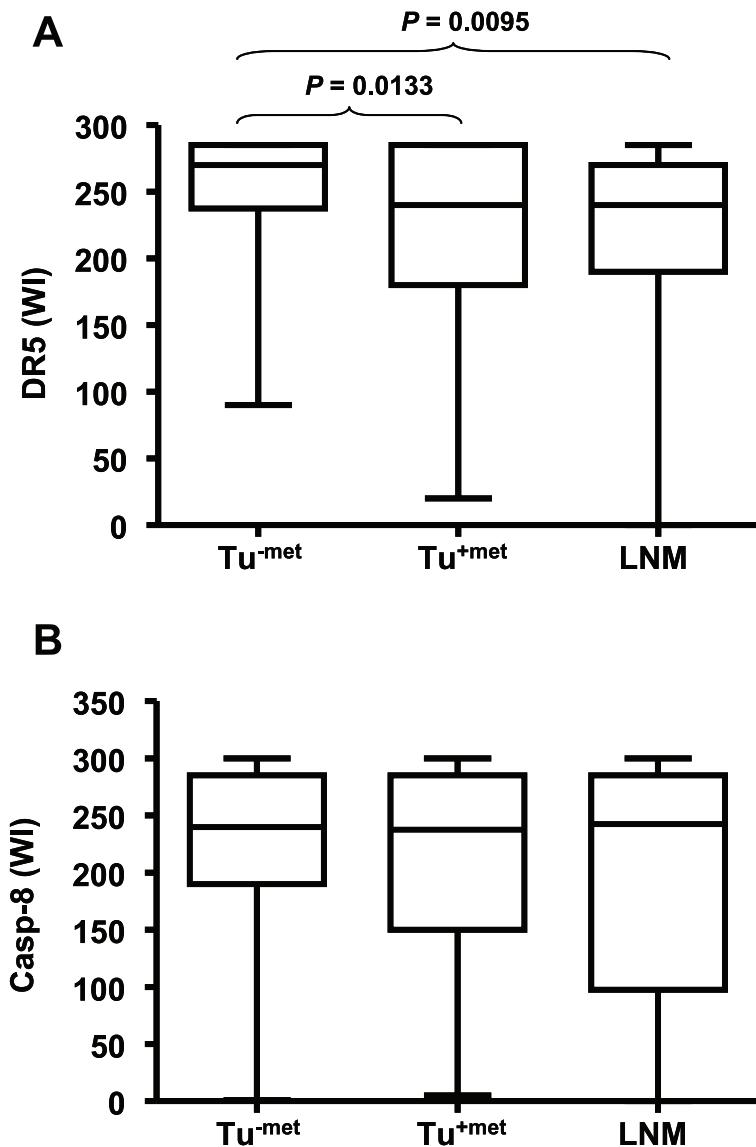


Figure 2. Comparison of DR5 (A) and caspase-8 (B) expression among different groups of HNSCC. Difference between two groups was evaluated with paired *t* test.
doi:10.1371/journal.pone.0012178.g002

Caspase-8 Expression and Its Correlation with Clinical Parameters

Similar to DR5, an analysis of the correlation between caspase-8 expression and the clinical parameters including gender, age at diagnosis, smoking status, tumor site, tumor size, tumor stage, nodal status, histologic grade, overall survival and disease-free survival was performed.

- 1) Caspase-8 and tumor site. Univariate analysis showed that there was a significant difference in tumor location and the WI of caspase-8. Tumors in Tu^{+met} group arising in the oral cavity and their matching LNM had significantly higher caspase-8 expression than tumors that arose in the oropharynx or larynx ($P < 0.05$).
- 2) Caspase-8 and histologic grade. By multivariable analysis, we found that caspase-8 expression was significantly reduced in PD primary tumors with metastasis (Tu^{+met}) compared to the MD and WD tumors in this group ($P < 0.05$). There were only three tumors characterized as WD in this group, so the WD

and MD tumors were combined and their WI compared to the PD tumors. By univariate analysis, the PD tumors in the matching LNM had significantly less caspase-8 expression as measured by the WI compared to the combined MD and WD tumors in this group (Fig. 3B).

- 3) Caspase-8 and patient survival. We examined the impact of caspase-8 on patient survival and found that caspase-8 expression correlated significantly with disease-free survival and overall survival. Specifically, Cox proportional hazards model showed that in tumors with no metastasis (Tu^{-met}), higher expression of caspase-8 was associated with better overall survival ($P = 0.0053$, HR = 0.994), while in tumors with LNM (Tu^{+met}), higher caspase-8 expression was associated with poorer overall survival ($P = 0.0347$, HR = 1.003). The Log-rank test with dichotomized caspase-8 level showed the same effects with overall survival (Figs. 5A and 5B). In addition, the same effect was also observed with disease free survival ($P = 0.0154$ and 0.0044 in Tu^{-met} and Tu^{-met} group

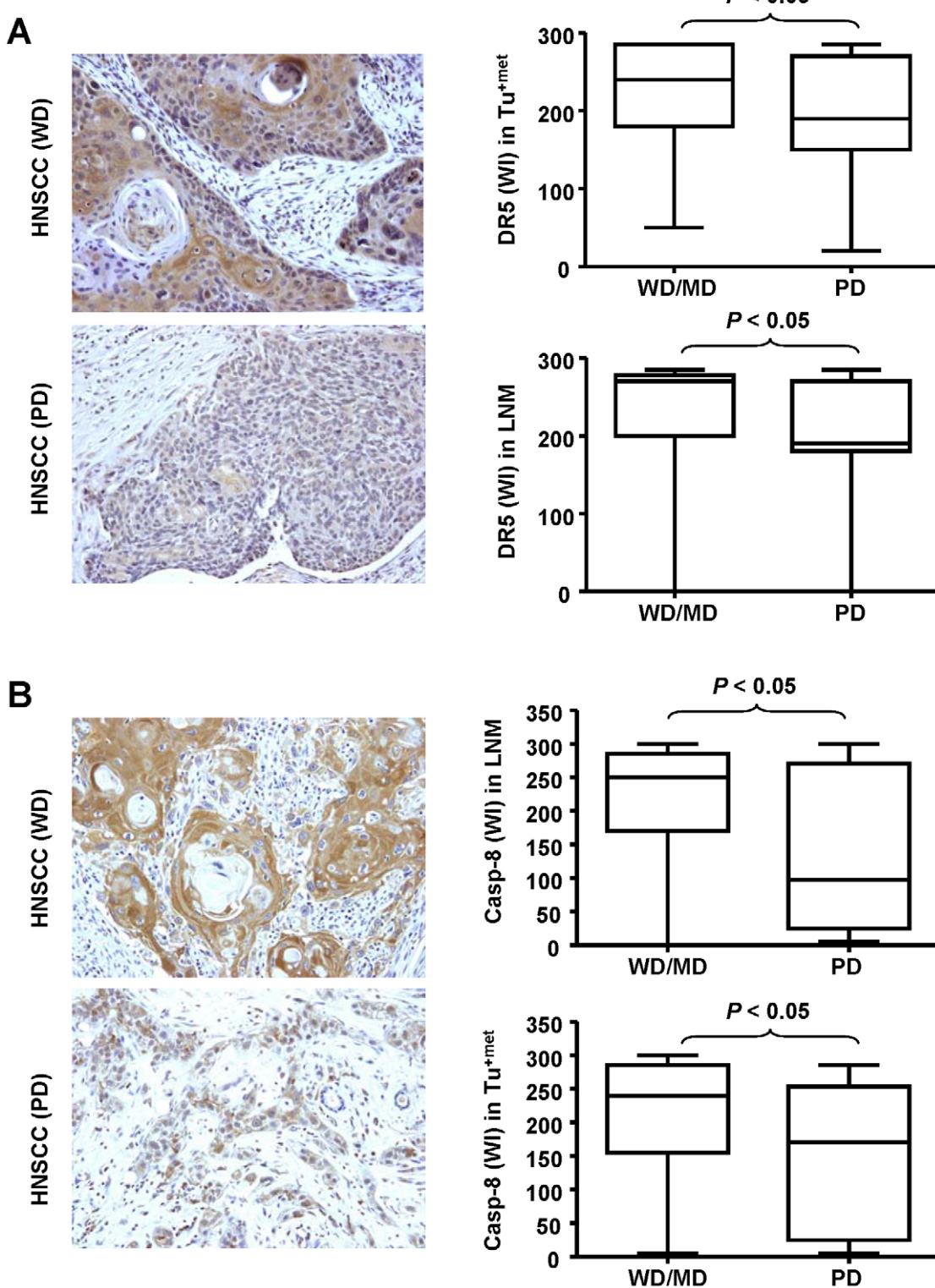


Fig. 3. Comparison of DR5 (A) and caspase-8 (B) between poorly differentiated (PD) and well differentiated (WD)/moderate differentiated (MD) HNSCC. Pictures are representative IHC staining of DR5 and caspase-8 (200X). Difference between two groups was evaluated with paired t test.

doi:10.1371/journal.pone.0012178.g003

correspondingly) (Figs. 5A and 5B). Multivariable analysis adjusting for age, tumor stage, gender, histologic grade, smoking, chemo and/or radiation therapy, and tumor site

showed that high caspase-8 (greater than mean) in the Tu^{-met} group was also significantly associated with better overall survival ($P=0.0026$, HR = 0.255; see supplemental Table S1).

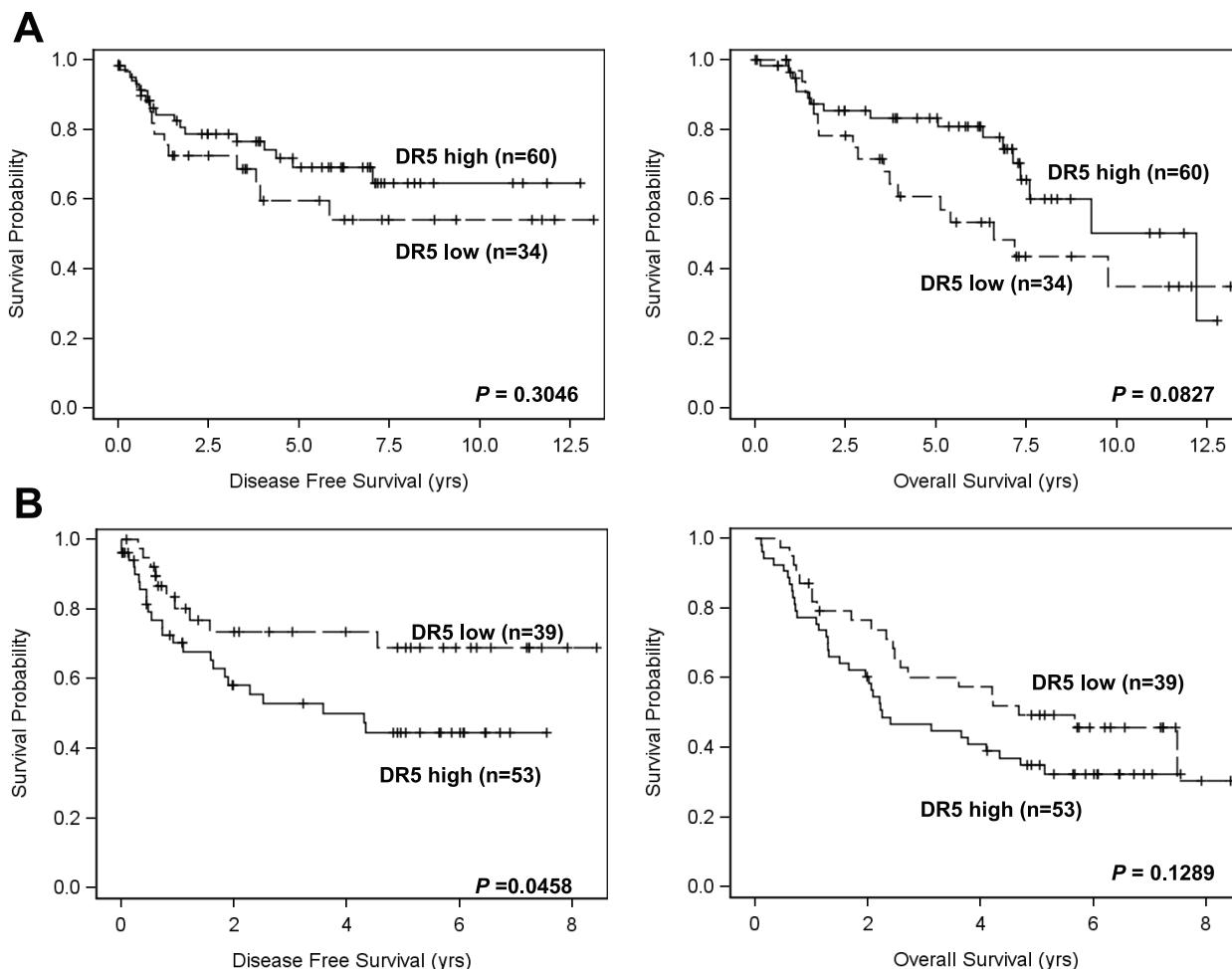


Figure 4. Impact of DR5 expression on disease-free survival and overall survival in HNSCC patients without LNM (Tu^{-met}) (A) and in HNSCC patients with LNM (Tu^{+met}) (B). Kaplan-Meier plots were generated according to high (greater than the mean value) and low (less than or equal to the mean value) levels of DR5.

doi:10.1371/journal.pone.0012178.g004

Impact of DR5 and Caspase-8 Combination on Patient Survival

Since both DR5 and caspase-8 are critical components in the extrinsic apoptotic pathway, we further analyzed the impact of DR5 and caspase-8 combination on HNSCC patient survival. The Log-rank test showed that the impact of the combined DR5 and caspase-8 on patient survival was identical to that of caspase-8 on patient survival. Specifically, in HNSCC with no LNM (Tu^{-met}), patients with high levels of both DR5 and caspase-8 had better overall survival and disease-free survival relative to patients with low levels of both DR5 and caspase-8 ($P < 0.0001$ and $P = 0.0124$, respectively) (Fig. 6A). In contrast, in HNSCC with LNM (Tu^{+met}), patients with higher levels of both DR5 and caspase-8 had worse overall survival and disease-free survival relative to patients with low levels of both DR5 and caspase-8 ($P = 0.0270$ and $P = 0.0065$, respectively) (Fig. 6B).

Discussion

The death receptor-mediated extrinsic apoptotic pathway plays an essential role in host immunosurveillance against tumor development, particularly metastasis [32–34]. In a genetic study, knockout of TRAIL receptor in mice does not affect primary tumor development, but enhances LNM [10], suggesting that

TRAIL receptor is important for suppressing tumor metastasis. In human melanoma samples, a reduced DR5 expression was reported to be associated with metastatic lesions [35]. In agreement, the current study revealed a significant downregulation of DR5 expression in primary tumors with LNM (Tu^{+met}) and their matching LNM compared to primary tumors with no metastasis (Tu^{-met}). Moreover, DR5 expression was significantly reduced in PD tumors compared to MD and WD tumors. Therefore, our data on DR5 from human HNSCC samples supports an inhibitory role of DR5 in regulation of metastasis. The mechanism of DR5 in regulation of metastasis is not known. However, DR5 is involved in mediating anoikis, a form of apoptosis triggered by loss of attachment of cells from the extracellular matrix [36,37]. Therefore, it is possible that the loss of DR5 we see in our metastatic HNSCC has contributed to the inhibition of anoikis, therefore allowing the tumor cells to escape apoptosis and migrate after detachment. It has been shown that in non-small cell lung carcinoma tissues, increased expression of DR5 correlates with PD tumors. Moreover, high DR5 expression is significantly associated with reduced overall survival [9]. However, our data clearly show that reduced DR5 expression correlates significantly with PD HNSCC. Furthermore, we did not find significant association between DR5 expression and overall

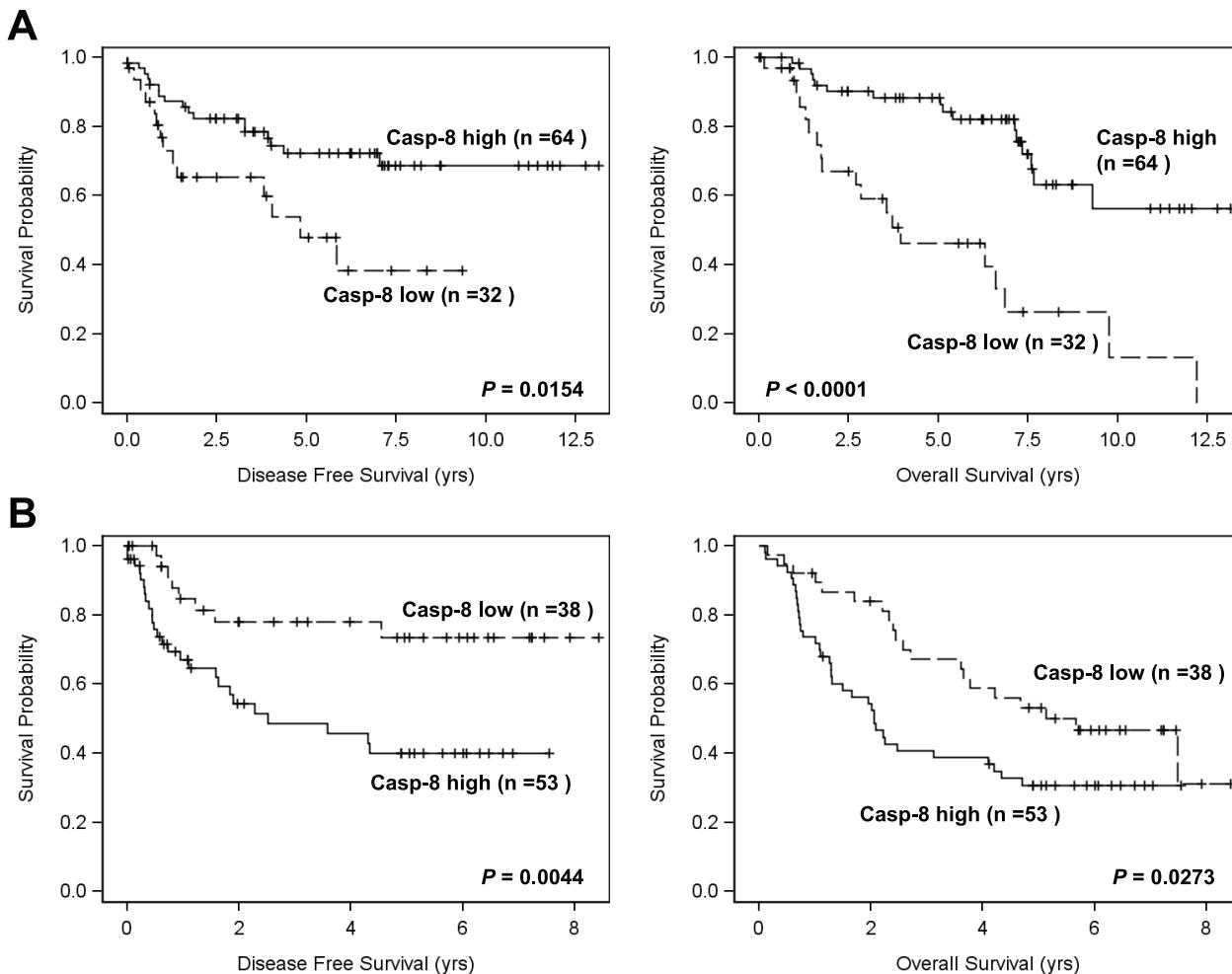


Fig. 5. Impact of caspase-8 expression on disease-free survival and overall survival in HNSCC patients without LNM (Tu^{-met}) (A) and in HNSCC patients with LNM (Tu^{+met}) (B). Kaplan-Meier plots were generated according to high (greater than the mean value) and low (less than or equal to the mean value) levels of caspase-8.

doi:10.1371/journal.pone.0012178.g005

survival or disease-free survival in Tu^{-met} group. In Tu^{+met} group, we found that the levels of DR5 were not associated with overall survival. However, higher DR5 was significantly associated with a worse disease-free survival. Despite the potential role of DR5 in negative regulation of metastasis as discussed above, our study did not show a survival advantage for Tu^{+met} HNSCC with high expression of DR5. In fact, one study has shown that TRAIL enhances the invasion of apoptosis-resistant pancreatic ductal adenocarcinoma cells *in vitro* and increases distant metastasis (e.g., liver) of pancreatic tumors *in vivo* [38], suggesting that activation of the DR5 signaling may facilitate metastasis under certain conditions. Moreover, the major mediator of DR5, caspase-8, has non-apoptotic functions that promote cell motility and migration as discussed below. Thus, whether DR5, like caspase-8, may also exert non-apoptotic functions, particularly in apoptotic resistant cells (see discussion below), should be further investigated.

We noted that DR5 staining in our study was primarily in the cytoplasm. However DR5 is known to be functional in inducing apoptosis as a membrane-bound protein. Given that DR5 expression levels do impact patient prognosis as demonstrated in this study, our data suggest that it may be interesting to study whether membrane-bound and cytoplasmic DR5 proteins exert

distinct functions (e.g., apoptotic vs. non-apoptotic) under different conditions.

It has been shown that cigarette smoke impairs tumor immune surveillance and promotes invasion of cancer cells including oral carcinoma cells and tumor metastasis in experimental systems [39–43]. In this study, we found after examining all patient samples together that a lower DR5 expression in HNSCC was significantly associated with smokers compared to non-smokers. Thus, it would be interesting to determine if tobacco carcinogens can downregulate DR5 expression and possibly add to the effect of tumor cells escaping apoptosis or contributing to metastasis.

In addition to DR5, DR4 is another TRAIL receptor that can initiate death signaling upon TRAIL binding or overexpression [4]. Depending on tumor types, expression of DR4 has variable impact on prognosis. For example, high DR4 expression has been shown to be associated with worse disease-free survival, worse overall survival and shorter time to recurrence in colon cancer [6], whereas DR4 expression did not impact patient survival in lung, cervical and ovarian cancers [7,44,45]. Moreover, in breast cancer, DR4, in contrast to DR5, has been shown to be more strongly expressed in better differentiated tumors, and correlated positively with surrogate markers of a better prognosis (hormone

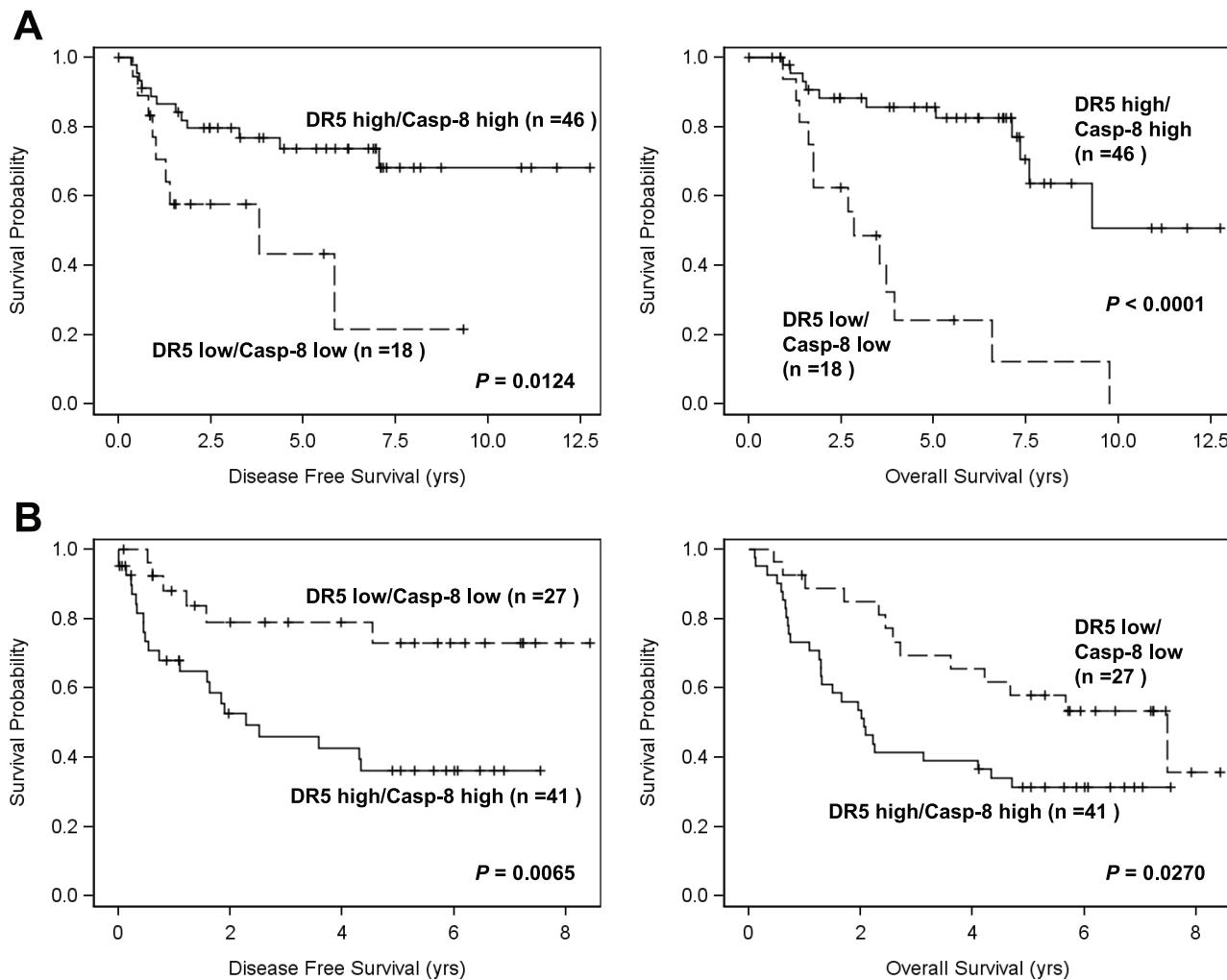


Figure 6. Impact of caspase-8 and DR5 combination on disease-free survival and overall survival in HNSCC patients without LNM (Tu^{-met}) (A) and in HNSCC patients with LNM (Tu^{+met}) (B). Kaplan-Meier plots were generated according to high (greater than the mean value) and low (less than or equal to the mean value) levels of DR5 and caspase-8.

doi:10.1371/journal.pone.0012178.g006

receptor status, Bcl-2, negative nodal status), but negatively with the expression of Her2/neu and the proliferation marker Ki67 [46]. In our study, we did not stain DR4 expression in our cohort of HNSCC tissues, largely due to antibody issues. Nonetheless, it will be interesting to study DR4 expression in HNSCC and its association with LNM and prognosis in the future.

The major function of caspase-8 is to mediate apoptosis induced by death receptors including DR5. It has been recently suggested that caspase-8 can play dual roles: one as an inducer of apoptosis and one as a promoter in metastasis [47]. Caspase-8 facilitates cell death initiated from the death receptor pathway after a death ligand (e.g., TRAIL) binds a death receptor (e.g., DR5) [48]. In addition, caspase-8 can also promote cell migration [27,49–51]. It has been suggested that caspase-8 can contribute to cell motility and adhesion by regulating calpain activity which controls cell migration including rac activation and lamellipodial assembly [50]. As well, the phosphorylation of procaspase-8 on tyrosine 380 and its interaction with the p85 alpha subunit of phosphatidylinositol 3-kinase was required to restore cell motility and adhesion in caspase-8 null cells [49]. However, there is evidence that a loss of caspase-8 is associated with increased metastasis. In neuroblas-

toma, a loss of caspase-8 prevented apoptosis by integrin-mediated cell death and therefore promoted metastasis [26]. In our study, caspase-8 expression trended towards a downregulation of expression in the metastatic group of patients, but this result was not statistically significant. Thus, it is unclear if the downregulation of caspase-8 that we observed in invasive HNSCC was playing a significant role in metastasis. It is possible that in primary tumors caspase-8 predominantly contributes to apoptosis and therefore can prevent metastasis, but in those tumor cells that escape apoptosis (i.e., are resistant to apoptosis), caspase-8 may be contributing to migration and metastasis. In our study, we found that in primary tumors with no LNM (i.e., Tu^{-met}) higher expression of caspase-8 correlated with better disease-free survival and overall survival, however, in tumors with LNM (i.e., Tu^{+met}) higher caspase-8 expression significantly correlated with worse disease-free survival and overall survival. Similar results were also generated when we analyzed the impact of caspase-8 and DR5 combination. Higher levels of both caspase-8 and DR5 in HNSCC with no LNM (Tu^{-met}) was significantly associated with better disease-free survival and overall survival, but was significantly correlated with poorer disease-free survival and overall survival in

HNSCC with LNM (Tu^{+met}). Thus, it is plausible to speculate that caspase-8 (as well as DR5) in primary HNSCC without LNM (Tu^{-met}) may be predominantly associated with its pro-apoptotic function and thus higher caspase-8 or caspase-8 plus DR5 provides protective advantage against cancer and correlates with better survival. Whereas in HNSCC with LNM (Tu^{+met}) which are resistant to apoptosis, caspase-8 and even DR5 may primarily exert their non-apoptotic function, i.e., activation of PI3K and promotion of migration, and thus higher caspase-8 expression negatively impacts patient survival. A limitation of the current study is the existence of potential bias due to usage of selected patient populations and retrospective design although no tumor pretreatment was the major criteria for tumor selection. Nonetheless, our interesting findings warrant further study to demonstrate the precise role of caspase-8 as well as DR5/caspase-8 pathway in regulation of HNSCC metastasis.

In summary, our IHC analysis of caspase-8 and DR5 in HNSCC suggest that a loss or downregulation of DR5 expression and possibly caspase-8 expression may be associated with more metastatic tumors and a loss of differentiation. The overall high expression of DR5 and caspase-8 in both primary and metastatic HNSCC suggests that DR5 or caspase-8 may be a good target for therapy of HNSCC. Various agonistic DR5 antibodies such as Conatumumab (AMG655), CS-1008 and Lexatumumab have

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BIOLOGY CONTRIBUTION

THE PROGNOSTIC VALUE OF TRAIL AND ITS DEATH RECEPTORS IN CERVICAL CANCER

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Purpose: Preclinical data indicate a synergistic effect on apoptosis between irradiation and recombinant human (rh) tumor necrosis factor-related apoptosis inducing ligand (TRAIL), making the TRAIL death receptors (DR) interesting drug targets. The aim of our study was to analyze the expression of DR4, DR5, and TRAIL in cervical cancer and to determine their predictive and prognostic value.

Methods and Materials: Tissue microarrays were constructed from tumors of 645 cervical cancer patients treated with surgery and/or (chemo-)radiation between 1980 and 2004. DR4, DR5, and TRAIL expression in the tumor was studied by immunohistochemistry and correlated to clinicopathological variables, response to radiotherapy, and disease-specific survival.

Results: Cytoplasmatic DR4, DR5, and TRAIL immunostaining were observed in cervical tumors from 99%, 88%, and 81% of the patients, respectively. In patients treated primarily with radiotherapy, TRAIL-positive tumors less frequently obtained a pathological complete response than TRAIL-negative tumors (66.3% vs. 79.0%); in multivariate analysis: odds ratio: 2.09, $p \leq 0.05$). DR4, DR5, and TRAIL expression were not prognostic for disease-specific survival.

Conclusions: Immunostaining for DR4, DR5, and TRAIL is frequently observed in the cytoplasm of tumor cells in cervical cancer patients. Absence of TRAIL expression was associated with a higher pathological complete response rate to radiotherapy. DR4, DR5, or TRAIL were not prognostic for disease-specific survival. © 2009 Elsevier Inc.

Cervical cancer, TRAIL, Death receptors, Radiotherapy, Immunohistochemistry.

INTRODUCTION

Cervical cancer is a major health problem, especially in non-industrialized countries. Although prevention and early detection are the most important factors in the fight against cervical cancer, improvement of current treatment is still needed. The choice of treatment depends on the stage of the tumor. For the smaller tumors confined to the cervix (Stage IA2 and IB1), the treatment of choice is surgery or radiotherapy with excellent 5 years survival rates (85%–95%) (1). Treatment of locally advanced cervical cancer (Stage

IB2–IVA) consists of radiotherapy in combination with cisplatin-based chemotherapy. Despite improvement in survival of cervical cancer, patients treated with chemoradiation the 5-year overall survival is still approximately 52%, and treatment may be accompanied by substantial morbidity (2, 3). Further improvement in survival by intensification of the standard treatment is limited by intrinsic and acquired tumor resistance to radiotherapy and/or chemotherapy and may increase short- and long-term side effects. Therefore, new alternatives are needed that can improve the antitumor effect with

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Supported by the Dutch Cancer Society by a personal grant to J. H. Maduro and Grants RUG 2000-2289 and 2005-3365.

Conflict of interest: none.

Acknowledgments—We thank the Radiotherapeutisch Instituut Friesland, the Department of Radiation Oncology at the Isala Kliniek in Zwolle, the Department of Pathology at the Martini Zieken-

huis in Groningen, Pathology Laboratory in Zwolle, Department of Pathology Leeuwarden, Department of Pathology at Bethesda Hospital in Hoogeveen, Department of Pathology at IJsselmeerkennhuizen in Lelystad, Pathology Laboratory Stichting Samenwerkende Ziekenhuizen Oost-Groningen in Winschoten and the Oost-Nederland Laboratorium of Pathology in Enschede for their cooperation. We also thank Tineke van der Sluis for her technical support.

Received Nov 7, 2008, and in revised form March 1, 2009.
Accepted for publication March 13, 2009.

acceptable or no increase of toxic side effects. Tumor resistance to (chemo-)radiation is commonly caused by a loss of the ability of tumor cells to go into apoptosis. Modulation of specific molecular pathways leading to increased cell death could potentially widen the therapeutic window (4). The extrinsic apoptotic pathway is initiated by activation of death receptors (DRs) expressed on the cell membrane. Several human DRs have been identified that belong to the tumor necrosis factor (TNF) receptor super family (5, 6). Apoptosis is triggered by the binding of specific TNF super family ligands, such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), to their cognate receptors DR4 and DR5, respectively. DR activation results in the formation of an intracellular death-inducing signaling complex composed of trimerized receptor molecules, recruited Fas-associated death domain molecules and procaspase 8 molecules (5, 6). After assembly of the death-inducing signaling complex, a caspase-8 initiated intracellular apoptotic cascade is activated, leading to cleavage of several substrates in the cytoplasm and nucleus and completion of the apoptotic program (5, 6).

Preclinical work from our own group indicates that the combination of targeting the TRAIL pathway by exposure to agonistic DR4 and DR5 antibodies or recombinant human (rh)TRAIL and irradiation works synergistically in cervical cancer cells (7). Moreover, in early clinical trials, these drugs also can be safely administered even when combined with chemotherapy (8, 9). We previously reported in a small series (25 patients) that cervical tumors frequently stain positive for DR4, DR5, and TRAIL (10). However, their role in relation to clinical outcome in cervical cancer is still unknown. Previous work by our group showed that DR4, DR5, and TRAIL staining in normal cervical tissue was associated with undifferentiated cells in the basal and parabasal layer, whereas in cervical cancer, the staining was more homogeneous (10). TRAIL expression has been found in a wide range of normal tissues but is able to induce apoptosis only in transformed and malignant cells (11, 12). Therefore, we concentrated on tumor specimens in this study.

We analyzed, the presence of DR4, DR5, and TRAIL in tumors of a large cohort of cervical cancer patients with Stage IA2–IVA disease treated with surgery and/or (chemo-)radiation. We combined classical clinical histopathological characteristics and immunostaining for TRAIL and its proapoptotic receptors to identify possible relations to each other and to treatment outcome. We also investigated DR4, DR5, and TRAIL expression in relation to response for patients treated with radiotherapy with or without chemotherapy.

MATERIALS AND METHODS

Patients

Clinicopathological characteristics of all cervical cancer patients referred to the Department of Gynecological Oncology of the University Medical Center Groningen are prospectively stored in a database since 1980. All staging and surgical procedures were performed at the University Medical Center Groningen. For the

present cohort study, all patients diagnosed with nonmetastatic invasive cervical cancer who were treated between January 1980 and December 2004 were identified. Eligibility was based on diagnosis of invasive cervical cancer Stage IA2–IVA treated with a Wertheim-Meigs operation or with radiotherapy plus or minus chemotherapy. We considered surgery as primary treatment in those patients in whom a Wertheim-Meigs operation was performed whether or not this was followed by radiation or chemoradiation. Primary radiotherapy was defined as radiotherapy or chemoradiation as the first treatment modality despite an additive surgical procedure. The clinicopathological and follow-up data were obtained during standard treatment and follow-up of the patients. For the present study, all relevant data were retrieved from our database into a separate anonymous database. The identity of the patients was protected by study-specific, patient codes. In case of uncertainties with respect to clinicopathological and follow-up data, the larger database could only be checked through the department's data managers. Follow-up data was collected up to November 2007.

Staging and treatment

Bimanual examination under general anesthesia was performed for clinical staging, in accordance with the Fédération Internationale de Gynécologie Obstétrique (FIGO) guidelines. Patients were treated according to the time period prevailing protocol, mainly based on FIGO staging. In general, this was a Wertheim-Meigs operation for Stage IB/IIA cervical cancer patients followed by external beam radiotherapy (EBRT) up to 45 Gray (Gy) in case of lymph node metastases, parametrial invasion, or positive resection margins. For the higher stage patients, primary treatment was either radiation (EBRT up to 45 Gy and low-dose-rate brachytherapy, two applications of 17.5 Gy) or chemoradiation.

EBRT was delivered by a linear accelerator. A box technique was used comprising an anterior, a posterior, and two lateral fields. The superior field border was the upper border of the fourth lumbar vertebra; the lowest field border was the lower margin of the obturator foramen (or in Stage IIIA, the distal vagina). The lateral margin of the anterior-posterior field was 2 cm lateral from the transverse diameter of the pelvic brim. The ventral border of the lateral fields is the upper margin of the symphysis and the dorsal margin the front of the os coccyx. All fields were given daily, 5 days a week. The dose was 1.8 Gy given to the center and planned on a contour outline of the patient. Low dose rate (¹³⁷Cesium) brachytherapy was applied with a standard applicator with a dose of 17.5 Gy to point A (reference location 2 cm lateral and 2 cm superior to the cervical os). If brachytherapy was impossible or inappropriate in cases of tumor extension into the parametria or lymph nodes, patients received an additional external boost of 25.2 Gy to a total dose of 70.2 Gy.

Concurrent chemotherapy before 1999 consisted of three 4-weekly cycles of carboplatin 300 mg/m² Day 1 and fluorouracil (5-FU) 600 mg/m² Days 2–5 intravenously (IV). After 1999, chemotherapy consisted of cisplatin 40 mg/m² IV once weekly for 6 weeks concomitant with external pelvic and intracavitary radiation.

In the period up to 1993, following irradiation all patients, if technically operable and in the absence of extra-uterine disease, underwent an additive hysterectomy, whereas in the period thereafter, only comparable patients with residual tumor identified by routine biopsy 6–10 weeks after completion of irradiation were operated.

Pathological response evaluation after primary radiotherapy

Pathological response to primary radiotherapy was evaluated in the hysterectomy material in the time period prior to 1994 and, in

the period thereafter, through biopsy 6–10 weeks after completion of irradiation. Pathological complete response was defined as absence of tumor cells in postirradiation tissue. Chemotherapy was included in the response analysis as an independent factor.

Tissue microarray (TMA)

From the patients meeting the inclusion criteria, we collected the paraffin-embedded tumor material and the hematoxylin-eosin (H&E)-stained slides obtained at diagnostic procedure or the specimens from patients at primary surgery. On the H&E-stained slides, representative tumor areas were marked avoiding areas of necrosis or severe leukocyte infiltration. From the corresponding paraffin blocks, three cores of 0.6 mm diameter were taken and placed in predefined array locations in a recipient blank paraffin block, using a precision instrument (Beecher Instruments, Silver Spring, MD). Eleven arrays were constructed, each containing three cores per tumor. Each array also contained internal control tissue such as normal cervix, skin epithelium, colon polyps, breast cancer, colon cancer, ovarian cancer, and several cervical cancer specimens.

Immunohistochemistry

Staining procedures for DR4, DR5, and TRAIL were performed as described previously (10, 13). Briefly, sections (4 µm) were deparaffinized in xylene and endogenous peroxidase was blocked by incubation in 0.3% hydrogen peroxide for 30 min. Only DR5 needed antigen retrieval by 15-min microwave treatment in 10 mM citric acid, pH 6.0 at 95°–100°C. All primary antibodies were diluted in phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 1% AB serum and added to the deparaffinized tumor material for 1 h at room temperature. Primary antibodies and dilutions were for DR4 goat anti-DR4 polyclonal antibody (1:50, clone C-20, Santa Cruz Biotechnology, Santa Cruz, CA), for DR5 rabbit anti-DR5 polyclonal antibody (1:100, Oncogene Research, Cambridge, MA) and for TRAIL goat anti-TRAIL polyclonal antibody (1:25, clone K18, Santa Cruz Biotechnology). 3,3'-Diaminobenzidine (DAB) was used as chromogen, and the slides were counterstained in hematoxylin. For the negative controls, the primary antibody was replaced by normal goat IgG (DR4 and TRAIL) or normal rabbit IgG (DR5).

Analysis of immunohistochemical staining

For DR4, DR5, and TRAIL staining, intensity was semiquantitatively scored: no staining (0), weakly positive (1), positive (2), or intense (3). For statistical analysis, all cases were initially studied separately and then dichotomized. Samples with scores 0 and 1 were regarded as negative and samples with score 2 and 3 as positive. Because of the relatively high amount of patients with no staining for TRAIL, we also divided the group in negative (0) and positive (1–3). (When referring to this classification this will be clearly stated in the results.) The cellular localization (nuclear, membranous, or cytoplasmatic) and patterns of staining (homogenous or heterogeneous) were also recorded. Two independent observers (K.H. and J.M.) scored the TMAs, and a concordance of more than 95% was found. The discordant cases were reviewed, and scores were reassigned on consensus of opinion. Patients were only included in the analysis in cases of minimally two representative tissue cores on the TMA.

Statistical analysis

Statistical analysis was performed with SPSS 14.0 for Windows (SPSS, Chicago, IL). A *p* value of ≤0.05 was considered statisti-

cally significant. The population was analyzed as a whole, and the group treated primarily with radiotherapy was analyzed separately. Associations between positive protein expression (DR4, DR5, and TRAIL, respectively; as dependent factor) and clinicopathological characteristics (age, stage, histology, tumor differentiation, lymphovascular invasion, and tumor volume; as independent factors) were calculated using logistic regression analyses. For the clinicopathologic analysis, the following covariates were used: age (continuous variable), FIGO stage (<IIB vs. ≥IIB), histology (adenocarcinoma vs. squamous cell carcinoma), tumor differentiation (poor versus good and moderate), lymphovascular invasion (present vs. absent), and tumor volume (≥4 cm vs. <4 cm). Variables that were significant (*p* ≤0.1) in univariate analysis were included in multivariate analysis in a stepwise manner. To determine factors predicting for response to radiotherapy, presence of pathological complete response to radiotherapy (as dependent factor) was evaluated in relation to clinicopathologic factors, protein staining, and the use of chemotherapy (as independent factors) with logistic regression analysis. Disease-specific survival (DSS) was defined as time period from date of diagnosis up to time point of death due to cervical cancer or last documented contact being alive. DSS was calculated for the whole population as well as for all patients in the primary radiotherapy subgroup. Differences in DSS according to clinicopathologic characteristics and to expression of DR4, DR5, and TRAIL were analyzed using the Cox regression analysis. Variables with a *p* value ≤0.1 in the univariate analyses were included in the multivariate analyses. Elimination of variables in a stepwise manner identified the statistically significant predictors on DSS by using a multivariate analysis.

RESULTS

Patient's characteristics

In total, we identified 765 patients, in 645 of whom sufficient tumor material was available to construct the TMA.

Clinicopathological data of the 645 patients with sufficient tumor material is summarized in Table 1. The median follow-up time was 55.7 months, and the median age at diagnosis 51.3 years. Primary treatment consisted of surgery in 313 patients (49%) and radiotherapy in 332 patients (51%). The baseline characteristics of the 120 patients in whom tumor material was missing differed from the study population regarding treatment modality primary radiotherapy (*p* <0.001) and FIGO staging (higher stage; *p* <0.001). This imbalance is accounted for by the fact that there was more tissue available from operated patients with lower tumor stage than from patients with only a biopsy taken before start of radiotherapy.

Immunohistochemical staining for DR4, DR5, and TRAIL

The results of the immunostainings are shown in Table 2. The number of patients with less than two representative tissue cores was 5.3% for DR4, 5.1% for DR5, and 4.7% for TRAIL. All three protein stainings were cytoplasmatic with no apparent membranous staining (Fig. 1). DR4 and DR5 were at least weak positive in 87.9% and 98.9% of the cases; for TRAIL, this was found in 79.8% of the cases. The expression of all the three proteins correlated with each other apart from TRAIL (negative [score 0] vs. positive [score 1–3]) and DR5 (negative [score 0–1] vs. positive [score 1–3]).

Table 1. Patient characteristics

Variables N = 645	Surgery n (%)	(chemo-) RT n (%)	Total n (%)
Patients	313 (49)	332 (51)	645 (100)
Age at diagnosis (in years)			
Median	43.0	54.1	47.7
Range	(17.5–86.2)	(20.6–92.0)	(17.5–92.0)
Follow Follow-up (in months)			
Median	64.9	45.9	55.7
Range	3.7–223.2	1.54–219.7	1.54–223.2
FIGO Sstage			
IA2	1 (0)	0 (0)	1 (0)
IB1	198 (63)	38 (11)	236 (37)
IB2	63 (20)	19 (6)	82 (13)
IIA	50 (16)	46 (14)	96 (15)
IIB	1 (0)	161 (48)	162 (25)
IIIA	0	7 (2)	7 (1)
IIIB	0	47 (14)	47 (7)
IVA	0	14 (4)	14 (2)
Histology			
Squamous	220 (70)	282 (85)	502 (78)
Adenocarcinoma	85 (27)	46 (14)	131 (20)
Other	8 (3)	4 (1)	12 (2)
Treatment			
Post operative radiotherapyRT	106 (34)		
Post operative radio-chemotherapy	18 (6)		
Chemotherapy		151 (45)	

Abbreviations: chemo = chemotherapy; RT = radiotherapy.

DR4, DR5, and TRAIL protein staining in relation to clinicopathologic characteristics

Table 3 shows the odds ratio (OR)s for the different immunostaining parameters in relation to known clinicopathologic characteristics in a uni- and multivariate logistic regression analysis. DR4 positive staining was associated with low tumor stage (<IIB) and presence of adenocarcinoma. DR5 positive staining was related to high tumor stage (\geq IIB) and to the presence of adenocarcinoma. No association was found between TRAIL expression and clinicopathologic characteristics.

Pathologic complete response to radiotherapy in relation to DR4, DR5, and TRAIL protein staining

To relate expression of DR4, DR5, and TRAIL and clinicopathologic characteristics to pathologic complete response to radiotherapy a uni- and multivariate logistic regression analysis was performed on 243 patients in whom either a rou-

tine hysterectomy was performed (until 1996) or in whom routine posttreatment biopsies were taken (after 1996; Table 4). The patient group eligible for posttreatment response evaluation was younger (median, 50.4 years) than the group with no posttreatment evaluation (median, 67.8 years; $p < 0.001$). For all other clinicopathologic characteristics, there was no difference between the patients with or without posttreatment evaluation, suggesting patients operability as the most important reason for not performing a posttreatment biopsy. Patients not fit for additive surgery were not evaluated with a posttreatment biopsy. In multivariate analysis, the absence of a pathological complete response in this group of 243 patients was related to positive staining for TRAIL (score 1–3), presence of adenocarcinoma and patients with FIGO Stage \geq IIB, ORs were, respectively, 2.09, 2.22, and 2.03 (all p values ≤ 0.05). Pathologic complete response rates were 66.3% vs. 79% for TRAIL positive vs. negative tumors, 54.3% vs. 73% for adenocarcinomas vs. squamous cell carcinomas, and 66.3% vs. 77.5% for FIGO Stage \geq IIB vs. FIGO Stage <IIB. Pathologic complete response to radiotherapy was not related to the concomitant use of chemotherapy. Results are shown in Table 4.

Table 2. Staining distribution

Tumor expression	DR4 (%)	DR5 (%)	TRAIL (%)
Negative	74 (11)	7 (1)	124 (19)
Weak positive	166 (26)	158 (25)	223 (34)
Positive	358 (56)	439 (68)	264 (41)
Strong positive	13 (2)	8 (1)	4 (1)
Missing cores	34 (5)	33 (5)	30 (5)
Total	645	645	645

Abbreviations: DR = death receptor; TRAIL = tumor necrosis factor factor-related apoptosis inducing ligand.

Disease-specific survival in relation to DR4, DR5, and TRAIL expression

In univariate Cox regression analysis, DR5 positive staining was associated with a worse DSS. In multivariate analysis for the whole group, age, high tumor stage, and tumor volume ≥ 4 cm correlated with a worse outcome. None of the immunostaining parameters (DR4, DR5, or TRAIL) correlated

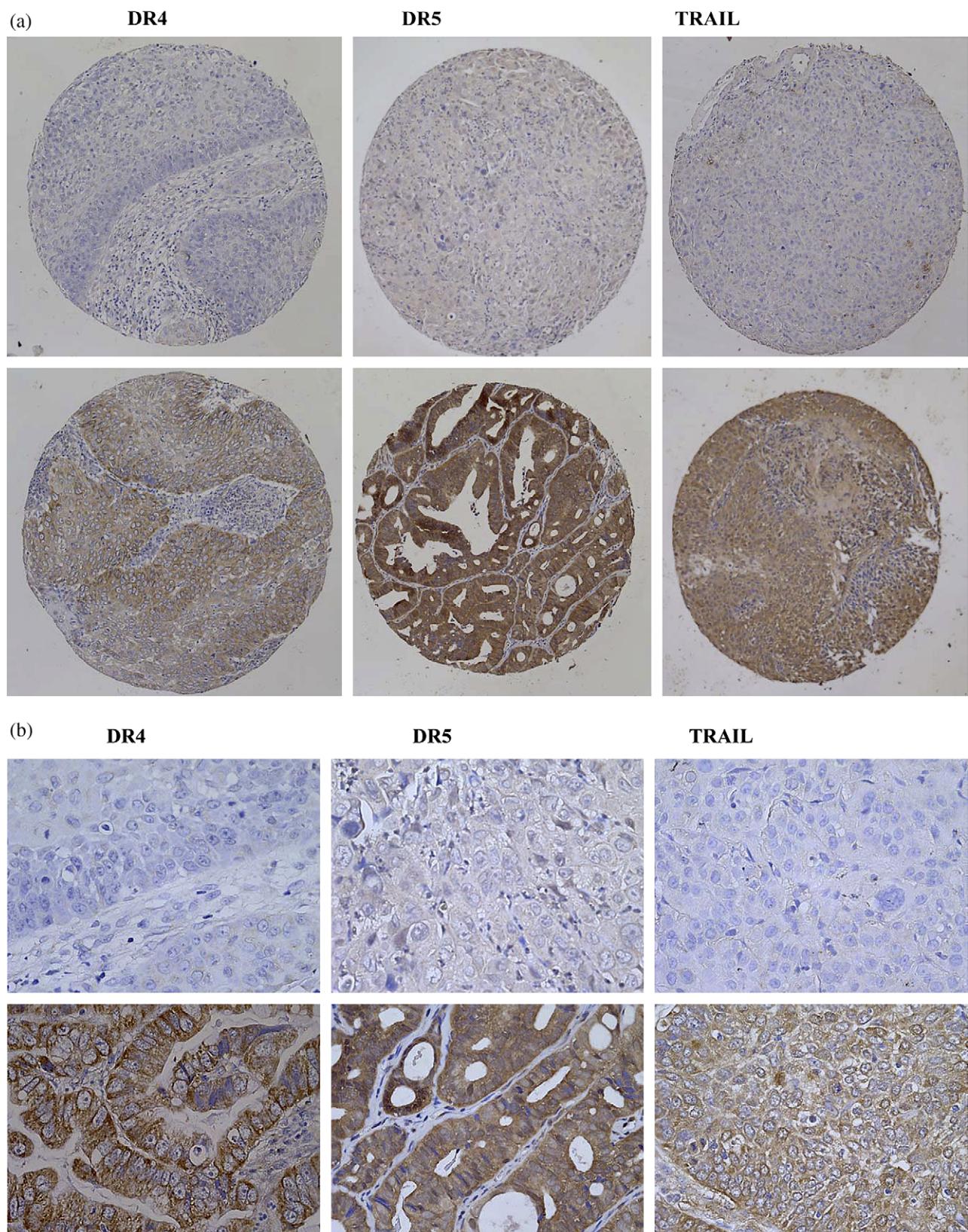


Fig. 1. Immunohistochemical staining for DR4, DR5, and TRAIL in a tissue microarray with tumors from cervical cancer patients. Representative examples for negative (upper panel) vs. positive (lower panel) staining. (a) 100 \times magnification, (b) 400 \times magnification. DR = death receptor; TRAIL = tumor necrosis factor-related apoptosis inducing ligand.

Table 3. Association between DR4 (aA), DR5 (bB), and TRAIL (cC) staining in relation to clinicopathologic characteristics

N = 645	A		Multivariate	
	Univariate		OR	95% CI
	OR	95% CI		
Age	0.98	0.97–0.99*	0.99	0.98–1.00†
Stage (IIB or higher)	0.52	0.37–0.73*	0.60	0.41–0.84†
Histology (adenocarcinoma)	2.76	1.74–4.39*	2.48	1.55–3.97†
Differentiation (poor)	0.76	0.45–1.29	—	—
Lymphovascular invasion (present)	1.34	0.93–1.92	—	—
Tumor volume (≥ 4 cm)	0.59	0.42–0.82*	—	—

N = 645	B		Multivariate	
	Univariate		OR	95% CI
	OR	95% CI		
Age	1.01	1.00–1.02*	—	—
Stage (IIB or higher)	3.23	2.09–4.97*	3.61	2.16–6.02†
Histology (adenocarcinoma)	1.73	1.05–2.84*	1.90	1.13–3.18†
Differentiation (poor)	0.922	0.64–1.34	—	—
Lymphovascular invasion (present)	0.51	0.35–0.75*	0.68	0.45–1.02
Tumor volume (≥ 4 cm)	1.43	0.99–1.43*	—	—

N = 645	C		Multivariate	
	Univariate		OR	95% CI
	OR	95% CI		
Age	1.00	0.99–1.01	—	—
Stage (\geq IIB)	1.25	0.90–1.74	—	—
Histology (adenocarcinoma)	0.91	0.61–1.36	—	—
Differentiation (poor)	0.84	0.60–1.18	—	—
Lymphovascular invasion	0.96	0.68–1.35	—	—
Tumor volume (≥ 4 cm)	1.05	0.76–1.46	—	—

Abbreviations: OR = odds ratio; CI = confidence interval.

Staining for DR4, DR5, and TRAIL with scores 0 and 1 were considered negative, and samples with score 2 and 3 were considered positive for DR4, DR5, and TRAIL. Bold factors with indicate a significant OR.

* $p \leq 0.1$.

† $p \leq 0.05$.

with disease outcome in multivariate analysis. The hazard rates (HRs) and confidence intervals (CIs) are reported in Table 5A. In the analyses of the primarily operated patients, the presence of positive pelvic lymph nodes (HR, 4.99; CI, 2.84–8.76) and tumor volume (HR, 1.83; CI, 1.07–3.13) were highly significant unfavorable prognostic factors.

In the primary radiotherapy group ($n = 332$), a worse DSS was related to high tumor stage (HR, 1.82; CI, 1.14–2.89), larger tumor volume (HR, 1.66; CI, 1.05–2.62), and persisting tumor after treatment (HR, 4.50; CI, 3.17–6.38). Although no effect on pathologic complete response chemotherapy contributed to a better DSS (HR, 0.62; CI, 0.43–0.87) in multivariate analysis (Table 5B).

DISCUSSION

This study shows that DR4, DR5, and TRAIL were cytoplasmically expressed in most Stage IA2–IVA cervical tumors. Patients with a TRAIL-positive tumor had an absolute

12.6% lower chance to obtain a complete pathologic response after radiotherapy. Absence of a complete pathologic response to radiotherapy was also related to presence of adenocarcinoma and higher FIGO stage. In multivariate analysis, DR4, DR5, and TRAIL immunostaining were not associated with DSS.

In the population studied, we found that classic clinicopathologic characteristics such as tumor stage (\geq IIB) and tumor size (≥ 4 cm) were the most important parameters affecting prognosis. Lymph node involvement was not taken into account in the analysis of the whole population because in most of the primarily irradiated patients, the presence of lymph node involvement is not known. In the analyses of the primarily operated patients, positive lymph nodes were associated with an unfavorable prognosis. These findings indicate the presence of a representative study population, and the distribution of patient characteristics (see Table 1) also mimics normal distribution in comparable cervical cancer populations from the Western world.

Table 4. Response to radiotherapy or chemoradiation: correlation between absence of complete response and clinicopathologic factors

N = 243	Univariate		Multivariate	
	OR	95% CI	OR	95% CI
Age	0.99	0.97–1.01	—	—
Tumor stage (\geq IIB)	1.75	0.95–3.25*	2.03	1.06–3.89†
Chemotherapy (yes)	0.77	0.44–1.34	—	—
Tumor histology (adenocarcinoma)	2.28	1.10–4.75*	2.22	1.05–4.70†
Tumor differentiation (poor)	0.69	0.29–1.18	—	—
Tumor lymphovascular invasion	1.06	0.49–2.26	—	—
Tumor volume (\geq 4 cm)	1.44	0.75–2.78	—	—
Tumor DR4 expression (positive)	1.13	0.65–1.98	—	—
Tumor DR5 expression (positive)	1.78	0.73–4.30	—	—
Tumor TRAIL expression (positive)	1.13	0.65–1.97	—	—
Tumor TRAIL expression ^{††} (positive)	1.91	0.94–3.87*	2.09	1.01–4.33†

Abbreviations: OR = odds ratio; CI = confidence interval.

Bold factors indicate a significant OR. In bold factors with a significant OR.

* $p \leq 0.1$.

† $p \leq 0.05$.

†† Score 0 (negative) versus vs. 1–3 (positive).

In our study, cytoplasmatic DR4, DR5, and TRAIL immunostaining were frequently observed, which was also the case in two much smaller studies not addressing the relation with DSS (10, 14). The biologic meaning of cytoplasmatic DR4, DR5, and TRAIL is not known, while for their activity they should be present at the cell membrane. Moreover, cytoplasmatic DR4 and DR5 do not exclude the presence of the DRs on the cell surface. Studies in ovarian, colon, and lung cancers have also demonstrated cytoplasmatic staining for DR4, DR5, and TRAIL (15–17). DR4, DR5, and TRAIL expression has been associated, in various tumors, with different clinical outcome. In patients with Stage III colon cancer treated with surgery and adjuvant chemotherapy, high cytoplasmatic DR4 expression at diagnosis was related to worse disease specific and overall survival (17). In a large cohort of breast cancer patients ($n = 655$) (18) and in 95 non-small cell lung cancer patients (16), high DR5 expression was associated with worse survival. In ovarian cancer, high TRAIL expression measured by real-time polymerase chain reaction was related to a better overall survival (19). In melanoma patients, DR4 and DR5 expression were not associated with clinical outcome (20). The diversity in the prognostic value of the DRs and TRAIL in various tumor types may be related to the various tumor types tested and the differences in treatment to which the patients were exposed. The lack of prognostic significance as observed in our study does not exclude functionality of the DRs. On the basis of preclinical data, it is likely that following chemo- and/or radiotherapy, the DRs are upregu-

Table 5. Disease-specific survival: (A): whole population, (B): primary radiotherapy group

N = 645	A			
	Univariate		Multivariate	
	HR	95% CI	HR	95% CI
Age	1.02	1.01–1.02*	1.01	1.00–1.02†
Tumor stage (\geq IIB)	3.20	2.41–4.25*	2.30	1.65–3.20†
Tumor histology (adenocarcinoma)	1.13	0.80–1.59	—	—
Tumor differentiation (poor)	1.26	0.94–1.67	—	—
Tumor lymphovascular invasion	0.96	0.70–1.31	—	—
Tumor volume (\geq 4 cm)	3.01	2.20–4.12*	2.01	1.41–2.86†
Tumor DR4 expression (positive)	0.93	0.69–1.23	—	—
Tumor DR5 expression (positive)	1.69	1.18–2.41*	—	—
Tumor TRAIL expression (positive)	1.11	0.83–1.47	—	—
Tumor TRAIL expression ^{††} (positive)	0.98	0.69–1.38	—	—

N = 332	B			
	Univariate		Multivariate	
	HR	95% CI	HR	95% CI
Age	1.00	0.99–1.01	—	—
Tumor stage (\geq IIB)	2.46	1.60–3.79*	1.82	1.14–2.89†
Tumor histology (adenocarcinoma)	1.43	0.93–2.20	—	—
Tumor differentiation (poor)	1.21	0.70–2.08	—	—
Tumor lymphovascular invasion	1.14	0.73–1.78	—	—
Tumor volume (\geq 4 cm)	2.16	1.40–3.34*	1.66	1.05–2.62†
Tumor DR4 expression (positive)	1.13	0.81–1.57	—	—
Tumor DR5 expression (positive)	1.21	0.75–1.94	—	—
Tumor TRAIL expression (positive)	0.99	0.71–1.38	—	—
Tumor TRAIL expression ^{††} (positive)	1.15	0.77–1.71	—	—
Residual tumor (yes)	5.05	3.62–7.04*	4.50	3.17–6.38†
Chemotherapy (yes)	0.70	0.50–0.97*	0.62	0.43–0.87†

Abbreviations: HR = hazard rate; CI = confidence interval.

Bold factors indicate HR rate.

* $p \leq 0.1$.

† $p \leq 0.05$.

†† Score 0 (negative) vs. 1–3 (positive).

lated at the cell membrane. More specifically, in our preclinical cervical cancer model, we showed by flow cytometry an upregulation of DR4 and DR5 membrane expression after irradiation (7). It might well be that during irradiation cytoplasmatic DR4 and DR5 are transferred to the membrane surface, thereby presenting them as potential targets for DR4/5 targeted drugs.

Historically, response rate to radiotherapy in cervical cancer has been measured in different ways, such as clinical examination or by postirradiation biopsies or

hysterectomies, jeopardizing meaningful comparison of response rates between studies. The uniqueness of this study lies in the fact that it analyzes response evaluation to radiotherapy in relation to DR4, DR5, TRAIL and classic clinicopathologic parameters. Pathologic complete response as assessed in our study gives the earliest insight in the biology of tumor in relation to irradiation. The importance of pathologic response measurement as a predictive marker for prognosis after irradiation for cervical cancer has been well established and was reviewed by Trott (21). A study in 556 cervical cancer patients showed that clinical measurement of response divided in no gross residual tumor and gross residual tumor also correlated well with clinical outcome (22). In our population, the pathologic complete response rate was 70%. The cervical cancer patients with tumors expressing TRAIL (75.7%) experienced less often a pathological complete response than those expressing no TRAIL (66.3% versus 78.9% OR in a multivariate analysis 2.09, p value ≤ 0.05). TRAIL expression, however, did not correlate with DSS, which in part may be caused by the fact that a proportion of patients with residual tumor after radiotherapy were salvaged by surgery, which in our institution is the standard of care for patients with resectable residual disease (23).

Not much is known about differences in radioresponsiveness between adenocarcinoma and squamous cell carcinoma in cervical cancer patients. In our study, patients with adenocarcinomas compared with squamous cell carcinomas obtained a pathologic complete response to irradiation less frequently, which has also been observed in a smaller FIGO Stage IB population (24). Despite the use of a different irradiation technique than ours, Rouzier *et al.* (25) also showed a near significant difference (p value 0.07) in complete pathologic response to radiotherapy between squamous cell carcinomas (62%) and adenocarcinomas (38%). It has been shown with measurements of MIB-1 and PC10 antigens in cervical cancer paraffin sections that cervical adenocarci-

nomas have no change, whereas squamous cell carcinomas have a transient increase in cycling cell population after 9 Gy of irradiation (26). The lower growth fraction in adenocarcinomas may be one reason for the radiation resistance of these tumors. This advantage in radioresponsiveness for squamous cell carcinomas did not translate into better DSS. As mentioned earlier, patients with residual disease after (chemo-)radiation may be salvaged by surgery. Nijhuis *et al.* previously showed in a similar population that 38% of the patients not having complete response after radiotherapy still achieve long-term complete remission after salvage surgery (23).

We observed that high DR4 and DR5 expression have an opposite correlation with FIGO stage. This finding suggests that depending on tumor stage, the DR route might be targeted differently. Both high DR4 and DR5 cytoplasmatic expression correlated positively with adenocarcinoma histology. The implication of this finding is not clear and needs to be elucidated.

Preclinical data indicated that the cytotoxic effect of radiotherapy is enhanced by rhTRAIL or its agonistic antibodies (7, 27). Irradiation induced both DR4 and DR5 membrane expression, whereas the enhancement of the cytotoxic effect was especially but not exclusively DR4 mediated (7). Moreover, in early clinical trials, rhTRAIL and its agonistic antibodies have been safely administered, even combined with chemotherapy (9, 28, 29). On the basis of the finding of our preclinical data (7), targeting DR4 in combination with (chemo-)radiation appears to be the most tentative treatment option for a clinical trial. The significance of DR4, DR5, and TRAIL expression as predictive factor for response should be prospectively investigated in a TRAIL route intervention study. In cervical cancer patients, in contrast to most other tumor types, it is relatively easily to perform serial biopsies during and after treatment allowing investigation of possible changes in expression of DR4, DR5, and TRAIL.

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An Apoptotic Molecular Network Identified by Microarray: On the TRAIL to New Insights in Epithelial Ovarian Cancer

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BACKGROUND. In a previous microarray expression analysis, the authors identified candidate genes that were expressed differentially between ovarian tumors with low malignant potential and invasive serous epithelial ovarian tumors. Among them, the apoptosis-related candidate genes tumor necrosis factor-related apoptosis-inducing ligand (*TRAIL*), caspase 8 (*CASP8*), FLICE-inhibitory protein (*FLIP*), and cytochrome C (*CYC*) were identified.

METHODS. For the current study, the authors conducted immunohistochemical analyses of a tissue array comprised of 235 serous tumors of different grades and stages to evaluate whether there was differential protein expression for these candidates and for the 4 death cell receptors of Trail: Dr4, Dr5, DcR1, and DcR2.

RESULTS. All proteins except DcR1 and DcR2 had significantly differential expression levels between grade 0 tumors (low malignant potential) and grade 2 and 3 tumors. Trail also showed differential expression between grade 0 tumors and grade 1 tumors. When all tumors were compared, the expression levels of Trail, Dr4, Dr5, DcR1, and Flip differed significantly between early-stage and advanced-stage disease. High Dr5 expression was associated with a poor prognosis in patients who had invasive tumors and in the subgroup of patients who had grade 3 tumors. Furthermore, the combinations of 2 proteins (Trail and Dr5, DcR2 and Cyc, Flip and Dr5, Flip and DcR2, DcR1 and Dr5 or Dr4 and Flip) revealed an association with patient prognosis.

CONCLUSIONS. The identification of new proteins in the initial diagnosis and prognosis of patients with epithelial ovarian cancer may lead to a better understanding of the disease, highlighting new potential therapeutic targets, and may be useful in patient management. *Cancer* 2007;110:297–308. © 2007 American Cancer Society.

KEYWORDS: epithelial ovarian cancer, low malignant potential/borderline tumors, diagnostic and prognostic markers, tumor necrosis factor-related apoptosis-inducing ligand (*TRAIL*) apoptosis.

Supported by a grant from the Canadian Institutes for Health Research (CIHR) (MOP-36056) to A.-M.M.-M., P.N.T., and D.M.P. Tumor banking was supported by the Banque de Tissus et de Données du Réseau de Recherche sur le Cancer of the Fonds de la Recherche en Santé du Québec, which is affiliated with the Canadian Tumor Repository Network.

V.O. was supported by studentships from the CIHR and the Canderel Fund of the Institut du Cancer de Montréal.

We are grateful to Louise Champoux, Lise Porte-lance, Manon de Ladurantaye, Marise Roy, and Stephanie Girard for technical assistance. We thank the gynecologic oncologists of the University of Montreal Central Teaching Hospital (CHUM) for providing specimens. We are grateful to laboratory members for thoughtful discussions.

We acknowledge the editorial assistance of Mr. Ovid Da Silva and the statistical assistance of Mr. Robert Boileau (Research Support Office, Research Center, CHUM).

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Received August 22, 2006; revision received March 27, 2007; accepted April 6, 2007.

Epithelial ovarian cancer (EOC) is a silent disease that usually is diagnosed at an advanced stage and, currently, represents the most fatal gynecologic malignancy.¹ EOC tumors are classified by different histopathologies, and the serous type is the most frequent.² Serous EOC tumors can be subdivided into those with low malignant potential (LMP) or borderline and invasive (TOV) tumors. In addition to a higher level of cellular atypia, the major morphologic criteria distinguishing these 2 classes is the presence of stromal invasion in TOV tumors, although at most microinvasion is observed in some LMP.^{3–5} Patients who have LMP tumors have an excellent 5-year survival rate (90–95%) compared with patients who have TOV tumors (30–40%). The degree of differentiation in malignant EOC defines the tumor grade, with LMP tumors (grade 0) being highly differentiated and TOV tumors classified as well-differentiated (grade 1), moderately differentiated (grade 2), or poorly differentiated (grade 3). The volume and extent of tumor spread define the clinical stage, varying from I to IV, with stage I limited to 1 or both ovaries, stage II associated with pelvic extension, stage III spreading into the abdominal cavity, and stage IV presenting with distant metastases.^{6,7}

After surgical removal of the tumor mass, patients with EOC usually receive a first line of platinum-based combined chemotherapy (for review, see Agarway and Kaye⁸). However, even if 80% of patients respond initially to treatment, most patients have recurrent disease and develop treatment resistance, with different latencies indicating the need for a better understanding of the disease and the identification of new therapeutic targets or treatment modalities. Some factors have been identified in drug resistance, such as the expression of multidrug resistance proteins, hypoxia in the tumor microenvironment, and resistance to apoptosis by overexpression of survival factors and down-regulation of death-signaling proteins.⁸

In a previous study, we defined molecular profiles that distinguished serous LMP tumors from grade 3 TOV tumors that included members of the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (Trail) signaling pathway. Trail (Tnfsf10, Apo2L) is a secreted protein that induces apoptosis after binding to its receptors Dr4 (Trail-R1, Apo2, Tnfrsf10A) and Dr5 (Trail-R2, Trick2, Killer, Tnfrsf10B). Two other receptors, DcR1 (Trail-R3, Trid, Tnfrsf10C) and DcR2 (Trail-R4, Trundd, Tnfrsf10D), allow the binding of Trail but are unable to transduce the apoptotic signal, thus acting as decoy receptors.⁹ Trail was seen as a promising therapeutic agent, because it induces apoptosis in cancer or trans-

formed cells but not in normal cells,^{10–13} whereas an in vitro study of EOC cell lines revealed higher sensitivity in normal cells than in cancer cells.¹⁴ It was suggested that this resistance to Trail-induced apoptosis by normal cells occurred in the presence of a higher level of decoy receptors at their surface compared with cancer cells.¹⁵ Preclinical studies of recombinant Trail in animal models have demonstrated that it is a potential therapeutic target for breast, colon, and ovarian cancer^{13,14,16,17} (for review, see Debatin and Krammer⁹). Trail also synergized with cytotoxic drugs and radiation to achieve antitumor activity in various cancers, including malignant gliomas; melanoma; leukemia; and cancers of the breast, colon, and prostate (for review, see Debatin and Krammer⁹).

In the current report, we focused on protein expression of Trail signaling members in ovarian cancer. By using immunohistochemistry (IHC), we assayed the expression levels of different candidate proteins on a tissue array that contained 235 serous tumor samples of different grades. Staining intensity was related to different clinical parameters, such as tumor grade, disease stage, and patient survival, to evaluate the usefulness of markers in the stratification of EOC tumors.

MATERIALS AND METHODS

Patients and Tissue Specimens

Tumor samples were collected after obtaining appropriate consent from patients who underwent surgery in the Division of Gynecologic Oncology at the Centre Hospitalier de l'Université de Montréal (CHUM). Histopathology, tumor grade, and disease stage, as defined by the International Federation of Gynecology and Obstetrics, were determined by an independent pathologist who reviewed and graded the tumor samples.⁷ Tissue selection criteria for this study were based on serous histopathology from chemotherapy-naïve patients, and all samples were collected between 1993 and 2003. Clinical data were extracted from the Système d'Archivage des Données en Oncologie, which includes entries on tumor grade and stage; treatment and clinical outcomes, such as the progression-free interval, as defined according to the Response Evaluation Criteria in Solid Tumors¹⁸; and survival.

Microarray Data

All Affymetrix HuGeneFL microarray data from this study are available publicly at URL: <http://www.genomequebec.mcgill.ca/ovarian/>.

TABLE 1
Description of the Serous Tissue Array

Patient characteristic	Tumor grade			
	0	1	2	3
No. of tumors	56	11	53	115
Mean age \pm SD y	49 \pm 14	41 \pm 15	60 \pm 12	62 \pm 10
Disease staging				
I-II (early)	21	1	3	10
III-IV (advanced)	35	10	50	114
Tumor residuum, cm				
>2	—	6	19	47
<2	—	5	23	44
Disease-free interval				
Mean \pm SD, mo	52 \pm 36	51 \pm 49	13 \pm 10	16 \pm 12
Censured patient	7/36	3/6	24/30	26/48
Survival				
Mean \pm SD, mo	58 \pm 33	56 \pm 47	28 \pm 14	28 \pm 16
No. of censored patients	0/41	2/7	12/35	50/62

SD indicates standard deviation.

Serous EOC Tissue Array

Two representative cores (0.6 mm in greatest dimension) from each tissue sample, after selection based on a review of the hematoxylin and eosin-stained slide, were arrayed on an empty paraffin block. The tissue array was composed of 56 LMP tumors (grade 0), 11 grade 1 tumors, 53 grade 2 tumors, and 115 grade 3 tumors (Table 1). Then the tissue array was sectioned, stained with hematoxylin and eosin, and received another pathology review to confirm its content.

Antibodies

For IHC analysis, anticaspase-8 (anti-Casp8) p20 rabbit polyclonal antibody (sc-7890), anti-FLICE-inhibitory protein (anti-Flip) mouse monoclonal antibody (sc-5276), anticytochrome C (anti-CYC) mouse monoclonal antibody (sc-13156), anti-Dcr2 goat polyclonal antibody (sc-11,638), anti-DcR1 goat polyclonal (sc-7193), anti-Dr4 goat polyclonal (sc-6823), and anti-TRAIL goat polyclonal antibody (sc-6079) were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif.). The specificity of the antibodies was tested by Western blot analysis.

IHC

The tissue arrays, cut into 5- μ m sections, were stained by an immunoperoxidase method as described elsewhere.¹⁹ Tissue sections were heated to 60°C for 30 minutes, deparaffinized in toluene, and rehydrated in an ethanol gradient. After 3% H₂O₂ treatment, slides were submerged in boiling citrate

buffer (0.01 M citric acid adjusted to pH 6.0) for 15 minutes, blocked with a protein blocking serum-free reagent (DakoCytomation Inc., Mississauga, Ontario, Canada), and incubated with the antibody for 60 minutes at room temperature. Tissues were incubated with either a secondary biotinylated antibody (DakoCytomation Inc.) or a rabbit antigoat biotin-conjugated antibody (1:300 dilution; sc-2774; Santa Cruz Biotechnology) for 20 minutes followed by incubation with streptavidin-peroxidase complex (DakoCytomation Inc.) for 20 minutes at room temperature. Liquid diaminobenzidine was applied to visualize the reaction (DakoCytomation Inc.), and nuclei were counterstained with hematoxylin. For negative controls, phosphate-buffered saline was used instead of the primary antibody. Protein expression was scored according to the extent (as a percentage of total malignant cells) and intensity (value of 0 for absence, 1 for low intensity, 2 for moderate intensity, and 3 for high intensity) of staining based on visualization. All slides were visualized independently by light microscopy at $\times 20$ magnification. All slides were analyzed independently in a blind study by 2 independent observers, and the interrater agreement was >90%. When strong differences in scoring between the 2 observers occurred, the core was reevaluated to reach a consensus between the 2 observers.

Apoptosis Assay

Apoptosis was assayed with anticleaved Casp3 antibody (no. 9661; Cell Signaling Technology, Beverly, Mass) according to the manufacturer's protocol. All sections were scored according to the extent (as a percentage of total malignant cells) and intensity (value of 0 for absence, 1 for low intensity, 2 for moderate intensity, and 3 for high intensity) of staining based on visualization. Slides were treated as described above (see IHC). All slides were analyzed in a blinded manner by 2 independent observers.

Statistical Analysis

The association between IHC staining intensity and clinical variables (tumor grade, stage, progression-free interval, and patient survival) was analyzed by using the Mann-Whitney *U* test. Comparisons of grades were performed between grade 0, grade 1, grade 2, and grade 3. The stages were divided into 2 groups: early (stages I-II) and advanced (stages III-IV). A Spearman correlation test was performed to evaluate the correlation between expression levels of the different proteins. The significance of the proteins in predicting the survival of patients with EOC was analyzed by using Kaplan-Meier survival curves

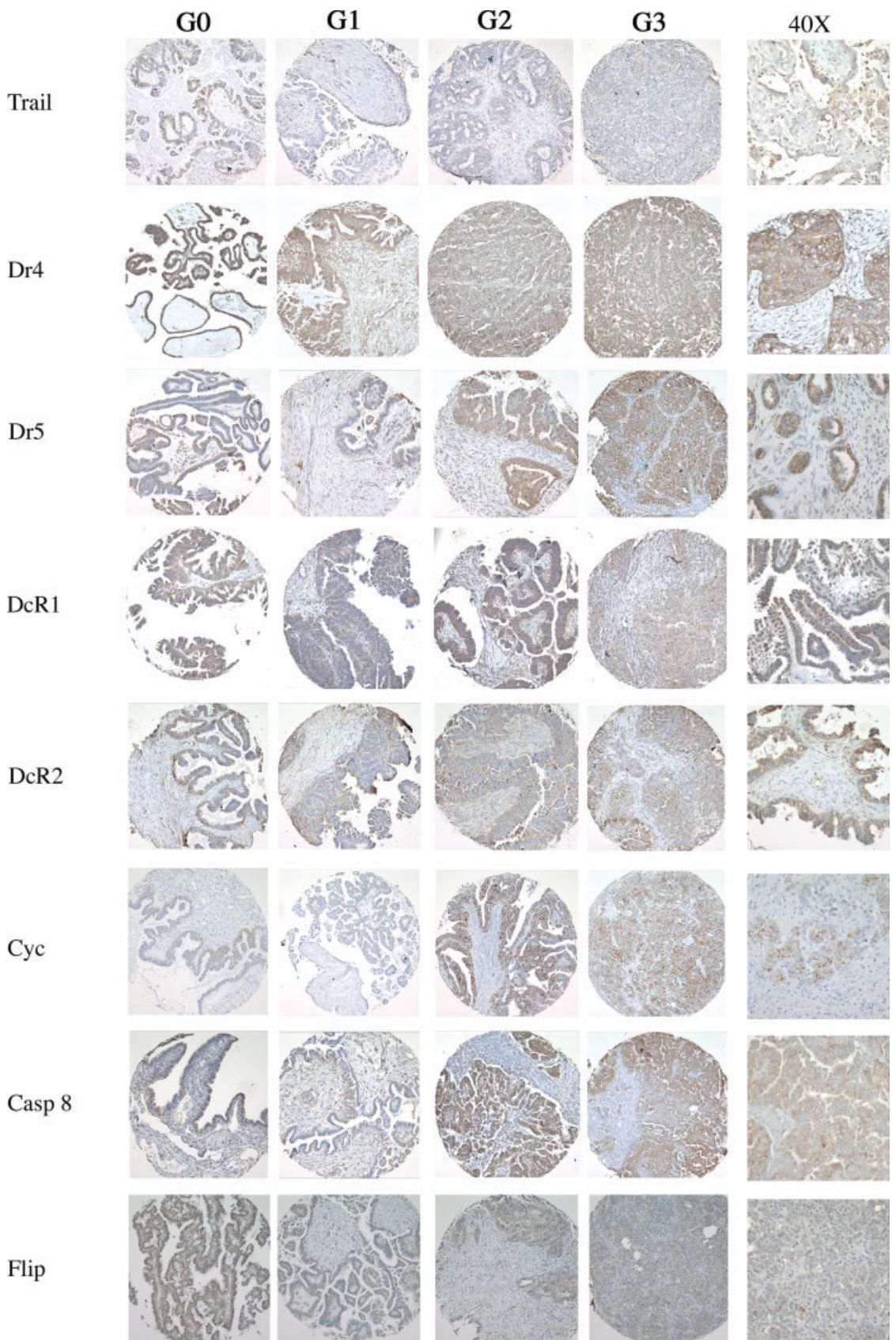


FIGURE 1

coupled to the log-rank test. The threshold of intensity used in the log-rank test to discriminate between the low- and high-intensity groups was based on the median intensity of TOV tumors (grades 1–3). Patients with follow-up <18 months were not included in this analysis. Statistical analysis was performed with SPSS software (version 11; SPSS Inc., Chicago, IL) with statistical significance set at $P < .05$.

RESULTS

Staining of Apoptosis-related Protein in Tumor Tissue

We previously performed molecular profiling of 6 LMP tumor tissues and 12 TOV tumor tissues by using Affymetrix GeneCHIP HuGeneFL microarray (Santa Clara, Calif).²⁰ A subset of differentially expressed candidate genes was implicated in death receptor apoptosis, including *TRAIL*, *CASP8*, *FLIP*, and *CYC*. To evaluate alterations at the protein level of these candidates in EOC, we undertook IHC using tissue microarray composed of 235 tumors of serous histopathology (Table 1). Because *TRAIL* was identified as a candidate gene, we were interested in determining whether the protein levels of its receptors (Dr4, Dr5, DcR1, and DcR2) also were modulated in EOC. Representative cores from each grade of tumor (grades 0–3) stained with the 8 different antibodies are presented in Figure 1. Trail staining was observed in the cytoplasm of epithelial cells and also diffusely in stromal cells. Staining was located mainly in the cytoplasm but was also associated with a cytoplasmic membrane staining in assays of Dr4, DcR1, and DcR2; whereas Dr5 presented additional nuclear staining. We noted exclusive cytoplasmic staining for Flip, Casp8, and Cyc, and the latter was distinguished by granular staining, consistent with the expected mitochondrial localization of this protein.

Association of Staining Intensity With Histologic Grade

Next, we attempted to determine whether protein expression was associated with tumor grade (Table 2). Statistically significant differences in protein expression, defined by the extent and intensity of staining, were observed for all proteins with the exception of DcR1 and DcR2 when grade 0 tumors (LMP) were compared with grade 2 and 3 tumors.

TABLE 2
Statistical Analyses of Candidate Expression Determined by Immunohistochemistry and Associated With Tumor Grade

Protein	Tumor grade	Median intensity	Significance: Mann-Whitney test					
			0–1	0–2	0–3	1–2	1–3	2–3
Trail	0	50	<.03*	.001*	<.001*	.64	.04*	.007*
	1	41						
	2	41						
	3	37						
Dr4 [†]	0	94	.39	.01*	<.001*	.50	.11	.03*
	1	92						
	2	89						
	3	84						
Dr5 [†]	0	55	.09	<.001*	<.001*	.33	.81	.12
	1	64						
	2	67						
	3	65						
DcR1 [†]	0	59	.91	.21	.35	.53	.70	.01*
	1	61						
	2	64						
	3	56						
DcR2 [†]	0	55	.30	.27	.23	.98	.66	.96
	1	57						
	2	62						
	3	57						
Casp8	0	42	.49	.003*	.003*	.05*	.05*	.55
	1	39						
	2	50						
	3	46						
Cyc	0	44	.58	.001*	<.001*	.02*	.006*	.59
	1	33						
	2	42						
	3	45						
Flip	0	75	<.001*	<.001*	<.001*	.68	.18	.19
	1	43						
	2	48						
	3	52						

Trail indicates tumor necrosis factor-related apoptosis-inducing ligand; Casp8, caspase-8; Cyc, cytochrome C; Flip, FlICE-inhibitory protein.

* Statistical significance was reached.

[†] Trail cell death receptors.

Dr5, DcR1, Casp8, and Cyc had higher expression levels in grade 2 and 3 tumors compared with the levels in grade 0 tumors ($P < .01$); whereas Trail, Dr4, and Flip had lower expression levels in higher grade tumors ($P \leq .01$) (Table 2). Trail and Flip were the only proteins that exhibited differential expression

FIGURE 1. Expression of apoptosis-related proteins in epithelial ovarian tumor tissues of different grades varying from grade 0 (G0) to grade 3. Representative images of immunoperoxidase-stained tissue cores are shown for each protein and grade (original magnification, $\times 20$). Tumor necrosis factor-related apoptosis-inducing ligand (Trail) staining was observed in the cytoplasm of epithelial cells, and diffuse staining was observed in stromal cells. Cytoplasmic and membrane staining was observed for the Trail receptors Dr4, DcR1, DcR2, and Dr5; whereas the latter receptor also presented nuclear staining. FLICE-inhibitory protein (Flip) and caspase 8 (Casp8) showed diffuse cytoplasmic staining, whereas cytochrome C (Cyc) showed a granular-like cytoplasmic staining. Higher magnification ($\times 40$) of the staining is presented in the right column.

TABLE 3
Expression of Apoptosis-related Protein Determined by Immunohistochemistry and Association With Disease Staging

Protein	Tumor stage	Median of intensity			<i>U</i> test for significance		
		All tumors	LMPs only	TOVs only	All tumors	LMPs only	TOVs only
Trail	Early	47	49	44	.01*	.94	.47
	Advanced	39	50	38			
Dr4 [†]	Early	93	95	88	.03*	.41	.75
	Advanced	87	93	86			
Dr5 [†]	Early	58	54	67	.03*	.53	.97
	Advanced	65	56	66			
DcR1 [†]	Early	65	60	74	.03*	.62	.01*
	Advanced	58	59	58			
DcR2 [†]	Early	58	63	52	.80	.15	.33
	Advanced	58	54	58			
Casp8	Early	43	43	48	.27	.64	.87
	Advanced	45	42	47			
Cyc	Early	39	33	45	.66	.87	.09
	Advanced	40	33	48			
Flip	Early	50	50	54	<.001*	.51	<.001*
	Advanced	39	55	42			

LMP indicates tumors with low malignant potential; TOVs, invasive tumors; Trail indicates tumor necrosis factor-related apoptosis-inducing ligand; Casp8, caspase-8; Cyc, cytochrome C; Flip, Flice-inhibitory protein.

* Statistical significance was reached.

[†] Trail cell death receptors.

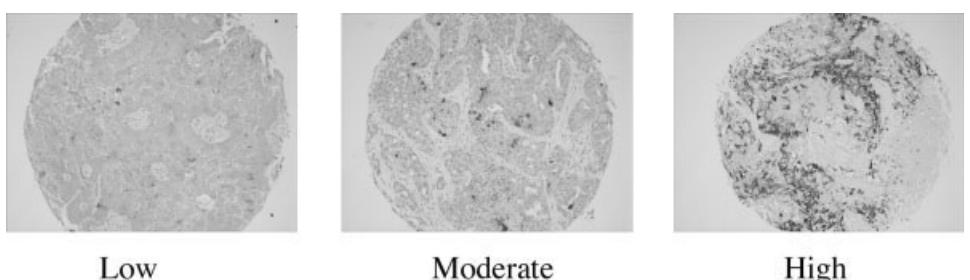


FIGURE 2. Expression of cleaved caspase-3 on epithelial ovarian tumor tissues. Representative images of immunoperoxidase-stained tissue cores for each of the 3 different staining intensities (low, moderate, and high) that were obtained with anticleaved caspase-3 (original magnification, $\times 20$).

between the 2 most differentiated groups of tumors (grade 0 > grade 1; $P = .03$ and $P < .001$, respectively) (Table 2). Statistically significant higher protein expression with increasing grade was observed when comparing grade 1 tumors with grade 2 and/or grade 3 tumors for Cyc ($P = .02$ and $P = .01$, respectively) and for Casp8 ($P = .05$ for grade 2 and 3 tumors), whereas lower expression levels of Trail were observed ($P = .04$ for grade 3 tumors) (Table 2). Grade 2 tumors expressed significantly higher levels of Trail and Dr4 compared with grade 3 tumors (Table 2).

Association of Staining Intensity and Clinical Stage

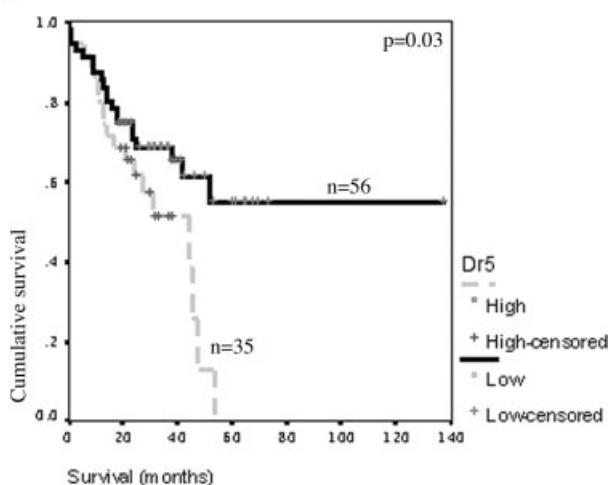
Because clinical staging is used to describe tumor extension, we assessed whether the staining intensity of apoptosis-related proteins was associated with

stage. We grouped clinical stages I and II as early-stage tumors (21 LMP tumors and 14 TOV tumors) and clinical stages III and IV as advanced-stage tumors (35 LMP tumors and 174 TOV tumors) (Table 1). When all tumors (grades 0–3) were analyzed together, we observed significantly higher levels of Dr5 expression in advanced-stage tumors ($P = .03$) compared with the expression levels of Trail, Dr4, DcR1, and Flip, which were higher in early-stage tumors ($P = .01$, $P = .03$, $P = .03$, and $P < .001$, respectively) (Table 3). When TOV tumors were considered separately, DcR1 and Flip showed higher expression levels in early-stage tumors compared with advanced-stage tumors ($P = .01$ and $P < .001$, respectively). However, because of the small number of early-stage tumors and the strong association with tumor grade, the

TABLE 4
Analysis of Apoptosis Monitored by Staining Intensity of Cleaved Capsase-3

Cleaved caspase-3 staining	Grade 0 samples		Grade 1 samples		Grade 2 samples		Grade 3 samples	
	No.	%	No.	%	No.	%	No.	%
None or weak	32	57	6	55	21	44	49	43
Moderate	18	32	5	45	23	48	54	48
High	6	11	0	0	4	8	10	9

A)



B)

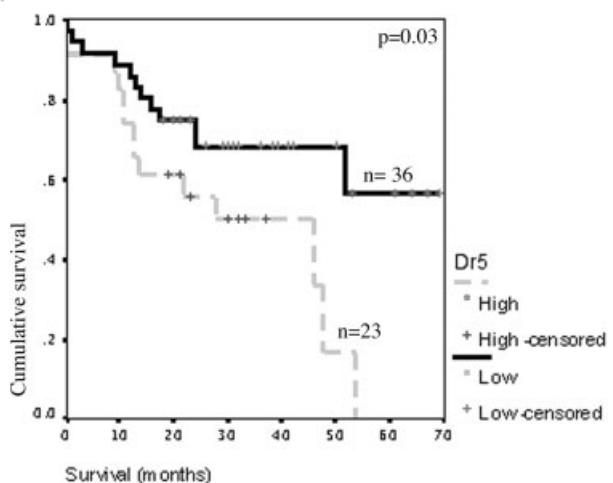


FIGURE 3. Relations between expression of the receptor Dr5 and the cumulative survival of patients with epithelial ovarian cancer. These Kaplan-Meier survival curves demonstrate poorer survival associated with high expression of Dr5 in invasive tumors (Grade 1–3; $P = .03$) (A) and in Grade 3 tumors only ($P = .03$) (B). Patients who had a follow-up of <18 months were not included in this analysis. The log-rank test was used to verify the significance of the difference in survival.

TABLE 5
Survival Analysis Performed Using a Kaplan-Meier Curve Coupled to a Log-rank Test

Protein	Median threshold of staining intensity	Survival (P^*)		
		Grades 1–3	Grade 2	Grade 3
Trail	38	.87	.91	.61
Dr4 [†]	68	.56	.93	.15
Dr5 [†]	67	.03 [‡]	.81	.03 [‡]
DcR1 [†]	59	.45	.85	.59
DcR2 [†]	59	.29	.17	.86
Casp8	47	.62	.3	.81
Cyc	46	.49	.23	.82
Flip	43	.14	.10	.79

Trail indicates tumor necrosis factor-related apoptosis-inducing ligand; Casp8, caspase-8; Cyc, cytochrome C; Flip, Flice-inhibitory protein.

* P value of the log-rank test coupled to the Kaplan-Meier curve.

[†] Trail cell death receptors.

[‡] Statistical significance was reached.

observed differential expression may have reflected the influence of grade rather than stage.

Analysis of Apoptosis

Because one of the hallmarks of cancer is resistance to cell death, we assessed whether our samples exhibited differential indices of apoptosis. One of the methods of evaluating apoptosis linked to signaling from cell death receptors, such as Trail receptors, is to measure the level of activation of the effector Casp3. We tested apoptosis levels by IHC using an antibody specific to cleaved Casp3 (Fig. 2) and visually scored staining as low, moderate, or high. Levels of apoptosis varied in each tumor grade (Table 4); and although, no statistically significant difference was observed, we did note a tendency toward lower activated caspase-3 expression in LMP tumors compared with grade 2 and 3 TOV tumors ($P = .08$ and $P = .09$). Next, we assessed whether the level of apoptosis, measured by the presence of cleaved Casp3, was correlated with the expression patterns observed with Trail signaling pathway members. Using a Spearman correlation test, we were unable to establish a significant correlation between any Trail signaling members and the level of cleaved Casp3 (data not shown).

Association of Apoptosis-related Proteins and Patient Survival

Then, we assessed whether the apoptosis-related proteins in our study could predict patient outcomes. We used the median staining intensity in TOV tumors for each protein as the threshold to perform

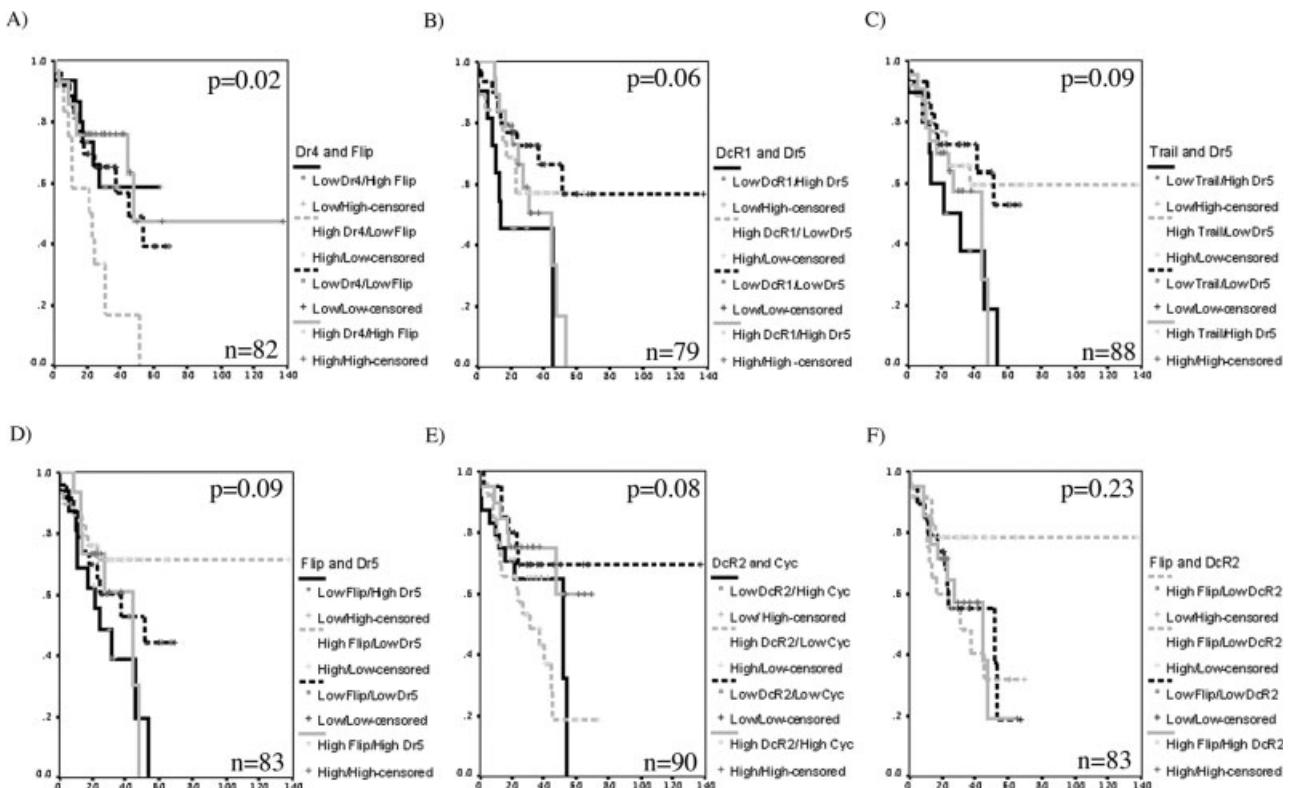


FIGURE 4. Relations between combinations of apoptosis-related proteins and the cumulative survival of patients with epithelial ovarian cancer. Patient survival is presented as Kaplan-Meier curves in which the log-rank test was used to define statistical difference between the different curves. These Kaplan-Meier curves illustrate all possible combinations of FLICE-inhibitory protein (Flip) and the tumor necrosis factor-related apoptosis-inducing ligand (Trail) receptor Dr4 (A), DcR1 and Dr5 (B), Trail and Dr5 (C), Flip and Dr5 (D), DcR2 and cytochrome C (Cyc) (E), and Flip and DcR2 (F). The x-axis represents patient survival in months, and the y-axis represents cumulative patient survival. Patients who had a follow-up of <18 months were not included in this analysis.

survival analyses based on Kaplan-Meier curves coupled with a log-rank test. We observed that only high expression of the receptor Dr5 was associated with poor survival in patients with invasive tumors ($P = .03$) (Fig. 3A, Table 5). Furthermore, because tumor grade was related to survival in patients with EOC, we performed an independent survival analysis for each tumor grade. Although no markers were associated with survival in the group of grade 2 tumors, high expression of Dr5 was associated with a poor patient prognosis in the subgroup of patients with grade 3 TOV tumors ($P = .03$) (Fig. 3B, Table 5).

Combination of Apoptosis-related Proteins to Predict Patient Survival

We evaluated whether tumors that exhibited certain patterns of protein expression were associated with tumor aggressiveness and influenced prognosis. We coupled Kaplan-Meier curves with a log-rank test to assess the significance of proteins in defining patient prognosis. The results of all protein analyses were combined in a pair-wise manner in which the 4

different combinations of expression levels were considered: high/high, high/low, low/high, and low/low. One combination presented a statistically significant association (Dr4 and Flip; $P = .02$) (Fig. 4A), whereas 4 others showed a trend toward an association with patient prognosis (DcR1 and Dr5; $P = .06$ [Fig. 4B]; Trail and Dr5; $P = .09$ [Fig. 4C]; Flip and Dr5; $P = .09$ [Fig. 4D]; and DcR2 and Cyc; $P = .08$ [Fig. 4E]). When all 4 combinations were compared, only Flip and DcR2 failed to show an association ($P = .23$) (Fig. 4F), although this result reflects the inability of markers to stratify patients in the early part of the curve (before 18 months). Subsequently, we refined the analysis to include only the 2 curves that defined the highest difference, and these selected, pair-wise combinations were compared further (Fig. 5). Tumors that expressed high levels of Dr4 and low levels of Flip conferred a worse prognosis compared with all other combinations of expression of these 2 markers ($P = .006$, $P = .01$, and $P = .04$) (Fig. 5A-C, respectively). Patients who presented with tumors that expressed low levels of both DcR1 and Dr5 had a

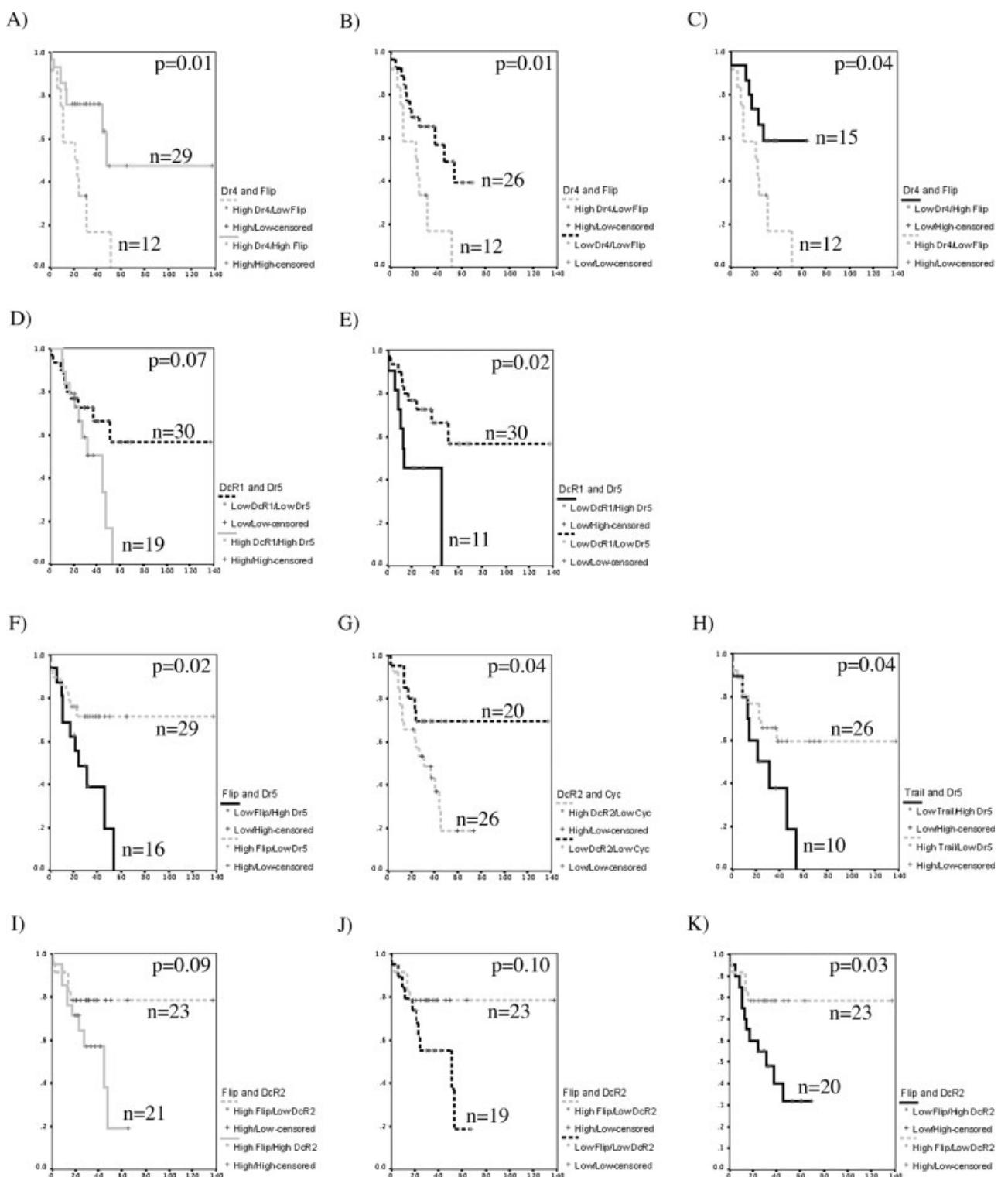


FIGURE 5. The relation between combinations of apoptosis-related proteins and the cumulative survival of patients with epithelial ovarian cancer. Patient survival is presented as Kaplan-Meier curves in which the log-rank test was used to define the statistical difference between the different curves. Kaplan-Meier curves of the combinations that provided the most significant patient prognosis are highlighted. Shown are combinations of the tumor necrosis factor-related apoptosis-inducing ligand (Trail) receptor Dr4 and FLICE-inhibitory protein (Flip) (A-C), DcR1 and Dr5 (D,E), Flip and Dr5 (F), DcR2 and cytochrome C (Cyc) (G), Trail and Dr5 (H), and Flip and DcR2 (I-K). The x-axis represents patient survival in months, and the y-axis represents cumulative patient survival. Patients with follow-up <18 months were not included in this analysis.

better prognosis compared with patients who presented with tumors that expressed high levels of both DcR1 and Dr5 ($P = .07$) (Fig. 5D) and also compared with patients who presented with tumors expressed low levels of DcR1 and high levels of Dr5 ($P = .02$) (Fig. 5E). Tumors that expressed high levels of Flip and low levels of Dr5 conferred a better prognosis compared with tumors that expressed low levels of Flip and high levels of Dr5 ($P = .02$) (Fig. 5F). Tumors that expressed low levels of both DcR2 and Cyc were associated with longer survival than tumors that expressed high levels of DcR2 and low levels of Cyc ($P = .04$) (Fig. 5G). Patients who had tumors that expressed high levels of Trail and low levels of Dr5 had longer survival than patients who had tumors that expressed low levels of Trail and high levels of Dr5 ($P = .04$) (Fig. 5H). Finally, although comparisons of all combinations of Flip and DcR2 were not significant, we did note that the combination of high expression of Flip and low expression of DcR2 seemed to confer a better prognosis compared with all other combinations of these 2 markers ($P = .09$, $P = .10$, and $P = .03$) (Fig. 5I–K, respectively).

DISCUSSION

For this report, we conducted a study on Trail signaling using tissue microarray and focused on the protein expression of Trail, its receptors (Dr4, Dr5, DcR1, and DcR2), other members of the pathway (Casp8, Cyc), the level of apoptosis in EOC, and correlated their expression with clinical factors, such as tumor grade and prognosis. In EOC, Trail signaling not only may influence the growth of cancer cells per se, but it may determine immunologic responses and could reflect the patient sensitivity or resistance to chemotherapy. Thus, it is the interplay of all of these factors that will influence disease endpoints and, ultimately, patient prognosis.

We demonstrated that Trail (Tnfsf10, Apo2L) was overexpressed in LMP tumors compared with every grade of invasive tumor, it was underexpressed in grade 3 TOV tumors compared with grade 1 and 2 TOV tumors, and it was overexpressed in early-stage disease versus advanced-stage disease when all tumors were included in the analysis. However, when LMP and TOV tumors were considered independently, no such association was observed. These results are consistent with those in the literature showing either Trail overexpression in early-stage EOC compared with advanced-stage EOC, or no association with disease stage.^{21,22} However, at the RNA level, TRAIL was overexpressed in poorly differ-

entiated (grade 2 and 3) tumors compared with well-differentiated (grade 1) tumors.²³ This discordance between protein levels and RNA levels was reported previously in breast cancer²⁴; it also was reflected in our microarray analysis, in which grade 3 TOV tumors expressed higher levels of TRAIL than LMP tumors, and in another published microarray study in which TOV tumors were compared with normal ovarian surface epithelium cells.²⁵ In colon cancer, at the protein level, results similar to ours were reported in which Trail expression was lower in adenomas compared with adenocarcinomas.²⁶

We observed the usual cytoplasmic and membrane staining of all Trail receptors, whereas nuclear staining also was observed for Dr5. Although it was unexpected, nuclear staining for a Trail receptor previously was reported in colon tumors.²⁶ We observed that grade 2 and 3 TOV tumors had higher expression of Dr5 compared with LMP tumors but did not demonstrate the differential expression of DcR2 and DcR1 compared with LMP tumors. Our results are consistent with an investigation of colon cancers in which Dr5 levels were higher in carcinoma cells compared with normal cells, and no difference was observed for DcR1 or DcR2.²⁶

One of the hallmarks of cancer is evasion from apoptosis.²⁷ This process can be accomplished in different ways, including a reduction of death receptor, ligand, and effector levels or the up-regulation of antiapoptotic proteins by genetic and/or epigenetic changes. In EOC, no mutations in Trail receptors were observed.²⁸ However, epigenetic silencing of TRAIL receptors was reported in different cancer types, including malignancies of the breast, lung, bladder, and cervix as well as lymphoma, leukemia, myeloma, neuroblastoma, and EOC^{29–32} (for review, see Debatin and Krammer⁹). Casp8, which is an effector of apoptosis, was inactivated by hypermethylation in a number of different tumors derived from neuroblastoma, brain tumors, Ewing sarcoma, and small lung cell carcinoma (for review, see Debatin and Krammer⁹). However, in EOC cell lines, no loss of expression of Casp8 was reported in Trail-resistant cell lines.³³

We observed that LMP tumors exhibited higher protein levels of Flip than grade 1, 2, or 3 TOV tumors. Flip is overexpressed in several tumors (Burkitt lymphoma, pancreatic carcinoma, melanoma, and neuroblastoma), and this increased expression is associated with resistance to chemotherapy in many types of cancers, including EOC^{10,34} (for reviews, see Debatin and Kramer,⁹ Fraser et al.,²⁵ and Ozoren and El-Deiry³⁶). Although a higher level of apoptosis was observed in our analysis of grade 2 and 3 TOV

tumors compared with grade 0 tumors, the difference was not statistically significant ($P = .08$ and $P = .09$, respectively), in agreement with another study in which the apoptotic index in ovarian tumors was higher in high-grade tumors compared with low-grade tumors.³⁷ In cell lines from different cancers, including EOC, sensitivity to Trail was associated with high expression levels of Dr4 and Dr5^{10,38} and reduced expression levels of DcR1 (for review, see Ozoren and El-Deiry³⁶). Other studies, including those on EOC cell lines, have revealed that only Dr4 levels, and not Dr5 levels, are correlated with Trail sensitivity^{10,33,39,40} and that the ratio of death and decoy receptors do not correlate with resistance to Trail.¹⁴

In the current work, we observed that high expression of Dr5 was associated with a worse patient prognosis either when TOV tumors of all grades were included or when only the subgroup of grade 3 TOV tumors was included, which is consistent with a previous report on lung cancer.⁴¹ In addition to its association with patient survival, Dr5 also was associated with tumor grade and disease stage, suggesting a role in EOC, although further molecular studies will be needed to address this point. We also demonstrated that, even if a protein alone is not associated significantly with patient prognosis, pairing its expression with another protein could result in a significant association. In this study, the combinations of Dr4/Flip, DcR1/Dr5, Flip/DcR2, Trail/Dr5, Flip/Dr5, and Cyc/DcR2 were associated with patient survival. It is noteworthy that the highest significance of any pair-wise combination appeared to involve combining Flip or Dr5 with another protein. We noted that Dr5 alone displayed greater significance for patient survival compared with all combinations that involved Dr5, although combinations that involved Dr4/Flip were as significant as Dr5 alone. These findings suggest that a strategy that sums the activities of different partners within a pathway may be more appropriate in designing nomograms for patient stratification. We have highlighted the advantage of looking at several proteins implicated in the same signaling pathway and the importance of holistic approach when dealing with a specific pathway, because the sum of the deregulation of each member is what generates the downstream effect of the pathway. In particular, this appeared to be reflected in the survival analysis; because the combination of 2 markers, instead of a protein alone, was required to significantly define patient prognosis.

In conclusion, to our knowledge, the current study is the first to simultaneously study both Trail and its 4 receptors in EOC. In this report, we demon-

strated that EOC tumors present the deregulation of apoptosis-related proteins that also are related to tumor differentiation. This deregulation is accompanied with a trend toward higher apoptosis levels in grade 2 and 3 TOV tumors compared with LMP tumors. Although we demonstrate that only Dr5 by itself was associated significantly with patient prognosis, our results indicated that a combination of markers together can provide relevant information on patient prognosis, suggesting that the interplay of members along a common signaling pathway influence the ultimate outcome. It remains to be determined in a larger series whether these proteins can be used clinically as markers of disease progression, either by themselves or in combination with proteins from other pathways.

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Resistance to TRAIL-induced apoptosis in ovarian cancer cell lines is overcome by co-treatment with cytotoxic drugs

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Received 5 November 2003

Available online 25 May 2004

Abstract

Background. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand, is a recently identified cytokine that preferentially kills transformed cells while sparing most normal cells.

Methods. We investigated the ability of TRAIL alone and TRAIL in combination with cytotoxic drugs to induce apoptosis in six ovarian cancer cell lines. To get some insight into the resistance to TRAIL, the expression of TRAIL receptors and selected downstream signaling elements was determined.

Results. TRAIL induced significant apoptosis (up to 80%) in three out of six ovarian cancer cell lines (MZ-26, CaOV-3, ES-2). In A2780 and A2780ADR cells, resistance to TRAIL-induced apoptosis correlated with their lack of DR4-expression. MZ-15 cells, which expressed the processed form of FLIP_L, p43 (FADD-like IL-1 β -converting enzyme (FLICE)-like inhibitory protein (FLIP)), and FLIP_S, were resistant to TRAIL in spite of the presence of DR4. When TRAIL-resistant cell lines were co-incubated with routinely used cytotoxic agents, TRAIL exerted a synergistic effect leading to apoptosis rates unachievable by incubation with cytotoxic agents alone.

Conclusion. The ability of TRAIL to induce apoptosis in ovarian cancer cells as well as to potentiate the activity of chemotherapeutic agents even in cell lines that are resistant to TRAIL-induced cytotoxicity is a powerful promise in the fight against this deadly disease.
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Keywords: Ovarian cancer; Apoptosis; TRAIL resistance; Chemotherapy

Introduction

Due to the lack of efficient screening methods, ovarian cancer is usually diagnosed at late stages, and therefore, the majority of patients require additional treatment after the surgical removal of tumors. After an initial response to

chemotherapy, most patients ultimately develop resistance, and substances to enhance and support the activity of cytotoxic drugs are desperately needed.

Several members of the tumor necrosis factor family such as tumor necrosis factor alpha (TNF α), Fas ligand (FasL), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) have been shown to induce apoptosis in susceptible cells (for review, see Refs. [1–3]). Unlike FasL and TNF α , TRAIL induces apoptosis preferentially in malignant cells while sparing normal cells. Apoptosis is induced by binding of TRAIL to its pro-apoptotic receptors DR4 or DR5, while binding to its soluble receptor osteoprotegerin (OPG) or to decoy receptors 1 and 2 (DcR1, DcR2), which lack a functional death domain, does not lead to cell death. Ligand binding to DR4 and DR5 causes receptor oligomerization and formation of a death inducing signaling complex (DISC) further downstream, which leads to the recruitment and

Abbreviations: TNF α , tumor necrosis factor alpha; FasL, Fas ligand; TRAIL, TNF-related apoptosis-inducing ligand; DR4, DR5, DcR1, DcR2, receptors for TRAIL; OPG, osteoprotegerin; DISC, death inducing signaling complex; FLIP, FADD-like IL-1 β -converting enzyme (FLICE)-like inhibitory protein; RT-PCR, reverse transcription polymerase chain reaction; HUVEC, human umbilical vein endothelial cells; FADD, FAS-associated death domain protein.

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activation of caspase 8. The FADD-like IL-1 β -converting enzyme (FLICE) inhibitory protein (FLIP) is an intracellular inhibitor of caspase 8 that binds to the DISC and potentially inhibits apoptosis mediated by all death ligands including TRAIL [4].

In the present study, we have evaluated the activity of TRAIL in a panel of ovarian cancer cell lines in terms of induction of apoptosis and expression of TRAIL-receptors DR4, DR5, DcR1, and DcR2 as well as of apoptosis-modulating proteins caspase 8, FLIP, and OPG. In addition, we have examined the cytotoxic effect of TRAIL in combination with the cytotoxic drugs most commonly administered in ovarian cancer.

Materials and methods

Cell lines

Epithelial ovarian cancer cell lines ES-2 (CRL-1978) and CaOV-3 (HTB-75) were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA), and cell lines A2780 and A2780ADR from the European Collection of Cell Cultures (ECACC, Salisbury, Wiltshire, UK). Through cooperation with the Department of Gynecology of the Medical School, Vienna, Austria, we received cell lines MZ-15 and MZ-26. All cell lines were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS and penicillin/streptomycin. The human umbilical cord endothelial cells (HUVECs) were a gift from Dr. Zyhdi Zhegu (Institute of Physiology/University of Vienna) and were obtained by primary culturing technique. The normal human chondrocytes were purchased from PromoCell bioscience alive GmbH (Heidelberg, Germany). Both benign cell lines were cultured in the cell type specific appropriate medium supplied by PromoCell bioscience alive GmbH. Experiments with primary culture cells were performed within two to four passages.

TRAIL-, paclitaxel-, topotecan-, carboplatin-, and adriamycin-mediated cytotoxicity and apoptosis

Recombinant human TRAIL was purchased from CHEMICON International (Temecula, CA, USA). Cells were seeded in 6-well plates at a density of 1×10^6 cells/well and allowed to adhere to the plate overnight. To assess the tumoricidal activity of TRAIL, recombinant human TRAIL was added at final concentrations of 100, 300, or 1000 ng/ml, and cells were incubated for additional 6, 12, 24, and 48 h. Apoptosis was measured by DNA fragmentation assay as described below.

To investigate the effect of TRAIL in the presence of paclitaxel, doxorubicin, topotecan, or carboplatin, cells were plated as described above and co-incubated with TRAIL (final concentrations: 100, 300, and 1000 ng/ml) and doxo-

rubicin (final concentrations: 0.5, 5, and 50 μ M; Pharmacia and Upjohn, Kalamazoo, MI, USA), paclitaxel (final concentrations: 0.5, 5, and 50 μ M; Bristol-Myers Squibb Co., Princeton, NJ, USA), topotecan (0.1, 1, and 10 μ g/ml; GlaxoSmithKline, Uxbridge, Middlesex, UK) or carboplatin (10, 100, and 1000 μ g/ml; Bristol-Myers Squibb, New York, NY, USA) for 6, 12, 24, and 48 h. Apoptosis was assessed by DNA fragmentation assay as described below.

DNA fragmentation assay

Using the Apo-Direct™ Kit (Phoenix Flow Systems, San Diego, CA, USA), the 3' OH termini in DNA breaks were measured by attaching fluorescent-tagged deoxyuridine triphosphate nucleotides, FITC-dUTP, in a reaction catalyzed by terminal deoxynucleotidyl transferase (TdT), and the amount of incorporated fluorescein was detected by flow cytometry. After incubation, treated and untreated cells were harvested, washed twice in phosphate-buffered saline (PBS), and fixed in 1% (w/v) paraformaldehyde in PBS (pH 7.2) for 15 min on ice. After two more washing steps, cells were resuspended in ice-cold 70% ethanol and stored at -20°C until further use. According to the manufacturer's instructions, cells were washed twice in wash buffer, resuspended in 50 μl staining solution (10 μl reaction buffer, 0.75 μl TdT, 8 μl FITC-dUTP, and 32 μl distilled water) and incubated for 1 h at 37°C . After one more washing step, cells were resuspended in 1 ml propidium iodine PI/RNase solution and incubated in the dark for 30 min at room temperature. Subsequently, cell samples were analyzed by flow cytometry on a FACScan (Becton Dickinson, CA, USA).

RT-PCR

The expression of DR4, DR5, DcR1, DcR2, FLIP, OPG, and caspase 8 was investigated by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was isolated from cell lines by standard procedures and digested with DNase to remove DNA contamination. First strand cDNA was generated from total RNA by incubating 2 μg of total RNA with oligo(dT)_{12–18} and 200 units SuperScript II RNase H Reverse Transcriptase (Life Technologies, Gaithersburg, MD, USA) at 42°C for 50 min. PCR reaction was performed with 5 μl of cDNA template in a final volume of 25 μl containing all four dNTPs (2 μl 10 mM dNTP Mix), 2.5 μl 10 \times PCR puffer, 0.2 μl *Taq* DNA polymerase (5 U/ μl), and 1 μl (10 pmol) of each primer. PCR conditions were as follows: 5 min denaturation at 94°C followed by 35 cycles, 30 s/ 94°C ; 30/60 °C; 30 s/ 72°C . The PCR-amplified products were run on a 1% agarose gel containing ethidium bromide and were visualized under ultraviolet light. Amplification of β -actin served as a positive control.

The sequences of specific primers used in this experiment were as follows:

Primer	Sequence	Product length
DR4.for	ATGGCGCCACCACCAAGCTAG	1407
DR4.rev	TCACTCCAAGGACACGGCAGA	
DR5.for	GGGAGCCGCTCATGAGGAAGTTGG	181
DR5.rev	GGCAAGTCTCTCTCCCAGCGTCTC	
DcR1.for	ACCCCTAAAGTCGTCGTCATC	205
DcR1.rev	TAATCCACACCCCTCTGTGCA	
DcR2.for	CTTTTCCGGGGCGTCATGTCCTTC	463
DcR2.rev	GTTTCTTCAGGCTGCTCCCTTTGTAG	
Caspase 8.for	TCTGGAGCATCTGCTGTCTG	426
Caspase 8.rev	CCTGCCTGGTGTCTGAAGTT	
FLIP.for	AATTCAAGGCTCAGAACGCA	226
FLIP.rev	GGCAGAAACTCTGCTGTTC	
OPG.for	GTGACGAGTGTCTATACTGCA	486
OPG.rev	ATCCTCTCTACACTCTCTGCG	
β-ACTIN. ATGGATGATGATATGCCGCG for	β-ACTIN. CTAGAACATTGCGGGGACATGGAGGGGCC rev	1127

Western blots

Protein expression of DR4, DR5, DcR1, DcR2, FLIP, and caspase 8 was determined by Western blot analysis. Cells were lysed in 1 ml of RIPA buffer (10 mM TRIS–HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% Na-Deoxycholate, 0.1% SDS) supplemented with Protease Inhibitor Cocktail Tablets (Roche Applied Science, Mannheim, Germany). The lysates were incubated at 4°C for 10 min and centrifuged at 15,000 rpm for 10 min. Protein concentration was determined using Protein Assay ESL (Roche Applied Science) according to the manufacturer's instructions. Equal amounts of protein were separated on 12% SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. The following primary antibodies were used: rabbit polyclonal anti-DR4 (NT) (ProSciInc, Poway, CA, USA); goat polyclonal anti-TRAIL-R2/DR5/TNFRSF10B (R&D Systems, Minneapolis, MN, USA); mouse monoclonal anti-caspase 8 (FLICE) Ab-1 (clone 8CSP01) (NeoMarkers Fremont, CA, USA); goat polyclonal anti-FLIP_L (C-19), goat polyclonal anti-FLIP_S (F-20), and goat polyclonal anti-Actin (I-19) (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA). Secondary antibodies: anti-goat IgG peroxidase conjugate (Sigma-Aldrich, Vienna, Austria), anti-mouse (NA 931) and anti-rabbit (NA 934) IgG HRP-linked (Amersham Pharmacia Biotech, Uppsala, Sweden). Protein determination was done by Protein Assay ESC (Roche Diagnostics, IN, USA). In case of quantitative experiments 40 μg of total protein was loaded and loading control performed by β-actin staining. We used ChemiGlow™ (Alpha Innotech, San

Leandro, CA, USA) to detect chemiluminescence on Western Blots.

Statistical analysis

The data were expressed as mean ± standard deviation calculated from three separate experiments, each performed in triplicate.

Data analysis for combination treatment

Synergism was determined by calculating the combination index (CI) using the median effect analysis for fixed drug dose combinations [5–8].

$$CIx = (D)_1/(Dx)_1 + (D)_2/(Dx)_2 + \alpha(D)_1(D)_2/(Dx)_1(Dx)_2$$

where CIx is the CI value for x% effect. $(Dx)_1$ and $(Dx)_2$ are the doses of agents 1 and 2 required to exert x% effect alone, whereas $(D)_1$ and $(D)_2$ are the doses of agents 1 and 2 that elicit the same x% effect in combination with the other agent, respectively. α describes the mode of interaction: $\alpha = 0$ for mutually exclusive (similar modes of action), $\alpha = 1$ for mutually nonexclusive drugs (independent modes of action). CI = 1 indicates additivity, CI < 1 synergism, and CI > 1 antagonism.

Results

TRAIL-induced apoptosis in MZ-26, ES-2, and CaOV-3 cells

Six ovarian cancer cell lines as well as two benign cell lines, human chondrocytes, and human umbilical cord endothelial cells (HUVEC) were incubated with three different concentrations of TRAIL in triplicate experiments. TRAIL sensitivity was defined as >25% apoptosis at 24 h after the initiation of incubation with TRAIL, as compared to control cells and quantitated by DNA fragmentation analysis. Three of the tested cell lines (MZ-26, ES-2, and CaOV-3) were sensitive to the cytotoxic effects of TRAIL, whereas the other three (A2780, A2780ADR, and MZ-15) showed less than 10% (9.75, 4.61, 4.86, respectively) apoptosis even upon incubation with the highest concentration of TRAIL (1000 ng/ml) (Fig. 1).

For all three apoptosis-sensitive (MZ-26, ES2, and CaOV3) cell lines, a clear dose- and time-dependent correlation between TRAIL concentration and the degree of apoptosis induction was observed with up to 80% (MZ-26), 50% (ES2), and 49% (CaOV3) apoptotic cells after 24 h of incubation with 1000 ng/ml TRAIL, respectively (Fig. 1).

The tested benign cell lines HUVEC and human chondrocytes were resistant to TRAIL after any incubation time and at any final TRAIL concentration.

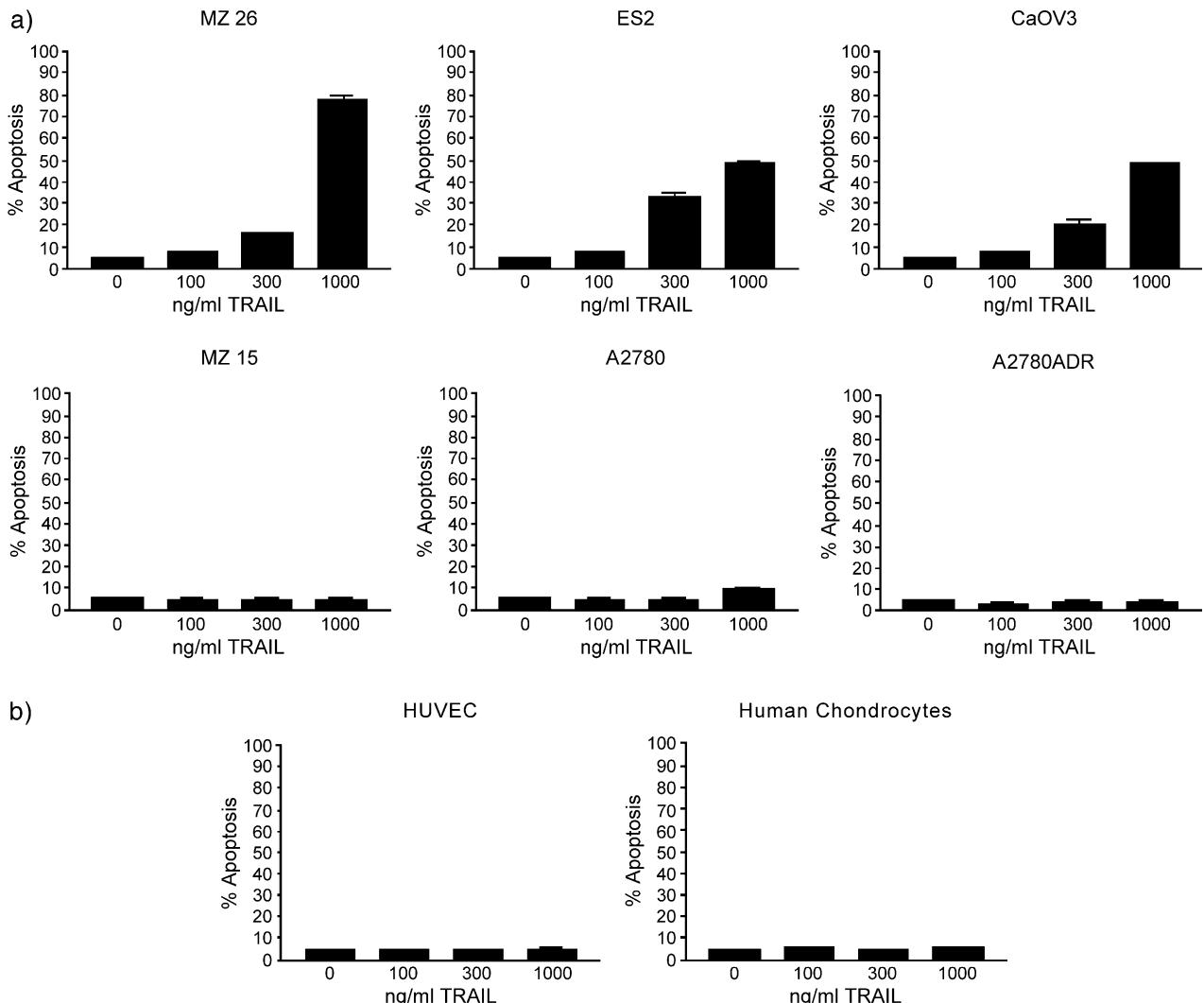


Fig. 1. Dose-response effect of TRAIL in human ovarian cancer cell lines (a) and controls (HUVEC, human chondrocytes; b). The cells were treated with different concentrations of TRAIL (0, 100, 300, and 1000 ng/ml) for 24 h. Apoptosis was measured by the DNA fragmentation assay using Apo-Direct™ Kit (Phoenix Flow Systems). Results are the mean of three independent experiments; bars, SD.

TRAIL receptor expression and resistance to TRAIL

Expression of TRAIL-receptors DR4, DR5, DcR1, and DcR2 was analyzed by RT-PCR and Western blotting (Figs. 2 and 3). TRAIL-sensitive cell lines MZ-26, ES2, and CaOV3 showed DR4 expression, whereas DR4 was undetectable in the TRAIL resistant cell lines A2780 and A2780ADR. This observation could not be confirmed in MZ-15 cells that expressed DR4, but were resistant to TRAIL.

DR5 expression as well as expression of decoy receptors DcR1 and DcR2 was seen in all cell lines and thus failed to predict for TRAIL-resistance.

Expression of genes regulating TRAIL-induced apoptosis

All ovarian cancer cell lines expressed caspase 8, which was described to be crucial in the TRAIL signaling cascade (Fig. 2). Likewise, all ovarian cancer cell lines expressed

mRNA of the caspase activation inhibitor FLIP at a comparable level when tested by RT-PCR (Fig. 2). On the protein level, a different picture was observed. Western Blotting revealed that protein expression of the short (c-FLIP(S)) and long (c-FLIP(L)) splice variants of FLIP varied between cell lines (Fig. 3): FLIP_S was only expressed by the TRAIL-resistant cell line MZ-15. In the same cell line, a 43-kDa cleavage product of FLIP_L was detected.

Transcripts of the soluble TRAIL receptor OPG were detected in MZ-15, ES-2, and CAOV-3 cells, but not in the cell lines MZ-26, A2780, and A2780ADR (Fig. 2).

Cytotoxic agents and TRAIL act synergistically in cell lines resistant to TRAIL alone

To determine whether TRAIL resistance could be overcome by co-incubation of ovarian cancer cell lines with

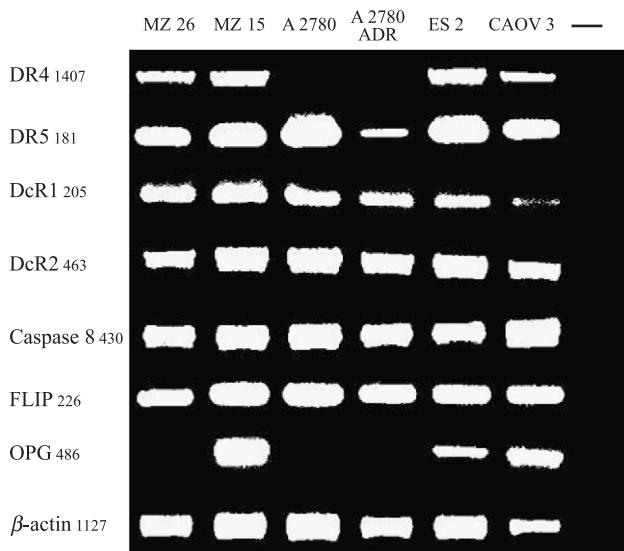


Fig. 2. Expression of TRAIL receptors DR4, DR5, DcR1, DcR2, caspase 8, FLIP, and OPG was assessed by RT-PCR in six ovarian cancer cell lines as described in Materials and methods.

cytotoxic agents and TRAIL, TRAIL-resistant cell lines A2780, A2780 ADR, and MZ-15 cells were treated either with paclitaxel (0.5, 5, and 50 μM), topotecan (0.1, 1, and 10 μg/ml), doxorubicin (0.5, 5, and 50 μM), and carboplatin (10, 100, and 1000 μg/ml) alone or concomitantly with 100, 300, and 1000 ng/ml TRAIL for 6, 12, 24, and 48 h.

Considerable dose- and time-dependent apoptosis induction by cytotoxic agents was shown in the three TRAIL-resistant cell lines, with maximum apoptosis seen after 24 h (Fig. 4). Only A2780ADR cells were completely resistant to doxorubicin treatment as expected because they were derived from A2780 by selecting for cells resistant to this drug.

In all three TRAIL-resistant ovarian cancer cell lines, co-incubation of TRAIL 300 ng/ml with the individual therapeutics was able to overcome TRAIL-resistance, leading to a strong synergic response (Fig. 4). No further increase of apoptosis was observed by using higher doses of TRAIL or extending incubation time to more than 24 h. Interestingly, the complete resistance of A2780ADR cells to doxorubicin could not be overcome by co-incubation with TRAIL and vice versa.

Discussion

Resistance to TRAIL appears to be a multifaceted phenomenon, and little conclusive evidence about its mechanism is available. In an attempt to gain some insight into the possible mechanism of apoptosis resistance observed in three of the tested ovarian cancer cell lines, we extended our experiments to the study of expression of TRAIL receptors and selected downstream signaling elements of potential functional significance.

All cell lines sensitive to TRAIL-induced apoptosis were positive for DR4 expression, whereas we were unable to detect DR4 in the TRAIL-resistant cell lines A2780 and A2780ADR. These findings are in accordance with accumulating evidence that missing or low DR4 expression correlates with TRAIL resistance [9,10]. Moreover, reconstitution of functional DR4 restored sensitivity in a TRAIL-resistant nasopharyngeal cancer cell line with a homozygously deleted DR4 gene [11]. Yet, MZ-15 cells expressed DR4 but were resistant to TRAIL, indicating different mechanisms of resistance. DR5 expression was seen in all cell lines, and so was DcR1 and DcR2 mRNA, indicating no correlation of their presence with TRAIL sensitivity or resistance.

Binding of TRAIL to its pro-apoptotic receptors turns on a signaling cascade that leads to the formation of the DISC, containing the FAS-associated death domain protein (FADD), as well as other still unknown proteins [3].

Caspase 8 binds to the DISC and is thereby processed into the active form. It was proposed that undetectable or reduced expression of caspase 8 could explain the resistance to TRAIL-induced apoptosis [12]. We tested our cell lines for caspase 8 expression and could not find any significant difference between TRAIL-resistant and TRAIL-sensitive cell lines (Fig. 2).

FLIP functions as an anti-apoptotic molecule by blocking apoptosis induced by death receptors and exists in a long (FLIP_L) Mr 55,000 and a short (FLIP_S) Mr 28,000 isoform [4]. Overexpression of either FLIP isoform has been reported to provide protection against TRAIL-induced apoptosis in different cell types [13–16]. When probed with FLIP_S antibody, only the TRAIL-resistant cell line MZ-15 was positive. In the same cell line, the FLIP_L antibody detected a 43-kDa cleavage product of FLIP_L. The presence of this 43-kDa cleavage product of FLIP_L in MZ-15 cells presumably leads to prevention of caspase 8 activation and subsequently to TRAIL resistance like it

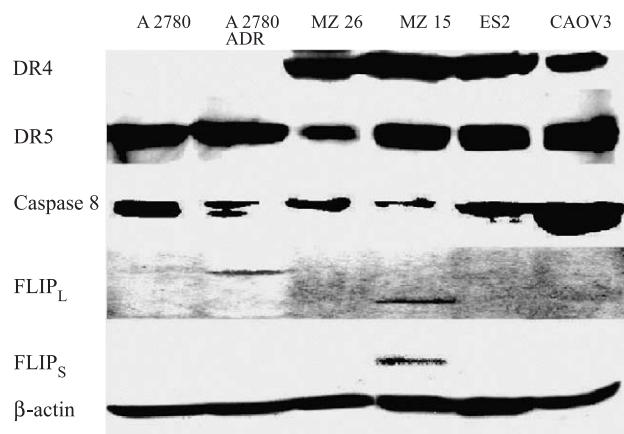


Fig. 3. Expression of DR4, DR5, FLIP_L, FLIP_S, caspase 8, and actin in ovarian cancer cell lines as determined by Western blotting. DR4, 57 kDa; DR5, 48 kDa; caspase 8, 55 kDa; FLIP_L, 55 kDa; FLIP_S, 30 kDa; and β-actin, 43 kDa.

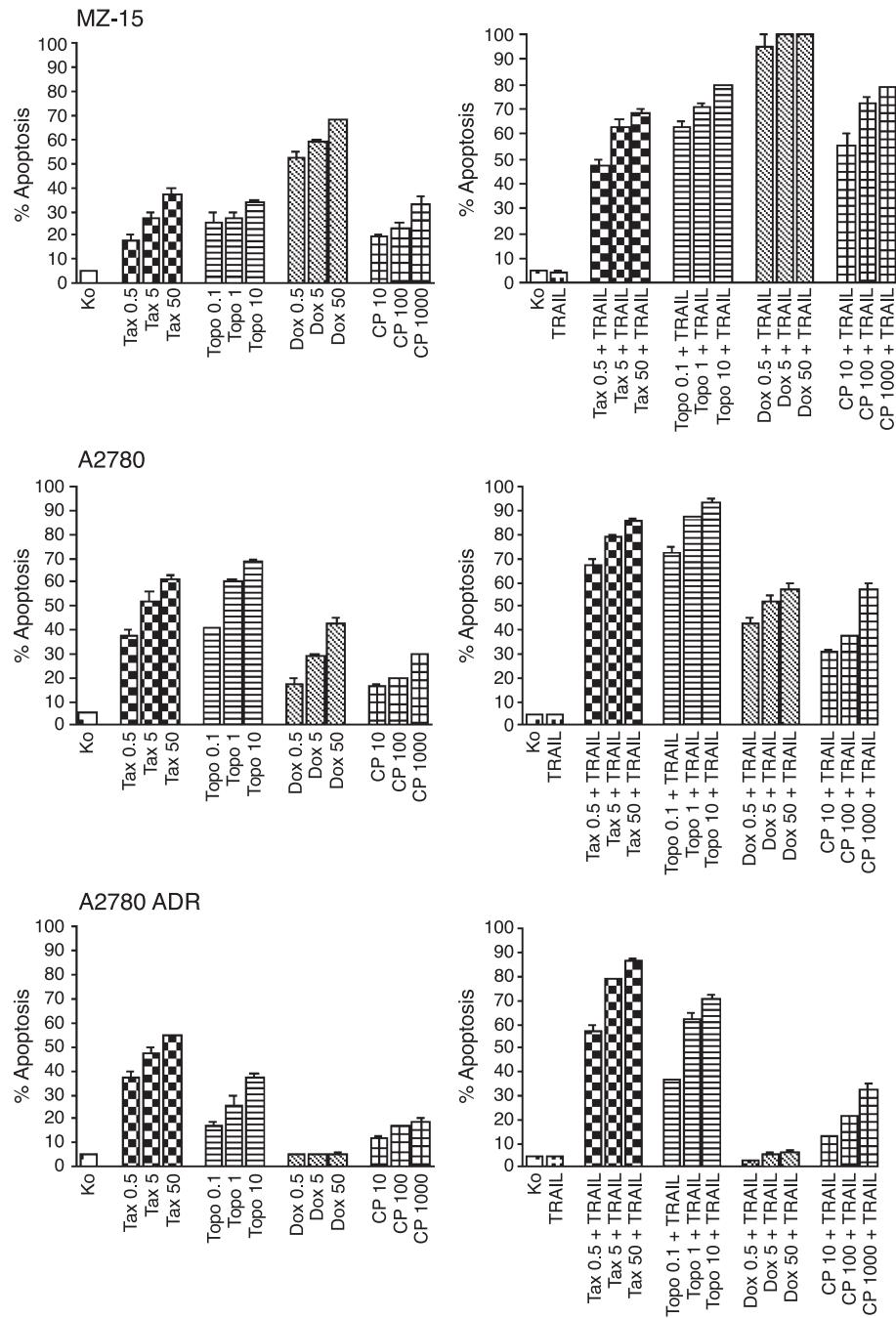


Fig. 4. Paclitaxel, doxorubicin, topotecan, and carboplatin sensitize TRAIL-resistant ovarian cancer cells for TRAIL-induced apoptosis. Apoptosis was measured by the DNA fragmentation assay. Results are the mean of three independent experiments; bars, SD.

has been demonstrated earlier for Fas-mediated apoptosis in neuroblastoma cells [13]. To our knowledge, a correlation between this 43-kDa cleavage product of FLIP_L and resistance to TRAIL induced apoptosis in a cancer cell line has never been demonstrated before. A2780ADR was the only cell line that expressed the unprocessed FLIP_L isoform. Whether this phenomenon contributes to the TRAIL resistance of the A2780ADR cell line is not clear because this particular cell line also lacks the expression of TRAIL receptor DR4.

Transcripts of the soluble TRAIL receptor OPG were detected in MZ-15, ES-2, and CAOV-3 cells, but not in the cell lines MZ-26, A2780, and A2780ADR. OPG is physiologically involved in the regulation of bone density and thus expectedly did not influence the response to TRAIL [17].

Apoptosis induced by TRAIL has recently been investigated in several ovarian cancer cell lines and has resulted in contradicting observations. These discrepancies are possibly due to different TRAIL preparations used, because it is well

known that various preparations of TRAIL cause different toxicities at least in normal cells (reviewed in 2). For example, the cell line SKOV-3 that was sensitive to TRAIL in two studies, which used recombinant human glutathione-S-transferase (GST)-TRAIL [18,19], was resistant in the next that used a recombinant TRAIL-protein without any fusion to heterologous sequences [20].

To investigate whether TRAIL resistance could be overcome by the addition of a cytotoxic agent, MZ-15, A2780, and A2780ADR were co-incubated with TRAIL and paclitaxel, topotecan, doxorubicin, or carboplatin, which are commonly used chemotherapeutic agents in the treatment of ovarian cancer. A strong synergic response was observed with either combination. The mechanism of the synergic effect between TRAIL and the chemotherapeutic agent is either the upregulation of pro-apoptotic molecules or the downregulation of anti-apoptotic molecules [21] and will hardly be associated with the change of a single molecule. This is once more demonstrated in our model, where the resistance to TRAIL is based on two different mechanisms, which could both be overcome by a variety of clinically used cytotoxic agents having different modes of action and targets.

While one priority of us and other groups is to elucidate the mechanism by which sensitivity to TRAIL is regulated, we think that one has to avoid a simplistic approach as demonstrated by the following experiment.

By adding doxorubicin to A2780 cells in a gene profiling experiment using Affymetrix GeneChip Human Genome U133A, compromised of more than 22,000 probe sets, 791 (3.55%) transcripts represented on the gene chip showed a higher expression compared to the control. For 315 transcripts, a three times higher expression was observed and 476 probes were present after doxorubicin treatment compared to their absence in the control. As from gene ontology, an estimated 11% of the probes are related to cell death regulation, we can demonstrate with this experiment that a considerable higher amount of genes is involved when TRAIL and a cytotoxic agent are co-incubated (unpublished data) than one might expect from the study of selected molecules.

To summarize, TRAIL had a synergic effect in almost all combinations with cytotoxic drugs, even though TRAIL by itself was unable to induce cell death in A2780, A2780ADR, and MZ-15 ovarian cancer cell lines. This phenomenon was independent from the underlying mechanism of TRAIL resistance as well as from the cytotoxic agent used. In the MZ-15 cell line, TRAIL resistance is probably based on the expression of the processed form of FLIP_L, p43 (FLIP) and FLIP_S, in A2780 as well as in A2780ADR cells on the absence of DR4 expression. Interestingly, in one cell line, which had developed complete resistance to a chemotherapeutic agent (A2780ADR to doxorubicin), the addition of TRAIL could not compromise this resistance.

Most recently, the expression of TRAIL in ovarian cancer tissue was linked to the prognosis of the disease

implicating an important role of the TRAIL pathway in ovarian cancer [22]. While detailed clinical and mechanistic studies are definitely needed to get more insight into the contribution of the TRAIL apoptotic pathway to ovarian cancer biology and the multifaceted phenomenon of TRAIL resistance, our data and the safety of TRAIL administered in vivo to athymic mice locoregionally [23] suggest that TRAIL-based tumor therapy may be an efficient strategy in the treatment of ovarian cancer, where tumor spread is restricted mostly to the peritoneal cavity and where therapeutics can be applied in relatively high concentrations locoregionally.

Acknowledgments

The authors thank Mrs. Maria Petutschnig, Cornelia Sax, and Waclawa Kalinowski for expert technical assistance. This work was supported by FWF grant P13696-Gen.

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Advances in Brief

Molecular Determinants of Response to TRAIL in Killing of Normal and Cancer Cells¹

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Abstract

The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL or Apo2L) is a potent inducer of death of cancer but not normal cells, which suggests its potential use as a tumor-specific antineoplastic agent. TRAIL binds to the proapoptotic death receptors DR4 and the p53-regulated proapoptotic KILLER/DR5 as well as to the decoy receptors TRID and TRUNDD. In the present studies, we identified a subgroup of TRAIL-resistant cancer cell lines characterized by low or absent basal DR4 or high expression of the caspase activation inhibitor FLIP. Four of five TRAIL-sensitive cell lines expressed high levels of DR4 mRNA and protein, whereas six of six TRAIL-resistant cell lines expressed low or undetectable levels of DR4 (χ^2 ; $P < 0.01$). FLIP expression appeared elevated in five of six (83%) TRAIL-resistant cell lines and only one of five (20%) TRAIL-sensitive cells (χ^2 ; $P < 0.05$). Two TRAIL-resistant lines that expressed DR4 contained an A-to-G alteration in the death domain encoding arginine instead of lysine at codon 441. The K441R polymorphism is present in 20% of the normal population and can inhibit DR4-mediated cell killing in a dominant-negative fashion. The expression level of KILLER/DR5, TRID, TRUNDD or TRID, and TRUNDD did not correlate with TRAIL sensitivity ($P > 0.05$). These results suggest that the major determinants for TRAIL sensitivity may be the expression level of DR4 and FLIP. TRAIL-resistant cells became susceptible to TRAIL-mediated apoptosis in the presence of doxorubicin. In TRAIL-sensitive cells, caspases 8, 9, and 3 were activated after TRAIL treatment, but in TRAIL-resistant cells, they were activated only by the combination of TRAIL and doxorubicin. Our results suggest: (a) evaluation of tumor DR4 and FLIP expression and host DR4

codon 441 status could be potentially useful predictors of TRAIL sensitivity, and (b) doxorubicin, in combination with TRAIL, may effectively promote caspase activation in TRAIL-resistant tumors.

Introduction

TRAIL³, a member of the TNF cytokine family and a type II membrane protein, was initially identified by homology to the C-terminal extracellular domain of other TNF family members, such as Fas ligand (FasL), TNF- α , and lymphotoxin α (1). TRAIL is a potent inducer of apoptosis in a variety of transformed or cancer cells of human and mouse origin but not normal cells (1, 2).

The therapeutic use of the Fas/FasL or the TNF- α /TNFR1 system in cancer treatment has been hampered by severe side effects (3). The systemic administration of TNF causes a septic shock-like response possibly mediated by nuclear factor- κ B activation, and the injection of agonist Ab to Fas can be lethal (3, 4). Compared to TNF- α or Fas, TRAIL may be a safer alternative because normal cells appear to be resistant, and it activates nuclear factor- κ B only weakly (5). Recently, evidence for the safety and potential efficacy of TRAIL therapy against breast and colon cancer was obtained in a severe combined immunodeficiency mouse model (6, 7). Additionally, in cell culture, the human leucine zipper (LZ)-TRAIL had no cytotoxic effects on normal cells, including human mammary epithelial cells, human renal proximal tubule epithelial cells, human lung fibroblasts, and human skeletal muscle cells but was toxic toward mammary adenocarcinoma cells (6). The *in vivo* experiments showed that the systemic administration of LZ-TRAIL into mice inoculated with breast cancer cells prolonged survival. These studies suggest that TRAIL may have a potential use for cancer treatment.

TRAIL can modulate an apoptotic response by binding to one of four cell-surface receptors: Death receptor (DR) 4 (TRAIL-R1; Ref. 8), KILLER/DR5 (TRAIL-R2, TRICK2; Refs. 9–12), TRID (DcR1, TRAIL-R3, or LIT; Refs. 5, 10, 13, and 14), and TRUNDD (DcR2 or TRAIL-R4; Refs. 15–17). DR4 and KILLER/DR5 have two cysteine-rich extracellular ligand-binding domains and a cytoplasmic death domain that signals downstream caspase activation (2, 18). KILLER/DR5 was identified as a candidate p53 target gene, linking DNA

Received 12/15/98; revised 10/29/99; accepted 10/29/99.

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¹ Supported in part by NIH Grants CA75138-01 and CA75454-01.

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³ The abbreviations used are: TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Ab, antibody; TNF, tumor necrosis factor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-PCR; TRUNDD, TRAIL decoy receptor containing a truncated death domain; TRID, TRAIL decoy receptor lacking an intracellular domain; KILLER/DR5, p53-regulated proapoptotic KILLER/death receptor 5; FLIP, FLICE inhibitory protein; PARP, poly ADP-ribose polymerase; FADD, FAS-associated death domain protein; CMV- β -gal, cytomegalovirus β -galactosidase; mAb, monoclonal Ab.

damage signaling from p53 to downstream caspase activation and cell death (9). The extracellular domain of TRID shares a homology with DR4 and KILLER/DR5, but it does not have a cytoplasmic death domain, and it is anchored to the membrane through a glycosyl phosphatidyl inositol linkage. TRUNDD has a substantially truncated cytoplasmic death domain. These two decoy receptors have been reported to protect cells from TRAIL-mediated apoptosis by competing with DR4 and KILLER/DR5 for binding to TRAIL (10).

The TRAIL-mediated biochemical signaling pathway leading to apoptosis is not yet clear. Previously, it was reported that the ectopic expression of FADD-DN (dominant-negative FADD, which blocks apoptotic signaling by the Fas/APO1 death receptor) does not efficiently block apoptosis triggered by TRAIL, and that overexpression of DR4 could induce apoptosis in FADD-deficient embryonic fibroblasts (19). These studies suggest that a FADD-independent pathway may link TRAIL to the caspase cascade (2, 19, 20). Moreover, it was shown that DR4 does not efficiently recruit FADD, TNF receptor-associated death domain (TRADD) protein, receptor interacting protein (RIP), or RIP-associated ICH-1/CED-3 homologous protein (RAIDD; Ref. 10). Although at present there is a missing link between TRAIL death receptors and caspase activation, it is clear that once TRAIL binds to its receptors, apoptosis ensues through the activation of caspases (5, 8, 10). Initiator caspases (caspases 8, 9, and 10) are composed of an N-terminal prodomain that contains the region for homotypic protein-protein interaction with adaptor molecules together with one large and one small subunit. When cells receive death-inducing signals, the prodomain is cleaved, and an active heterodimeric tetramer containing two small and two large subunits is formed. It was reported that caspases 3 and 8 became activated when HeLa cells were treated with TRAIL (21) and also that in TRAIL-sensitive breast cancer cell lines, caspase 3 cleavage was observed (22). In addition, a recent report that T lymphocytes that have catalytically inactive caspase 10 are TRAIL-resistant implicates caspase 10 in TRAIL-mediated apoptosis (23).

Although the efficacy and potential use of TRAIL in cancer treatment has been suggested, little is known about the factors that determine the sensitivity of cancer cells to killing by TRAIL. Recently, there were some reports on the determinants of TRAIL sensitivity in breast cancer cells (22), melanoma (24), and brain tumors (25, 26). The results have been somewhat controversial in that some reports showed no correlation between TRAIL sensitivity and the expression level of proapoptotic death receptors, whereas others demonstrated a correlation between them.

We investigated the expression level of various TRAIL receptor family members as determinants for TRAIL sensitivity and whether a DNA-damaging chemotherapeutic drug such as doxorubicin might have additive effects with TRAIL in killing cancer cells. We report here that the expression of the proapoptotic TRAIL receptors, in particular DR4, and the caspase activation inhibitor FLIP may be major determinants of TRAIL sensitivity. In addition to the expression level of DR4, a polymorphism found in the death domain region of DR4 prevents DR4-mediated cell killing in a dominant-negative fashion. Finally, we also report that a DNA damaging agent such as doxorubicin can sensitize cells to TRAIL-mediated cell killing.

Our results provide essential preclinical information that may be useful in the design of clinical trials using recombinant TRAIL in the therapy of human cancer.

Materials and Methods

Cell Lines. Human lung fibroblast WI38 and human foreskin fibroblast HS27 cells were obtained from the American Type Culture Collection (Rockville, MD). The human ovarian cancer cell line SKOV3, the human breast cancer cell line SKBr3, and the human nasopharyngeal squamous cancer cell line FADU were also obtained from the American Type Culture Collection. The human lung cancer cell lines H460 Neo/E6, the human colon cancer cell lines HCT116 Neo/E6, the human ovarian cancer cell lines PA1 Neo/E6, and the human colon cancer cell line SW480 were maintained as described previously (27). The J82 human bladder cancer cell line was a gift from T. McGarvey and B. Malkowicz (University of Pennsylvania, Philadelphia, PA), and the A875 human melanoma cell line was a gift from D. George (University of Pennsylvania, Philadelphia, PA).

Assessment of Cell Viability. Recombinant soluble human TRAIL was purchased from Kamiya Biomedical Co. (Seattle, WA), and the anti-FLAG M2 mAb was purchased from Sigma (Saint Louis, MI). Three thousand cells were seeded into each well of a 96-well plate. After 24 h, the cells were treated with TRAIL (200 ng/ml) and cross-linked with the anti-FLAG M2 mAb (2 µg/ml). Cell viability was measured by using the MTT assay at 16 h after treatment (28). When normal cells were treated with both doxorubicin and TRAIL, the cells were treated with increasing concentrations of chemotherapeutic drugs alone (doxorubicin, 0, 0.1, 1, 10, and 100 µg/ml) or in combination with TRAIL (20 ng/ml) cross-linked with the anti-FLAG M2 Ab (2 µg/ml). To assess the long-term effect of TRAIL, a total of 5×10^4 of each cell line were seeded in triplicate into 24 wells, and at 24 h, cells were treated with TRAIL (50 ng/ml) and the anti-FLAG M2 Ab (2 µg/ml). The media containing TRAIL and Ab was changed every 48 h, and the culture was maintained for 7 days, at which time the remaining cells were stained with Coomassie Blue.

Semiquantitative RT-PCR. Total RNA was isolated from cell lines as described (29). cDNA was generated from 2 µg of total RNA in a final volume of 20 µl using SuperScript II (Life Technologies, Inc., Gaithersburg, MD) and random primers. The sequences of specific primers used in this experiment were as follows: DR4 F, 5'-CGATGTGGTCAGAGCTGGTACAGC-3'; DR4 R, 5'-GGACACGGCAGAGCCTGTGC-CATC-3'; KILLER/DR5 F, 5'-GGGAGCCGCTCATGAG-GAAGTTG G-3', KILLER/DR5 R, 5'-GGCAAGTCTCTCT-CCAGCGTCTC-3'; TRID F, 5'-GTTTGTTGAAAGACTT-CACTGTG-3', TRID R, 5'-GCAGGGTTCTGCTCTGT-GGAAAC-3'; TRUNDD F, 5'-CTTCAGGAAACCAGAGCTT-CCCTC-3', TRUNDD R, 5'-TTCTCCGTTGCTTATCACGA-3'; GAPDH F, 5'-ACCACAGTCCATGCCATCAC-3', GAPDH R, 5'-TCCACCACCCCTGTTGCTGTA-3'.

To analyze the expression level of the death receptors, 2 µl (out of 20 µl) of synthesized cDNA was amplified in a total volume of 50 µl containing 200 µM each of all four dNTPs, 2 µCi $\alpha^{32}\text{P}$ -dCTP (3000 Ci/mmol), 2 µM each of death receptor-

specific primer set along with 2 μ M each of the GAPDH primers, and 1 unit of *Taq* DNA polymerase (Perkin-Elmer). The cycle numbers that showed linear growth of product were initially determined for each PCR product by analyzing a 10- μ l sample from multiple identical amplification reactions (Fig. 2A and data not shown). In the case of DR4 and KILLER/DR5, 23 cycles were chosen; for TRID and TRUNDD, 24 cycles were chosen; and in the case of GAPDH, 18 cycles were chosen. During PCR, 10 μ l of the reaction were removed at the indicated cycle numbers. PCR conditions were as follows: 1 cycle, 5 min/95°C; 23 or 24 cycles, 30 s/95°C, 30 s/55°C (for DR4, KILLER/DR5, and TRUNDD), 52°C (for TRID), or 30 s/72°C. Nondenaturing PAGE (7%) was performed, and the gel was fixed, dried, and autoradiographed. Band intensities were quantitated by using a Phosphorimager Storm 840 (Molecular Dynamics, Sunnyvale, CA).

Genomic DNA Isolation and Cycle Sequencing. Whole blood (20 ml) from 10 normal healthy volunteers was drawn, and genomic DNA was isolated using the Blood and Cell culture DNA maxi kit (QIAGEN Inc., Valencia, CA). The DNA (50 ng) was used as a template for the amplification of the DR4 death domain region spanning nucleotide 1322. Sequences of primers used in PCR are as follows: DR4 11, 5'-CTCTGATGCTGT-TCTTGAC-3'; DR4 12, 5'-TCACTCCAAGGACACGGCAGA-3'. After amplification, each PCR product was visualized and purified from an agarose gel using the QIAquick gel extraction kit (QIAGEN Inc.) and was then used as a DNA sequencing template. Cycle sequencing was performed using a SequiTHERM cycle sequencing kit (Epicentre Technologies, Madison, WI) according to the manufacturer's instructions.

Site-directed Mutagenesis and Sequencing. Site-directed mutagenesis was performed using a Quick change site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. To change a base in the death domain region of DR4 (A to G at nucleotide 1322 of DR4), plasmids that contained either the full-length DR4 (f/DR4 (A) in pCEP4, Invitrogen, Carlsbad, CA) or the cytoplasmic domain of DR4 (CD/DR4 (A) in pcDNA3.1-Myc, His; Invitrogen, Carlsbad, CA) were used as templates. The sequences of the primer pairs used for changing the base were as follows: DR4DDMUT F, 5'-GGAAGAGAGACATGCAAGAGAGAAGATTCAAGGCC-3'; DR4DD MUT R, 5'-GGTCCTGAATCTTCTCTTGCATGTCTCTTCC-3'. The sequences of the mutagenized plasmids were confirmed. Sequencing of expression plasmids was performed using a T7 DNA sequencing kit (United States Biochemicals, Cleveland, OH) according to the manufacturer's instructions.

The mutagenized f/DR4 or CD/DR4 was used for transfection into SW480 colon cancer cells as previously described (30). After 24 h of transfection, cell lysates were prepared from each transfected followed by Western immunostaining for confirmation of expression after mutagenesis.

Evaluation of Cell Death Induced by Transfected DR4.

For cell death evaluation, cotransfection of the CMV- β -gal marker gene and the DR4 mutant constructs generated was performed as previously described (31). Briefly, 1 \times 10⁵ of SW480 cells were plated per well in 24-well plates and transfected with 2 μ g of the corresponding parental vectors, f/DR4 (A), CD/DR4 (A), f/DR4 (G), or CD/DR4 (G), with CMV- β -gal

at 10% of the total amount of DNA. At 24 or 48 h later, cells were fixed and stained with 5-bromo-4-chloro-3-indolyl- β -galactopyranoside to quantify the number of blue cells. To determine whether polymorphic DR4 has a dominant-negative effect on cell killing, SW480 cells were transfected with variable ratios of CD/DR4 (A) to CD/DR4 (G), f/DR4 (A) to CD/DR4 (G), or f/DR4 (A) to f/DR4 (G) (4:1, 1:1, and 1:4) along with CMV- β -gal.

Abs and Western Blot Analysis. Western blot analysis was carried out as previously described (32). Blotted membranes were immunostained with anti-PARP (1:2000; Boehringer Mannheim, Mannheim, Germany), anti-caspase 3 (E-8, 1:500; Santa Cruz Biotechnologies, Inc., Santa Cruz, CA), anti-caspase 7 (1:500; PharMingen, San Diego, CA), anti-caspase 8 (C-20, 1:500; Santa Cruz Biotechnologies, Inc.), anti-caspase 9 (1:500; IIMGENEX, San Diego, CA), anti-caspase 10 (N-19, 1:500; Santa Cruz Biotechnologies, Inc.), anti-caspase 2 (H-19, 1:500; Santa Cruz Biotechnologies, Inc.), anti-DR4 (1:500, PharMingen), anti-DR5 (1:500; IMGENEX), anti-FLIP (1:500; IMGENEX), anti-Myc (9E10, 1:500; Santa Cruz Biotechnologies, Inc.), or antiactin (I-19, 1:200; Santa Cruz Biotechnologies, Inc.).

Statistical Analysis. The statistical correlation between the expression level of TRAIL death receptors and TRAIL-mediated apoptosis was performed using regression analysis and the correlation between the expression of FLIP and TRAIL sensitivity, or the expression of DR4 and TRAIL sensitivity was performed using the χ^2 test.

Results

Normal Cells as Well as a Newly-defined Subset of Cancer Cells Are Resistant to TRAIL-mediated Apoptosis. We evaluated the cell killing effect of TRAIL on various normal and cancer cell lines. As previously reported by others (1, 3), normal cells (fibroblasts) were resistant to TRAIL treatment (Fig. 1, A and B). In contrast, cancer cells showed a variable response to TRAIL (Fig. 1). HCT116, H460, PA1, SKBr3, and SW480 were sensitive to TRAIL. TRAIL sensitivity was defined as a <75% cell viability at 16 h after TRAIL treatment is measured by the TRAIL MTT assay. A875, FADU, J82, and SKOV3 cells were found to be resistant to TRAIL. Human Papillomavirus E6-expressing HCT116, H460, and PA1 cells were relatively more resistant to TRAIL than the neocounterparts (Fig. 1A). Long-term (7 days) TRAIL treatment of cell lines (Fig. 1B) showed nearly the same result as the short-term (16 h) MTT assay results. Based on the observations from the long-term TRAIL treatment assay, certain fractions of cells showed resistance to TRAIL, although the majority of the cells were killed by TRAIL treatment.

Taken together, those results suggest that there is a subgroup of TRAIL-resistant cancer cells and that to a degree, wild-type p53 may modulate TRAIL responsiveness. We further explored the molecular basis of TRAIL resistance in cancer cells.

Correlation between TRAIL Receptor Expression and TRAIL Sensitivity. To determine whether there is any correlation between TRAIL sensitivity and the expression level of TRAIL receptors, a semiquantitative RT-PCR assay was per-

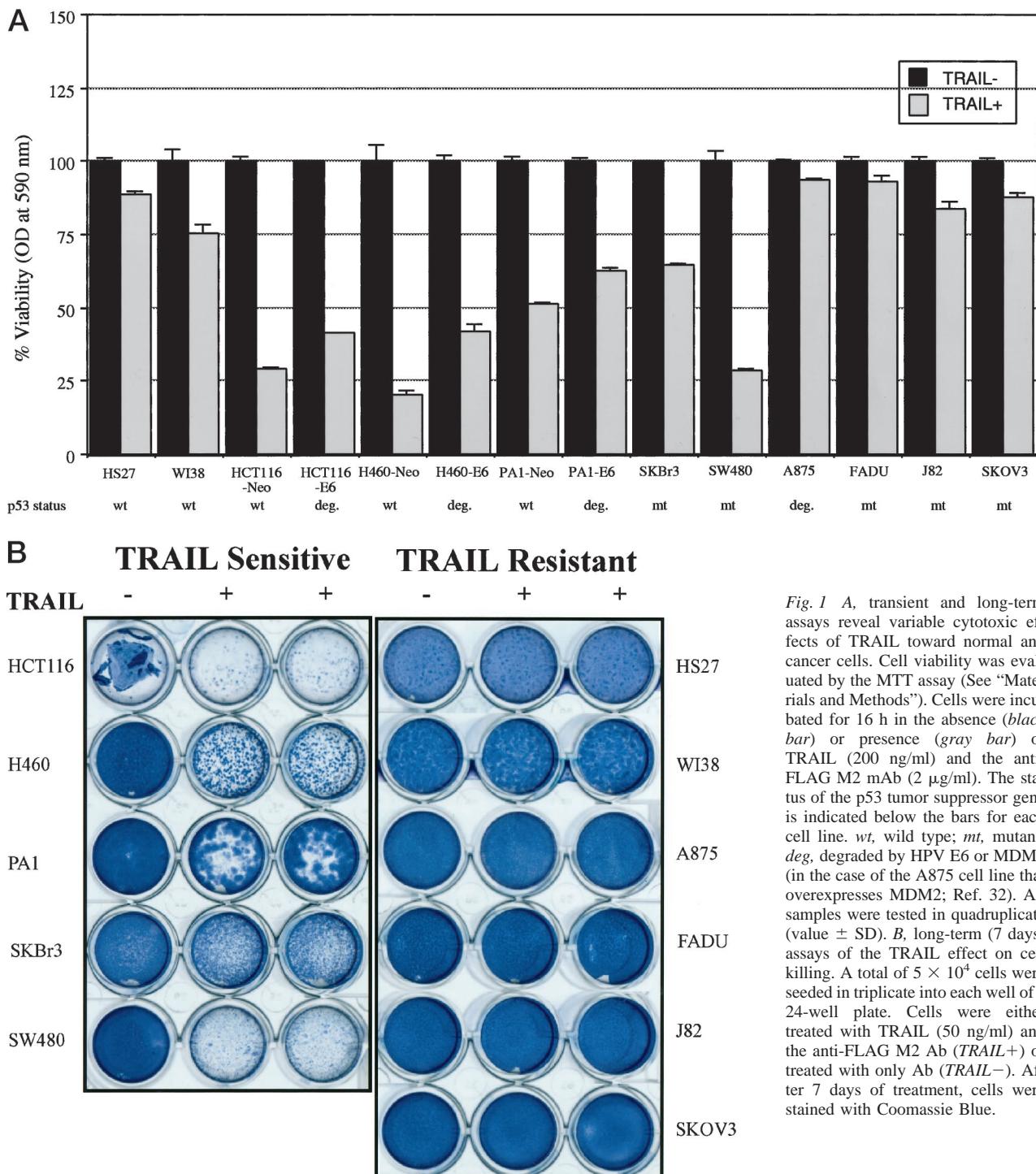


Fig. 1 *A*, transient and long-term assays reveal variable cytotoxic effects of TRAIL toward normal and cancer cells. Cell viability was evaluated by the MTT assay (See “Materials and Methods”). Cells were incubated for 16 h in the absence (black bar) or presence (gray bar) of TRAIL (200 ng/ml) and the anti-FLAG M2 mAb (2 µg/ml). The status of the p53 tumor suppressor gene is indicated below the bars for each cell line. *wt*, wild type; *mt*, mutant; *deg*, degraded by HPV E6 or MDM2 (in the case of the A875 cell line that overexpresses MDM2; Ref. 32). All samples were tested in quadruplicate (value ± SD). *B*, long-term (7 days) assays of the TRAIL effect on cell killing. A total of 5×10^4 cells were seeded in triplicate into each well of a 24-well plate. Cells were either treated with TRAIL (50 ng/ml) and the anti-FLAG M2 Ab (*TRAIL+*) or treated with only Ab (*TRAIL-*). After 7 days of treatment, cells were stained with Coomassie Blue.

formed (Fig. 2). The number of PCR cycles required for linear amplification and detection was initially determined for each death receptor (Fig. 2A). KILLER/DR5 was expressed in all cell lines tested (Fig. 2, *B* and *C*), and its mRNA expression level did not correlate with TRAIL sensitivity (Fig. 3B). In contrast, the expression level of DR4 varied among different cell lines (Fig. 2B). For example, in normal fibroblast cells, DR4 expression was very low or not detectable (Fig. 2B, *Lanes 1* and *2*). Cancer

cell lines except J82 and SKOV3 that expressed DR4 were sensitive to TRAIL regardless of p53 status (Fig. 1, Fig. 2B, and Fig. 3A; see below). PA1, A875, and FADU cells did not express detectable DR4 protein (Fig. 2B, *Lanes 5*, *6*, and *9*). DR4 protein was highly expressed in HCT116, H460, and SW480 cells (DR4 in Fig. 4, *Lanes 3*, *4*, and *7*), and they were the most sensitive cell lines to TRAIL (Fig. 1, *A* and *B*). The antiapoptotic TRAIL receptors, TRID and TRUNDD, were also

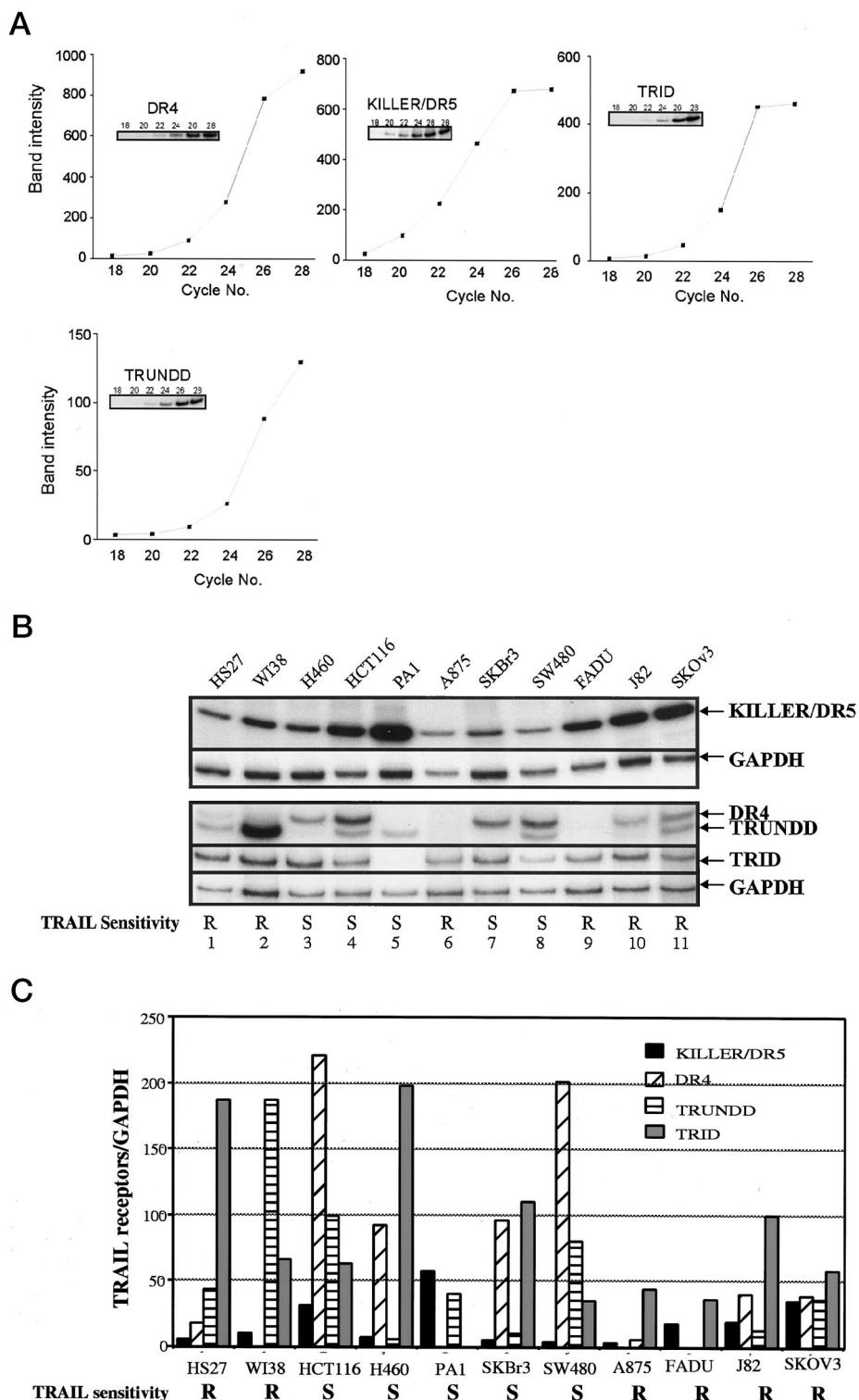


Fig. 2 Expression level of TRAIL death receptor genes in normal and cancer cells. **A**, kinetics of amplification of mRNA using a semiquantitative-labeled RT-PCR assay (see "Materials and Methods"). Autoradiograms are shown in the inset for each experiment, with PCR cycle numbers shown above different lanes. **B**, expression of TRAIL receptor genes using the semiquantitative RT-PCR assays as described in the text. **C**, relative expression of TRAIL receptors normalized with GAPDH expression.

expressed in cancer cells. TRID was expressed in all of the cell lines except PA1 cells, whereas TRUNDD was not expressed in H460, A875, SKBr3, and FADU cell lines (Fig. 2B, Lanes 3, 6, 7, and 9). The high expression of TRID or TRUNDD in the

normal cell lines HS27 or WI38 is consistent with previous results implicating high decoy receptor expression as a mechanism of TRAIL resistance. However, neither TRID nor TRUNDD levels adequately explain the observed patterns of

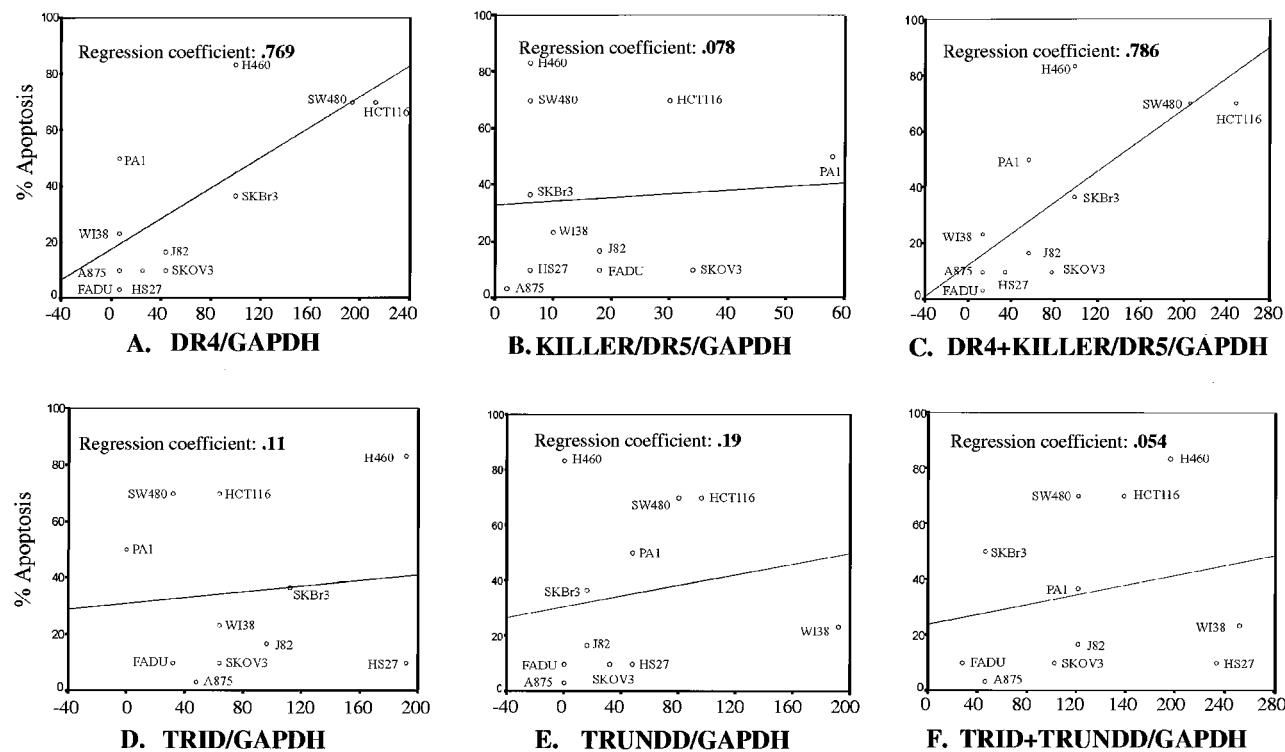


Fig. 3 Regression analysis of the relation between TRAIL-mediated apoptosis and the expression level of death receptors normalized to GAPDH expression. A, B, D, and E, the result obtained from regression analysis between TRAIL-mediated apoptosis *versus* the expression level (determined by RT-PCR) of each TRAIL death receptor. C and F, the result obtained from regression analysis between TRAIL-mediated apoptosis *versus* the sum of the expression level of the proapoptotic TRAIL death receptors and the antiapoptotic TRAIL death receptors. The regression coefficient for the relation between apoptosis and expression of DR4 or DR4+KILLER/DR5 was 0.769 and 0.786, respectively ($P = 0.006$ and 0.004 , respectively).

TRAIL sensitivity in the panel of cancer cells (Fig. 3, D-F). The presence of DR4 alone ($r = 0.769; P = 0.006$) or DR4 and KILLER/DR5 ($r = 0.786, P = 0.004$) appeared to correlate better with TRAIL sensitivity of cancer cells than the expression of decoy receptors (Fig. 3, A and C).

FLIP Expression Correlates Well with TRAIL Resistance. Cellular FLIP is an inhibitor of caspase activation and may be overexpressed in human cancer cells (33). We determined whether the expression level of FLIP might correlate with TRAIL sensitivity. We detected FLIP expression in five of six TRAIL-resistant cell lines including normal cells A875, J82, and SKOV3 (FLIP in Fig. 4, Lanes 1, 2, 8, 10, and 11) but only in one (PA1) of five TRAIL-sensitive cell lines (FLIP in Fig. 4, Lane 5). These results suggest that high expression of FLIP may be the another important determinant of TRAIL resistance ($\chi^2; P < 0.05$).

K441R Polymorphism Found in the Death Domain of DR4. Contrary to our expectation that DR4-expressing cells should be sensitive to TRAIL, J82 and SKOV3 were resistant to TRAIL treatment. Previously, there was a report indicating that Fas carrying a mutation in the death domain region could act as a dominant-negative inhibitor of Fas-induced cell killing (25). To investigate whether there is a DNA sequence change in the death domain of DR4 in J82 and SKOV3 cells, RT-PCR and DNA sequencing was performed. Sequencing results showed that there is an A-to-G alteration in nucleotide 1322 of DR4 both

in SKOV3 and J82 cells (Fig. 5A and data not shown). This A-to-G transition resulted in the conversion of the amino acid lysine (codon 441) to arginine. To determine whether this alteration is present in normal populations, genomic DNA was isolated from total blood drawn from 10 normal healthy volunteers, and PCR cycle sequencing was performed. The results revealed that 2 (donor 1 and 10) of 10 (20%) normal individuals have the base change (Fig. 5B), and thus, we refer to the alteration as a polymorphism. The polymorphism was found in donors 1 and 10, and SKOV3 was heterozygous in all cases (Fig. 5B).

Effect of the K441R Polymorphism in the Death Domain of DR4 on DR4-mediated Cell Killing. To determine whether the K441R polymorphism has any effect on DR4-mediated cell killing, we generated DR4 mammalian expression constructs containing the polymorphism by using site-directed mutagenesis (Fig. 6, A and B). Upon transfection, we found that polymorphic DR4 was less effective in cell killing than its wild-type counterpart (Fig. 6, C and D). In addition, polymorphic DR4 showed an inhibitory effect toward cell killing by wild-type DR4. A potent dominant-negative effect of the K441R polymorphism was observed when the cytoplasmic DR4 (CD/DR4) was expressed. The CD/DR4 (G) rather than f/DR4 (G) showed a nearly complete inhibition of DR4-mediated cell killing (Fig. 6D).

These results suggest, at least in terms of TRAIL sensitiv-

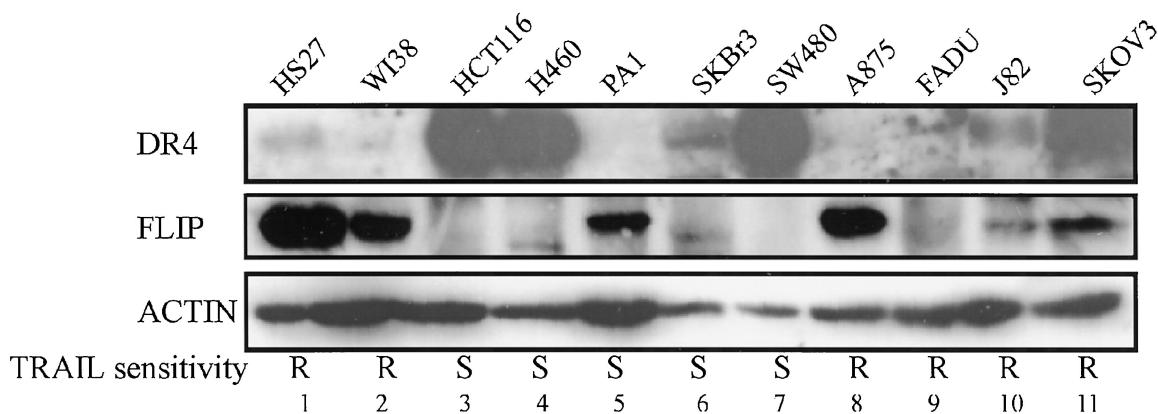


Fig. 4 Protein expression of DR4 and FLIP. Cell lysates were prepared from each cell line, and an equal amount of protein was loaded on a 15% SDS-PAGE gel. Western immunoblotting was performed with anti-DR4 and anti-FLIP Ab. Actin was used as an internal control for protein loading.

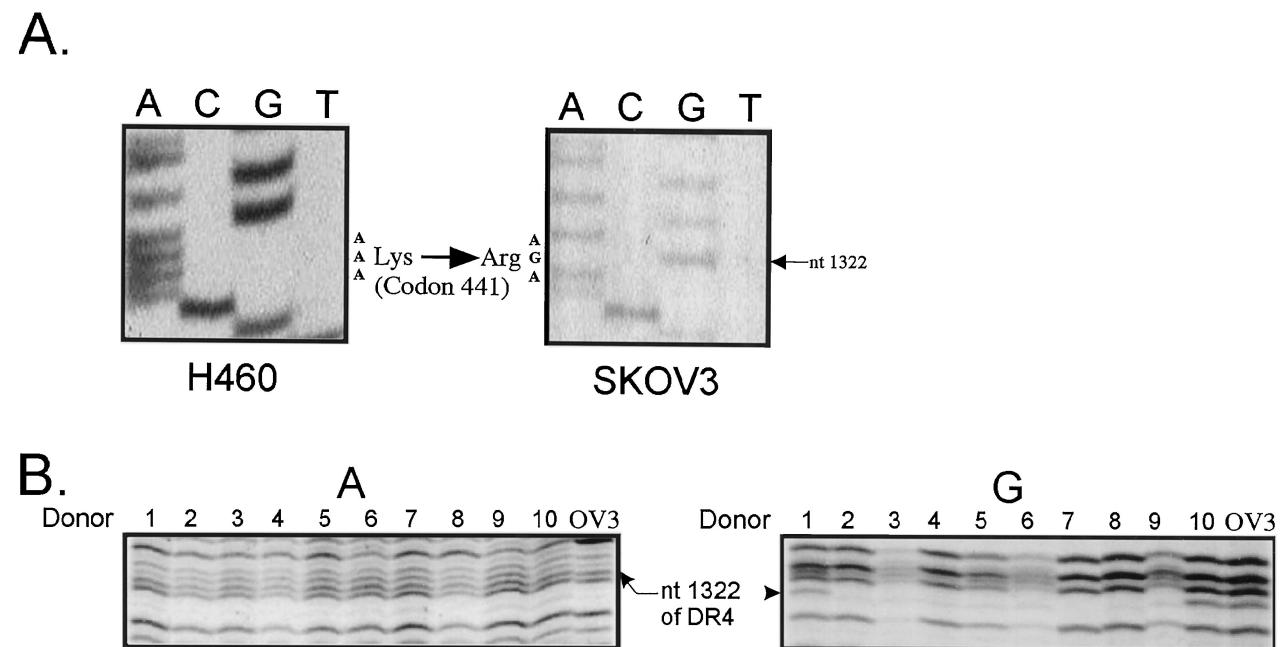


Fig. 5 K441R polymorphism found in the death domain of DR4. A, A-to-G transition at nucleotide 1322 of DR4 in SKOV3 cells. RT-PCR was performed as described in the text. PCR products were cloned into a TA cloning vector (Invitrogen) followed by sequencing using cloned plasmid as a template. Approximately 50% of the clones contained the K441R polymorphism. TRAIL-sensitive DR4-expressing cell lines such as H460 (and HCT116, data not shown) have A at nucleotide 1322, but resistant cell lines such as SKOV3 (and J82, data not shown) have G encoding arginine instead of lysine at codon 441. B, A-to-G transition is found in a normal population. PCR amplification using genomic DNA isolated from whole blood of normal healthy donors as a template was performed and followed by cycle sequencing. Samples from each termination mix were loaded together for easy comparison. Donors 1 and 10 showed A-to-G transition, and also, they were heterozygous. SKOV3 also shows an A-to-G transition and is heterozygous.

ity, that the K441R polymorphism in the death domain of DR4 makes cells relatively resistant to TRAIL treatment, although they express DR4 on their cell surface. Thus, this polymorphism found in J82 and SKOV3 could contribute to TRAIL resistance.

Cell Killing by Combination of Doxorubicin and TRAIL in TRAIL-resistant Cell Lines. Normal cells such as HS27 and WI38 are resistant to TRAIL in part due to a low or

undetectable expression of DR4, a high expression level of decoy receptors, and a high expression level of FLIP (Fig. 2 and Fig. 4). However, when these cells were treated with the combination of doxorubicin and TRAIL, viability was dramatically reduced (Fig. 7A) and PARP cleavage became evident (Fig. 7B). Western immunostaining (Fig. 7C) showed that there was a significant induction of KILLER/DR5 protein expression. This

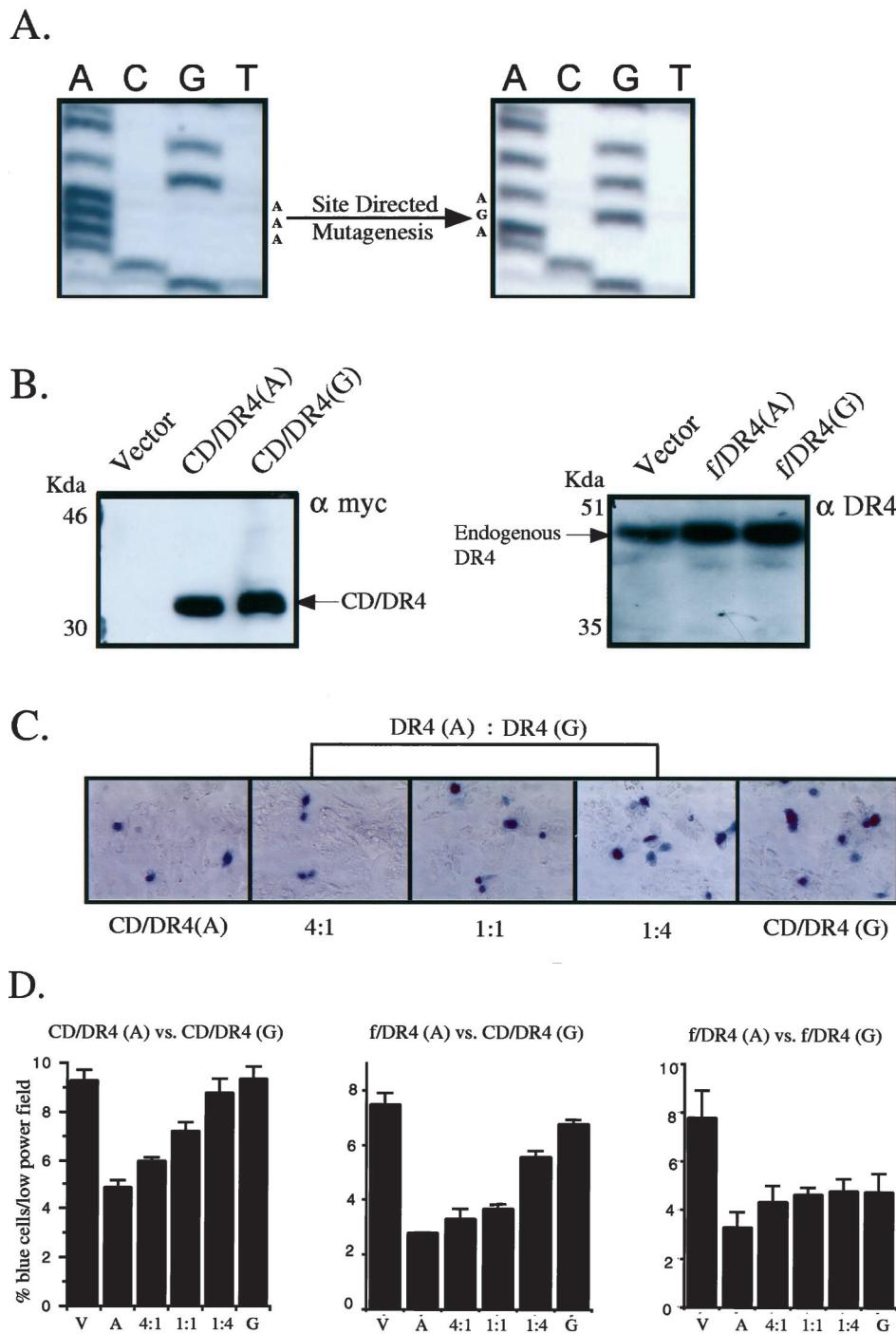
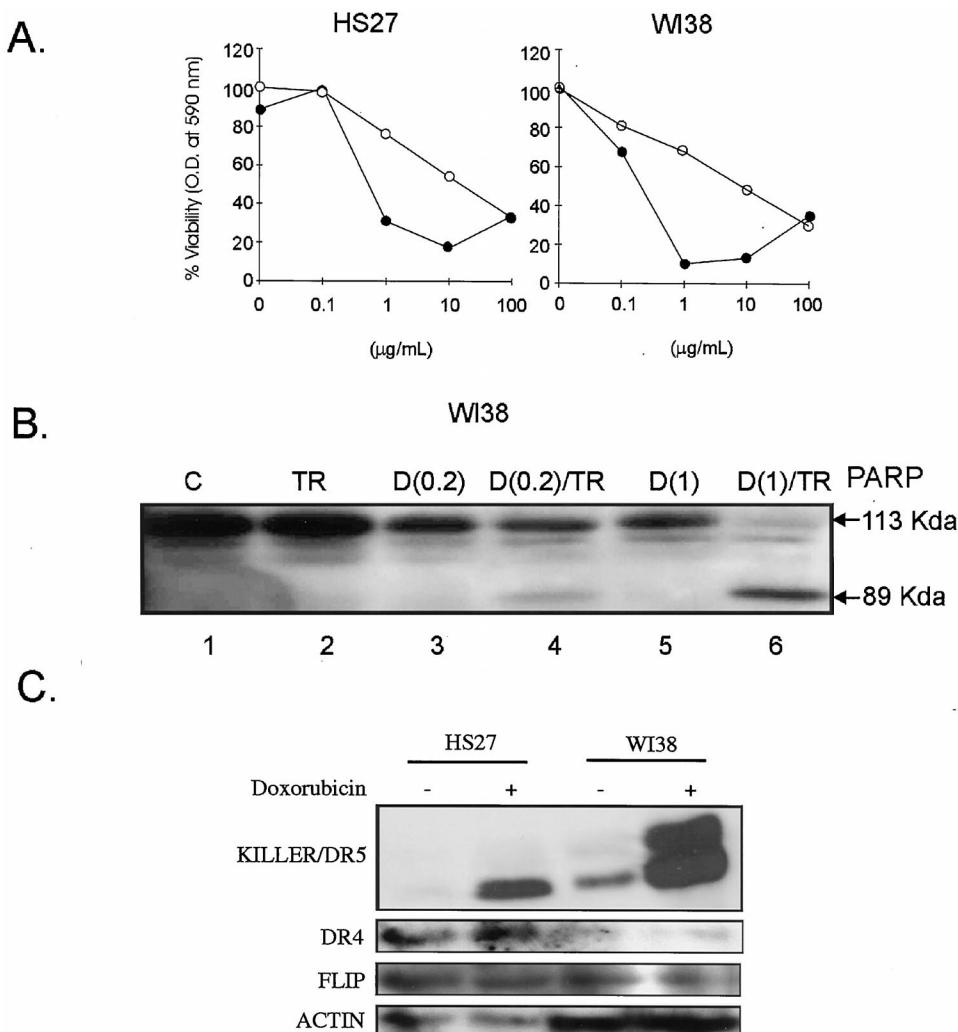


Fig. 6 Functional effect of the polymorphism on the DR4-mediated cell killing. *A*, site-directed mutagenesis of a DR4 expression plasmid. F/DR4 (A) or CD/DR4 (A) that can express a full-length or cytoplasmic domain of DR4 cloned in pCEP4 or pcDNA 3.1, respectively, was used for mutagenesis. Mutagenesis was confirmed by sequencing. The resulting constructs were named f/DR4 (G) or CD/DR4 (G). *B*, Western blot analysis to confirm the protein expression of CD/DR4 and f/DR4 constructs before and after mutagenesis. SW480 cells were transfected with each DR4 expressing construct. At 20 h after transfection, cell lysates were prepared, and Western immunoblotting was performed using anti-DR4 for f/DR 4 or anti-Myc for CD/DR4. Arrow, myc-tagged CD/DR4. *C*, SW480 cells were cotransfected with variable ratios of CD/DR4 (A) to CD/DR4 (G), as indicated, and CMV-β-gal (at 10% of the total DNA) for 48 h. Cells were then stained for the β-galactosidase activity with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. The same high power fields ($\times 320$) are shown under phase-contrast microscopy. *D*, dominant-negative effect of polymorphic DR4 on wild-type DR4. The number of blue cells per low power field ($\times 100$) was quantified after transfection of SW480 cells as described in *C*. All samples were tested in quadruplicate (value \pm SD). V, vector; A, wild-type DR4; G, polymorphic DR4.

induction of KILLER/DR5 by doxorubicin may sensitize normal cells to TRAIL-mediated cell killing. These results suggest that an increase in the ratio of expression between proapoptotic and antiapoptotic molecules may reset the responsiveness of the cells from resistant to sensitive. There was no change in the level of DR4 or FLIP expression after doxorubicin treatment (Fig. 7C).

p53 function was compromised in all of the TRAIL-resistant cancer cell lines tested in this study either by mutation (J82, FADU, and SKOV3) or by the overexpression of MDM2 (A875; Ref. 32). Thus, an exposure to a DNA damaging agent such as doxorubicin might not be expected to result in the p53-dependent KILLER/DR5 induction observed in the normal cells. Nevertheless, when those cells were treated with both

Fig. 7 KILLER/DR5 but not DR4 induction after doxorubicin exposure correlates with an enhanced sensitivity of normal cells to TRAIL-mediated apoptosis. *A*, effect of combined treatment of doxorubicin and TRAIL on viability of HS27 or WI38. Cells were treated with varying concentrations of doxorubicin in the absence (open circles) or presence (solid circles) of TRAIL (20 ng/ml) and anti-FLAG M2 mAb (2 µg/ml) for 16 h. Cell viability was evaluated by MTT assay. *B*, cleavage of PARP occurs upon treatment of WI38 with TRAIL and doxorubicin. *C* represents control cells (*Lane 1*); *TR* represents cells treated with TRAIL only (*Lane 2*); *D(0.2)* represents cells treated with doxorubicin (0.2 µg/ml; *Lane 3*); *D(0.2)/TR* represents cells treated with doxorubicin (0.2 µg/ml) and TRAIL (*Lane 4*); *D(1)* represents cells treated with doxorubicin (1 µg/ml; *Lane 5*); and *D(1)/TR* represents cells treated with doxorubicin (1 µg/ml) and TRAIL (*Lane 6*). *C*, Western blot analysis revealed that there was an induction of KILLER/DR5 but no change in DR4 or FLIP expression after doxorubicin treatment. Actin was used as an internal control for protein loading.



doxorubicin and TRAIL, PARP cleavage became evident (PARP in Fig. 8C, *Lanes 4, 8, 12, and 16*).

Because there were no changes in the expression level of DR4, DR5, or FLIP after doxorubicin treatment in TRAIL-resistant cancer cell lines (data not shown), we investigated the effect of TRAIL or doxorubicin on the activation of caspases. In terms of doxorubicin sensitivity, TRAIL-resistant cancer cell lines can be divided into doxorubicin-sensitive (FADU) and doxorubicin-resistant (A875, J82, and SKOV3) cells (Fig. 8C and morphological data not shown).

In doxorubicin-sensitive FADU cells, caspase 8 was activated by doxorubicin treatment alone (caspase 8 in Fig. 8C, *Lane 7*). Caspase 9 was also activated by doxorubicin treatment alone in FADU cells (caspase 9 in Fig. 8C, *Lane 7*). Unexpectedly, however, although there was activation of caspases 8 and 9 ("initiator" caspases) in doxorubicin-treated FADU cells, we did not observe complete procaspase 3 ("executioner" caspase) depletion (caspase 3 in Fig. 8C, *Lane 7*). In the doxorubicin-resistant cell lines (A875, J82, and SKOV3), caspase activation was not observed after exposure to either doxorubicin alone or

TRAIL alone (Fig. 8C). Interestingly, caspases 8, 9, and 3 became activated after exposure to the combination of doxorubicin and TRAIL (caspases 8, 9, and 3 in Fig. 8C, *Lanes 4, 12, and 16*). In contrast to TRAIL-resistant cancer cells, cleavage of caspases 8, 9, and 3 was observed after TRAIL treatment of the TRAIL-sensitive HCT116 colon cancer cell line (Fig. 8A). When HCT116 was treated with TRAIL, PARP cleavage was evident by 4 h after TRAIL addition, and caspases 8, 9, 3, and 7 became activated at approximately the same time point (4 h after the TRAIL addition; Fig. 8B).

Discussion

The cytokine TRAIL is a promising agent for cancer therapy and is presently under investigation (6, 7). The importance of TRAIL as a potential anticancer agent is that it appears to be a potent cancer-specific cytotoxic drug and is not as toxic as other cytokines. TNF- α or Fas have not been successful in clinical trials when administered systemically because of toxicity (3, 4).

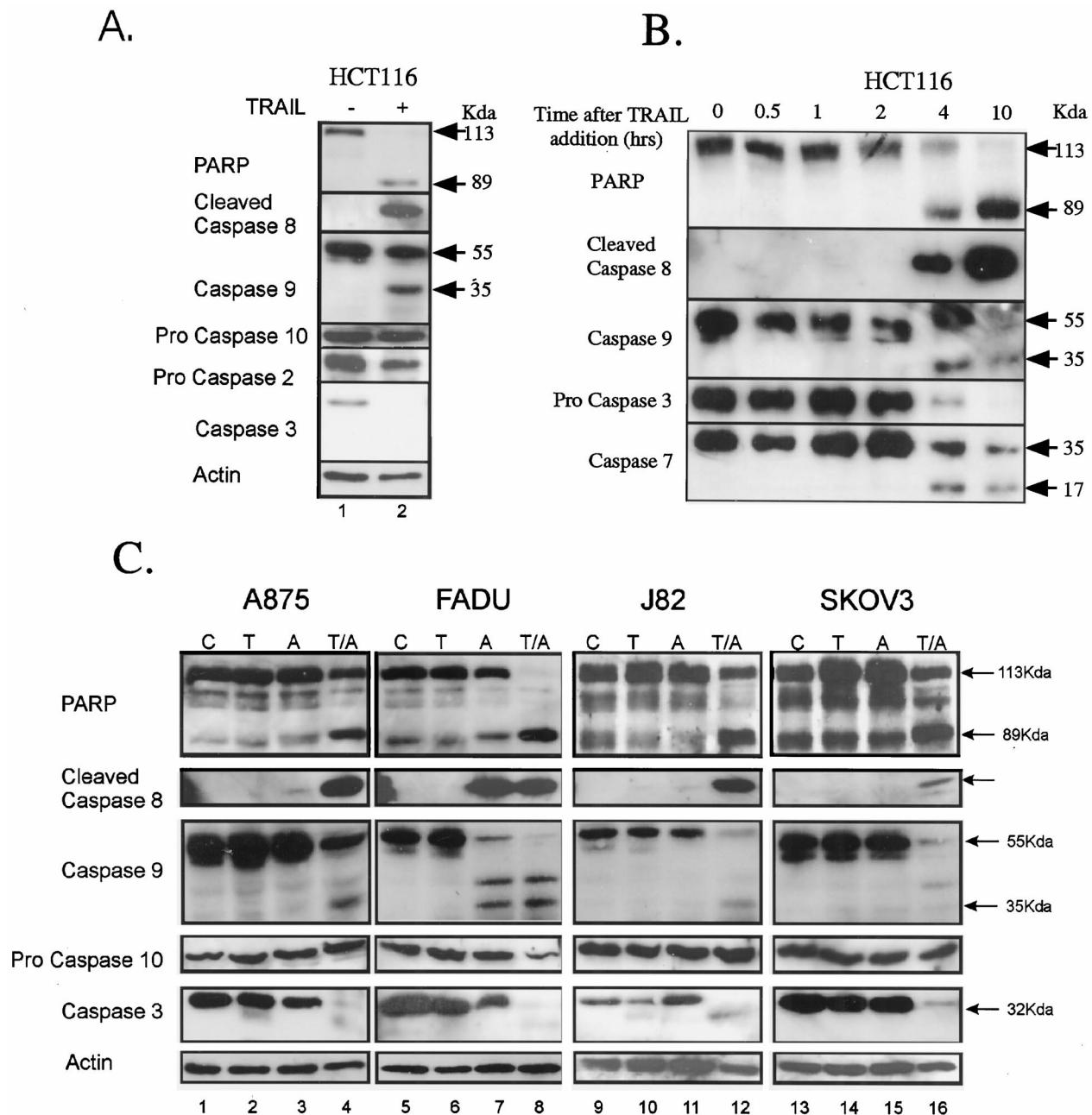


Fig. 8 Caspase activation after treatment by TRAIL alone or combined treatment using doxorubicin and TRAIL in TRAIL-sensitive and TRAIL-resistant cells. A, TRAIL-sensitive HCT116 cells were treated with TRAIL (200 ng/ml) and cross-linked with the anti-FLAG M2 Ab (2 μ g/ml). B, time course activation of caspases in HCT116 after treatment of TRAIL (200 ng/ml) cross-linked with anti-FLAG M2 Ab (2 μ g/ml). Lysates were prepared at the indicated times shown above the figure. C, TRAIL-resistant cells were treated with TRAIL (200 ng/ml) cross-linked with the anti-FLAG M2 Ab (2 μ g/ml) alone (T), doxorubicin (5 μ M) alone (A), or with both (T/A) for 16 h. Cell lysates were prepared, and an equal amount of cellular protein was used for Western immunoblotting. C represents mock treatment.

Our results provide novel basic information relevant to TRAIL therapy of cancer in the following respects. First, we report that TRAIL resistance is mainly determined by the expression of its proapoptotic death receptors, especially DR4 ($r = 0.769$, $P = 0.006$). In fact, cell lines that were resistant to TRAIL were found to have a relatively low or undetectable

expression level of DR4. Normal cell lines, such as HS27 and WI38, which are resistant to TRAIL, have extremely low expression of DR4 mRNA or protein (Fig. 2B, Fig. 3A, and Fig. 4), and a subgroup of TRAIL-resistant cells also have low or undetectable DR4 expression (Fig. 2B and Fig. 4). For DR4 expression alone, a χ^2 analysis revealed that this parameter is a

highly significant predictor of TRAIL sensitivity when expression is high *versus* low or undetectable ($P < 0.01$). For the χ^2 analysis, high expression was defined as DR4/GAPDH > 50 as shown in Fig. 2C. It is important to note that mRNA levels do not always correlate with protein levels and that the strength of the correlation between DR4 expression and TRAIL sensitivity (Fig. 2 and Fig. 3) might be stronger or weaker if the measured DR4 protein levels (Fig. 4) were actually quantitated. The expression of KILLER/DR5, however, does not correlate well with TRAIL sensitivity (Fig. 2 and Fig. 3B). Our observation is supported by a recent report that TRAIL sensitivity in melanoma cells correlates well with the expression level of DR4 (24). Contrary to our observation, J82 and SKOV3 expressed DR4 (Fig. 2B and Fig. 4) but were resistant to TRAIL treatment. A previous report that mutation in the death domain region of Fas can act as in a dominant-negative fashion in cell killing (25) prompted us to examine the death domain region of DR4 in J82 and SKOV3 cells. Indeed, J82 and SKOV3 have an A-to-G alteration at codon 441 in the death domain region of DR4 (Fig. 5A). However, that change is also found in 20% (2 of 10) of a normal population and thus, we refer to the DR4 K441R alteration as a polymorphism. Polymorphic DR4 acted in a dominant-negative manner in DR4-mediated cell killing (Fig. 6, C and D). We make no claim about any disease susceptibility associated with the K441R polymorphism in the DR4 gene. However, the presence of the K441R DR4 polymorphism in cancers may reduce their sensitivity to TRAIL, at least *in vitro*.

It is important to note the differences observed when full-length *versus* cytoplasmic domain expression constructs were used to express DR4. In particular, Fig. 6, C and D demonstrates that the cytoplasmic domain of DR4 does not itself induce cell death when it contains 441R. In addition, this variant of the cytoplasmic is capable of completely inhibiting death induced by the 441K allele. However, full-length DR4 containing the K441R mutation does not share these properties. Instead, full-length DR4 containing the 441R allele induces apoptosis in $\sim 50\%$ of transfected cells and poorly inhibits killing by the full-length 441K allele (Fig. 6D, right). These results suggest that the polymorphic 441R allele may contribute but cannot alone explain the observed resistance to TRAIL in certain cancer cell lines (J82 and SKOV3). These cell lines express somewhat increased levels of FLIP (Fig. 4), which may also contribute to their resistance to TRAIL (see below).

Second, the inhibitor of caspase activation FLIP may confer resistance to TRAIL at a point downstream of the death receptors. We found that 83% (five of six cell lines) of TRAIL-resistant cell lines showed a detectable expression of FLIP, whereas only one of five (20%) TRAIL-sensitive lines expressed FLIP (Fig. 4; χ^2 ; $P < 0.05$). However, the fact that FLIP-expressing PA1 cells are sensitive to TRAIL suggests that even in the presence of FLIP, cells can be killed if there is enough of an input signal for inducing apoptosis.

We measured the expression level of five genes (DR4, KILLER/DR5, TRID, TRUNDD, and FLIP) and tested for correlations with TRAIL sensitivity. The expression of two of the parameters (DR4 and FLIP) appeared to independently correlate with TRAIL sensitivity. From the regression analysis shown in Fig. 3, the P value for the DR4 correlation with TRAIL sensitivity is 0.006 (see legend of Fig. 3). Thus, we

would have had to test 167 variables to reach the 0.006 level of significance at random for DR4 due to the effect of multiple testing. Moreover, the design of our study was hypothesis driven, with a biological basis giving a reasonable pretest probability of certain correlations. For example, we tested biologically plausible determinants of TRAIL sensitivity. One of the concerns with multiple correlations arises when one tests a very large number of variables (without a hypothesis), such as in a questionnaire with several hundred questions or perhaps a query of an expression of several thousand genes on a DNA microarray chip, and then develops the hypothesis based on any observed correlations at the $P < 0.05$ level. Of course, if one tests enough variables, there is a random chance that a few will appear to be significant but will actually be meaningless. Thus, because we believed that correcting for multiple testing artifacts would not significantly alter our P s or conclusions, we have not corrected our calculations for the effects of multiple comparisons. Thus, there is a small chance that our analysis may be limited by the effects of multiple comparisons, and it remains to be seen if others will find a similar significance of DR4 and FLIP expression levels using larger sample sizes and testing fewer variables.

Third, the targeted destruction of p53 to generate otherwise isogenic cancer cell lines revealed that TRAIL sensitivity could be modulated somewhat by p53 (Fig. 1). This is a preliminary observation that requires further investigation. It is clear from our data that wild-type p53 is not required for the apoptotic response to TRAIL.

Fourth, the combination of doxorubicin and TRAIL can kill TRAIL-resistant cancer cells, although each treatment alone cannot effectively kill the cells. The mechanism(s) of this additive killing is not clear yet. We have ruled out changes in the expression level of death receptors or FLIP as a basis for enhanced cell killing by doxorubicin plus TRAIL (data not shown). The fact that FADU cells show caspase 8 and 9 activation upon doxorubicin treatment suggests that the caspase activation axis from caspase 8 through Bcl2 inhibitory protein (Bid) to caspase 9 might be intact in FADU cells but not in other TRAIL-resistant cell lines (Fig. 8C). As recently reported (22) and observed in our experiments, doxorubicin and TRAIL could activate caspases in augmenting the killing effect. However, although TRAIL resistance can be overcome by combined treatment with doxorubicin, careful consideration should be given to the dose of doxorubicin given the observed sensitization of normal cells to TRAIL-mediated apoptosis (Fig. 7).

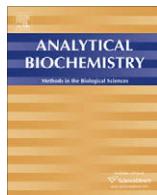
Fifth, among TRAIL-sensitive cancer cells, a certain fraction appears to be resistant to TRAIL-mediated killing (Fig. 1B). A recent report also showed that subclones of TRAIL-sensitive cancer cells display a variable response to TRAIL, although the expression level of TRAIL death receptors or FLIP was not changed (24). We do not know the underlying mechanism of this TRAIL resistance yet.

Our findings suggest that although TRAIL may be useful as a therapeutic agent in cancer, particular attention to molecular determinants of sensitivity needs to be considered to optimize such therapy. TRAIL does not appear to have harmful effects toward normal cells and can kill cancer cells irrespective of p53 status if wild-type DR4 is expressed on their cell surface. Our results also indicate that doxorubicin can sensitize cells to

TRAIL-mediated cell killing *in vitro*, thereby raising hopes that such a strategy may be useful in cancer therapy.

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Identification of suitable reference genes for gene expression studies of human serous ovarian cancer by real-time polymerase chain reaction

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ARTICLE INFO

Article history:

Received 8 June 2009

Available online 19 July 2009

Keywords:

Ovarian cancer

Real-time PCR

Reference gene

GeNorm

NormFinder

ABSTRACT

Quantitative real-time RT-PCR (RT-qPCR) has proven to be a valuable molecular technique in gene expression quantification. Target gene expression levels are usually normalized to a stably expressed reference gene simultaneously determined in the same sample. It is critical to select optimal reference genes to interpret data generated by RT-qPCR. However, no suitable reference genes have been identified in human ovarian cancer to date. In this study, 10 housekeeping genes, ACTB, ALAS1, GAPDH, GUSB, HPRT1, PBGD, PPIA, PUM1, RPL29, and TBP as well as 18S rRNA that were already used in various studies were analyzed to determine their applicability. Totally 20 serous ovarian cancer specimens and 20 normal ovarian epithelial tissue specimens were examined. All candidate reference genes showed significant differences in expression between malignant and nonmalignant groups except GUSB, PPIA, and TBP. The expression stability and suitability of the 11 genes were validated employing geNorm and NormFinder. GUSB, PPIA, and TBP were demonstrated as the most stable reference genes and thus could be used as reference genes for normalization in gene profiling studies of serous ovarian cancer, while the combination of two genes (GUSB and PPIA) or the all three genes should be recommended as a much more reliable normalization strategy.

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Quantitative real-time reverse transcription-polymerase chain reaction (RT-qPCR)¹ has become an established and powerful technique for gene expression studies. Relative quantification is a crucial and frequently used method to assess RT-qPCR data, while target gene expression levels are associated with a stably expressed internal reference gene determined in the same biological sample at the same time. Identification of suitable reference genes is an important problem involved in this approach. The expression of an ideal reference gene should be stable, unregulated, and invariable under the conditions of experiment [1–3].

In the literature, the widely used reference genes include housekeeping genes, such as glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and β-actin (ACTB) as well as ribosomal RNA (18S rRNA and 28S rRNA). However, some studies revealed that the expression levels of the commonly used reference genes did not always remain invariable; they varied across tissues [4] and cell types [5], as well as metabolic conditions and treatments [6]. Recently, some authors have discussed the identification of candi-

date reference genes for the relative quantification of expression data in cancers [7–10]. It has been suggested that all genes are regulated under some conditions and there is probably no universal reference gene with a constant expression in all tissues [11].

Ovarian cancer is the leading cause of death from gynecological malignancy. An estimated 21,650 new cases of this malignancy were diagnosed and 15,520 deaths attributed to this disease in the United States during 2008 [12]. The most frequent subtype of ovarian cancer is the serous subtype, which accounts for approximately 60 to 80% of ovarian cancer cases [13]. The majority of the patients (about 75%) are related to extraovarian spread at the time of diagnosis. The 5-year survival rate for women diagnosed with early-stage disease is approximately 95%, while survival rates drop to less than 30% when diagnosis is delayed until late stages. The condition has not been improved over the past few decades. In recent years, gene expression studies in malignant ovarian tissue and normal tissue counterpart have been performed to find new predictive and prognostic molecular markers associated with ovarian cancer. RT-qPCR is a frequently used tool to detect these markers. Thus, a scan on the normalization strategies used in quantitative gene expression studies of ovarian cancer is necessary. Combining the MeSH terms “ovarian cancer” and “real-time PCR,” we performed a PubMed search of articles published from January 1, 2004 to

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¹ Abbreviation used: RT-qPCR, quantitative real-time reverse transcription-polymerase chain reaction.

May 1, 2009 and got 119 available articles that use 21 various reference genes. Surprisingly, only 11 (9.2%) out of 119 studies used multiple reference genes for data normalization. Within the other 108 (90.8%) studies that applied a single reference gene, GAPDH was the most frequently used normalizer (52 times, 43.7%), followed by ACTB (23 times, 19.3%) and 18S rRNA (11 times, 9.2%). Apart from the three genes, all the other cited genes accounted for only 0.8 to 3.4%, such as HPRT1, GUSB, RPL29, PBGD, and TBP. Moreover, housekeeping genes and rRNAs were used as reference genes without any preliminary evaluation of suitability. The search results also clearly reveal that a systematic study on the selection of appropriate reference genes for gene expression studies in ovarian cancer has not been carried out until now and it is urgent to perform a specific evaluation of the currently used reference genes.

Therefore, the goal of our study was to identify the most suitable gene or set of genes as reference genes in gene expression studies of ovarian cancer. In the current study, we validated the stability of a panel of 11 putative reference genes in ovarian cancer tissues and normal ovarian epithelial tissues, each from 20 patients. The 11 candidate genes are commonly used as endogenous controls in the context of, but not restricted to, ovarian cancer: ACTB, ALAS1, GAPDH, TBP, HPRT1, RPL29, PBGD, PPIA, PUM1, GUSB, and 18S rRNA. Some of them have been identified as optimal reference genes in some other cancer types, such as TBP [14] and HPRT1 [3]. We were able to evaluate gene expression stability between ovarian cancer and normal ovarian epithelium employing geNorm software [15,16] and NormFinder [11,17].

Materials and methods

Patients and samples

Primary tumor samples ($n = 20$) were obtained from untreated ovarian cancer patients (mean age 56 years, range 39–71 years; 5 premenopausal and 15 postmenopausal) underwent tumor resection surgery. Normal ovarian epithelial tissue samples ($n = 20$) were derived from postmenopausal women who required bilateral adnexitomy when undergoing surgery because of other gynecological diseases (uterine prolapse, hysteromyoma, endometrial polyps, and ovarian simple cyst). All tumors were primary serous ovarian carcinoma. Histological analysis of H&E-stained sections showed that all cancer samples contained at least 90% tumor cells without necrosis. All the normal ovarian samples were verified to be free of any pathology. Tumor stage and grade were determined according to the International Federation of Gynecology and Obstetrics standards (FIGO). One of the 20 tumors was classified as stage I, 5 tumors stage II, 11 tumors stage III, and two tumors stage IV. The histological grading was one G1, 4 G2, and 15 G3. All of the specimens were collected at the Women's Hospital, School of Medicine, Zhejiang University, China. After excision, specimens were immediately snap-frozen in liquid nitrogen within

about half an hour and stored at -80°C until RNA extraction. Informed consent was obtained from each woman, and the study received the approval of the Ethical Committee for Clinical Research of Women's Hospital, School of Medicine, Zhejiang University.

RNA extraction and cDNA synthesis

Preserved tissue samples (50–100 mg) were homogenized in 1 ml TRIzol reagent (Invitrogen, Carlsbad, CA, USA) using a bench-top homogenizer (Polytron PT1600E, Kinematica AG, LittauLuzern, Switzerland) and total RNA was isolated from homogenized tissues according to the manufacturer's protocol. An additional step of RNase-free DNase I ((TaKaRa Biotechnology, Japan) treatment was performed. Concentrations of the isolated RNA and the 260/280 absorbance ratio were measured with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, DE, USA). The integrity of RNA samples was confirmed by electrophoresis on a 1% agarose gel. The criterion to include RNA samples was $260/280 \approx 2$ (1.9 to 2.2) and $28S/18S \geq 1.7$. The concentration of RNA was adjusted to 0.5 $\mu\text{g}/\mu\text{l}$ with nuclease-free water. One microgram total RNA was reverse-transcribed to cDNA using a PrimeScript RT reagent kit (perfect real-time) (TaKaRa Biotechnology, Japan) in a total volume of 20 μl according to the manufacturer's instructions. The kit uses PrimeScript RTase, which is based on reverse transcriptase originated from M-MLV, and offers a fast, complete, and high-yield cDNA synthesis for real-time PCR. Briefly, RNA samples, buffer, RNase free water, enzyme mix, random 6 mers, and oligo (dT) were mixed and incubated for 15 min at 37°C , followed by 5 s at 85°C to inactivate enzymes. Primer complementary DNA was stored at -80°C until use.

Real-time quantitative PCR

Eleven putative reference genes were selected for investigation to identify the most stable reference gene that could be used for normalization in RT-qPCR studies of ovarian cancer: ACTB, ALAS1, GAPDH, TBP, HPRT1, RPL29, PBGD, PPIA, PUM1, GUSB, and 18S rRNA (Table 1). They belong to different abundance and functional classes. Oligonucleotide primers were designed with primer5 software according to the sequences obtained from GeneBank database (Table 2). All primers except for 18S rRNA spanned at least one intron to minimize inaccuracies due to genomic DNA contamination in RNA samples. The specificity of the primer sequences were confirmed by BLAST searches. SYBR green real-time PCR was performed with an Applied Biosystems 7900HT Fast Real-time PCR system using the SYBR Premix Ex Taq (perfect real time) (TaKaRa Biotechnology). The PCR volume was 20 μl , containing 1 μl cDNA. The following cycling conditions were used [95°C for 10 s, (95°C for 10 s, 60°C or 63°C for 30 s) \times 40 cycles]. All reactions were run in duplicate and all 40 samples were analyzed in the same run in order to exclude between-run variations. No tem-

Table 1
Putative reference genes evaluated.

Gene symbol	GeneBank Accession No.	Gene name	Genomic localization	Molecular function
18S	NR_003286	18S ribosomal RNA	22p12	Ribosome subunit
ACTB	NM_001101	Beta-actin	7p15-p12	Cytoskeletal structural protein
ALAS1	NM_000688	Aminolevulinate, delta-, synthase 1	3p21.1	5-Aminolevulinate synthase
GAPDH	NM_002046	Glyceraldehyde-3-phosphate dehydrogenase	12p13	Oxidoreductase in glycolysis and gluconeogenesis
GUSB	NM_000181	Beta-glucuronidase	7q21.11	Galactosidase
HPRT1	NM_000194	Hypoxanthine phosphoribosyltransferase 1	Xq26	Metabolic salvage of purines
PBGD	NM_000190	Porphobilinogen deaminase	11q23	Hydroxymethylbilane synthase
PPIA	NM_021130	Peptidylprolyl isomerase A	7p13	Cyclosporin binding protein
PUM1	NM_001020658	Pumilio homolog 1 (Drosophila)	1p35.2	RNA binding
RPL29	NM_000992	Ribosomal protein L29	3p21.3-p21.2	Structural constituent of ribosome
TBP	NM_003194	TATA box binding protein	6q27	General transcription factor

Table 2

Details of primers and amplicons for the 11 evaluated genes.

Gene	Forward primer sequence[5' → 3']	Genomic position	Reverse primer sequence[5' → 3']	Genomic position	Amplicon length
18S	CGGCTACCACATCCAAGGAA	1st Exon	GCTGGAATTACCGGGCT	1stExon	186 bp
ACTB	AGAAAATCTGGCACCAACC	3rd Exon	TAGCACAGCCTGGATAGCAA	4th Exon	173 bp
ALAS1	GGCAGCACAGATGAATCAGA	4th Exon	CCTCATCGTTTACACT	5th Exon	150 bp
GAPDH	GACAGTCAGCCGCATCTTCT	1st Exon	TTAAAAGCAGCCCTGGTAC	3rd Exon	127 bp
GUSB	AGCCAGITTCCTCATCAATGG	6th Exon	GGTAGTGGCTGGTACGGAAA	7th Exon	160 bp
HPRT1	GACCAGTCAACAGGGGACAT	4th Exon	CCTGACCAAGGAAAGCAAAG	6th Exon	132 bp
PBGD	AGTGTGGTGGGAACCGAGC	9th Exon	CAGGATGATGCCACTGAAC	10, 11th Exon	144 bp
PP1A	AGACAAGTCCCCAAAGAC	2nd Exon	ACACCCCTGACACATAAA	4th Exon	118 bp
PUM1	CAGGCTGCCTACCAACTCAT	16th Exon	GTTCGGAACCATCTCATTC	17, 18th Exon	211 bp
RPL29	GGCGTTGTTGACCTATTTC	1st Exon	GTGTGTTGTTGTTCTTGG	2nd Exon	120 bp
TBP	TGCACAGGAGCCAAGAGTCAA	4th Exon	CACATCACAGCTCCCCACCA	5th Exon	132 bp

plate controls (no cDNA in PCR) were included in each assay run for each gene. A melting curve was constructed for each primer pair to confirm product specificity.

Threshold cycles (C_t values), the cycle number at which the fluorescence signal of the sample exceeds background fluorescence, were determined for quantitative comparison of the amplification of the candidate genes. C_t values were transformed to relative quantities for analysis considering the PCR efficiencies of the candidate reference genes according to the equation, $E^{(\min C_t - \text{sample } C_t)}$, in which $\min C_t$ = lowest C_t value over a range of samples for a given primer pair and E = amplification efficiency (2 = 100%) [16].

PCR efficiency

A 10-fold dilution series was created from a random pool of cDNA from our sample groups ranging from $\times 1$ dilution to $\times 100,000$ dilutions. PCR were performed as described above in triplicate. The PCR efficiency and correlation coefficients (R^2) of each primer pair were generated using the slopes of the standard curves. The efficiencies were calculated by the formula: efficiency (%) = $(10^{(-1/\text{slope})} - 1) * 100$.

Date analysis

Statistical analyses were performed with SPSS 15.0 program. The distribution fitting procedure according to the D'Agostino-Pearson omnibus normality test and Student's t -tests were applied. Since type II error is uncontrollable in a two-sided t -test, we also performed an equivalence test suggested by Haller et al. [18]: whether the difference of the expected logarithmized expression level δ is bounded by a determined number ε is concerned in this test. The hypothesis is defined as $H_0: \delta \notin [-\varepsilon; \varepsilon]$ versus $H_1: \delta \in [-\varepsilon; \varepsilon]$. If the confidence interval (CI) for the difference δ of the expected logarithmized expression values is included by the determined deviation area, a candidate reference gene can be considered as equivalent in expression on level α . The analyst constructs a $100(1 - 2\alpha)\%$ confidence level (CI) for the difference between the two mean values of two groups and compares it with the determined deviation area, $[-\varepsilon; \varepsilon]$. If the CI for the difference of the expected logarithmized expression values in two groups is completely contained within the interval $[-\varepsilon; \varepsilon]$, candidate reference genes can be considered as equivalent in expression on level α . The lower border and the upper border of the CI can be calculated according to the formula [18]

$$\text{CI}(\delta) = [\delta_L; \delta_H], \quad (1)$$

$$\delta_{LU} = (\bar{X}_1 - \bar{X}_2) \mp S \sqrt{\frac{1}{N_1} + \frac{1}{N_2}} \times t_{1-\alpha, N_1+N_2-2} \quad (2)$$

$$S = \sqrt{\frac{(N_1 - 1) \times S_1^2 + (N_2 - 1) \times S_2^2}{N_1 + N_2 - 2}} \quad (3)$$

In which S_1 and S_2 are the standard deviation of the logarithmized expression values in groups 1 and 2; N_1 and N_2 are the number of samples in the two groups; $t_{1-\alpha, N_1+N_2-2}$ means $1 - \alpha$ quantile of the t distribution with $N_1 + N_2 - 2$ degrees of freedom. As noted above, if $[\delta_L; \delta_H] \subset [-\varepsilon; \varepsilon]$, H_0 could be rejected. In addition, $[\delta_L; \delta_H]$ must contain 0.

Correlations between gene expression level (a continuous scale variable) and tumor stage and FIGO stage as well as menopausal status were characterized by the Spearman's rank test. Association between gene expression and patient age was assessed applying Pearson's test. A constant level $\alpha = 0.05$ were used for rejection of null hypothesis in all statistical tests. $P < 0.05$ was considered statistically significant.

For stability comparisons of candidate reference genes, the software geNorm, version 3.5, and NormFinder programs were applied according to the recommendations. The program geNorm is available on the internet <http://medgen.ugent.be/genorm/>. It calculates the expression stability measure (M) for candidate reference genes and by stepwise exclusion of the gene with highest M value in each step allows ranking of the tested genes according to their expression stability. It also provides a way to determine how many reference genes were needed for accurate normalization. NormFinder is a Microsoft Excel add-in and calculates a stability value for each individual candidate reference gene and ranks the genes according to their expression stability value in certain samples derived from a designed experiment [11]. The stability value is based on the combined estimate of intra- and intergroup variation of gene expression. A low stability value indicates a low combined variation and reveals high expression stability. Employing NormFinder, a best combination of two reference genes is also calculated.

Results

Quality control

To avoid erroneous conclusions, only RNA samples with high quality were included in this study. The selected RNA samples isolated from 20 malignant and 20 nonmalignant specimens all exhibited a high quality. The mean $A_{260/280}$ ratio of the RNA samples was 2.01 ± 0.045 (range from 1.95 to 2.12) and reflected pure and protein-free RNA. The integrity of RNA samples was characterized by the 28S/18S ratio (>1.7) on a 1% agarose gels.

The amplification efficiencies and correlation coefficients (R^2) of the 11 candidate genes were generated using the slopes of the standard curves obtained by serial dilutions. Correlation coefficients (R^2) ranged from 0.995 to 0.999 and PCR efficiencies from 98 to 108% (Supplementary Fig. 1). The amplification specificity for each qRT-PCR analysis was confirmed by melting curve analysis (Supplementary Fig. 2). Furthermore, PCR products were separated by 1.5% agarose gel to confirm an expected single band at the right amplicon size.

Expression levels of candidate reference genes

The 11 candidate reference genes displayed a wide expression range, with C_t values between 9.54 and 33.03. All genes showed a normal distribution pattern proved by the D'Agostino-Pearson fitting procedure in both the malignant and the nonmalignant tissue samples. As shown in Fig. 1, the nonmalignant and the malignant samples were separately shown as box plots with ranges as whiskers to demonstrate the total expression ranges. Among these genes, 18S rRNA is the most abundant transcript with mean ($\pm SD$) C_t values of 10.67 ± 0.9 in malignant and 11.33 ± 0.85 in nonmalignant samples. PBGD is the lowest expressed gene with mean ($\pm SD$) C_t values of 26.83 ± 1.2 in malignant and 29.98 ± 2.2 in nonmalignant samples. Significant differences in gene expression between malignant and nonmalignant samples were observed for all candidate reference genes, except GUSB ($P = 0.121$), PPIA ($P = 0.27$), and TBP ($P = 0.49$). Interestingly, compared with nonmalignant samples, the expressions of 18S rRNA, ACTB, ALAS1, GAPDH, HPRT1, PUM1, and RPL29 were all significantly increased in malignant samples with a very low P value ($P < 0.001$).

We used a fold change of 3 in equivalence test as suggested by Haller et al. [18]. Thus, the $[-\varepsilon; \varepsilon] = [\log_2 1/3; \log_2 3] = [-1.58496, 1.58496]$. The CI (δ) values for GUSB, PPIA, and TBP were $[-0.11283; 1.11183]$, $[-0.17239; 1.35239]$, and $[-0.36503; 0.87403]$, respectively, which were completely contained within $[-1.58496, 1.58496]$ and contain 0. Thus, the expressions of GUSB, PPIA, and TBP are equivalent in the tumor and control groups. The CI (δ) value for 18S, $[0.09086; 1.23634]$, was part of the determined deviation area but did not contain 0 and thus the null hypothesis could not be rejected. The CI (δ) values for ACTB [2.19612; 3.88787], ALAS1 [2.30874; 3.66726], GAPDH [3.27525; 4.87175], HPRT1 [0.58475; 2.46525], PBGD [2.15847; 4.13653], PUM1 [1.68741; 3.26359] and RPL29 [2.99850; 4.18250] were not bounded by the determined deviation area, and therefore the eight candidate reference genes were not equivalently expressed in the two groups. Hence, the possibility of a type II error (false negatives) in the t -test was excluded and the results were further confirmed through this approach.

Additionally, the expression of these candidate genes did not depend on age (correlation coefficient = -0.111 to 0.345 ; $P = 0.136$ to 1.000), menopausal status (correlation coefficient = -0.132 to 0.208 ; $P = 0.379$ to 1.000), FIGO stage (correlation coefficient =

-0.336 to 0.203 ; $P = 0.147$ to 0.922), and tumor degree (correlation coefficient = -0.093 to 0.255 ; $P = 0.279$ to 0.977).

Expression stability of candidate reference genes

Including the 3 suitable genes, all 11 candidate reference genes were included in the program geNorm and ranked according to their M values (Fig. 2a). The M value is the average pairwise variation of an individual gene with all other control genes. The M values for GUSB, PPIA, TBP, 18S rRNA, HPRT1, PUM1, RPL29, ALAS1, and ACTB were lower than the geNorm default threshold of 1.5, while the two remaining genes, GAPDH and RPL29, showed M values greater than the threshold. GUSB and PPIA (both $M = 0.842$) were identified as the two most stable genes according to geNorm analysis. After excluding the 8 genes noted that differ in expression between groups, the geNorm analysis was repeated. The most stable genes were still PPIA and GUSB, followed by TBP, which was in accord with the previous finding. To determine the optimal number of genes required for RT-qPCR date normalization, geNorm calculates the pairwise variation (V_n/V_{n+1}) between sequential normalization factors (NF) (NFn and $NFn + 1$). A large variation means that the added gene has a significant effect and should preferably be included for calculation of a reliable normalization factor [16]. As shown in Fig. 2b, the pairwise variation (v) on normalization with the three most stable reference genes, PPIA, GUSB, and TBP, and introduction of the fourth one was 0.188. The trend of the value became roughly stable after the addition of the eighth gene.

To compare the result generated from geNorm, another free tool available on the internet to validate the expression stability was also used. The stability data calculated by that program was a combined estimate of intra- and intergroup expression variations of the genes studied. It could reveal the expression differences of the genes observed between normal and tumor groups. The result of the analysis by NormFinder appeared to be similar to the one determined by geNorm (Table 3). The three genes GUSB, PPIA, and 18S rRNA achieved the best stability values, and the best combination of two genes was that of GUSB and PPIA.

Discussion

It is well recognized that the reference gene should be properly validated for a particular experiment to ensure that gene expres-

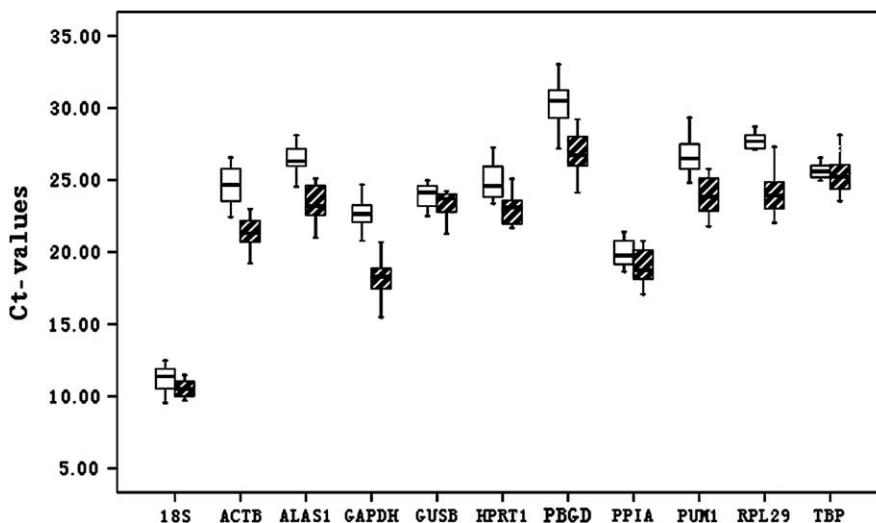


Fig. 1. Expression levels of candidate reference genes in normal ovarian epithelia and serous ovarian cancer samples. Values are given as real-time PCR cycle threshold numbers (C_t values). Boxes (blank, normal; cross-striated, cancer) represent the lower and upper quartiles with medians; whiskers represent the ranges for the data of the 20 samples in each group.

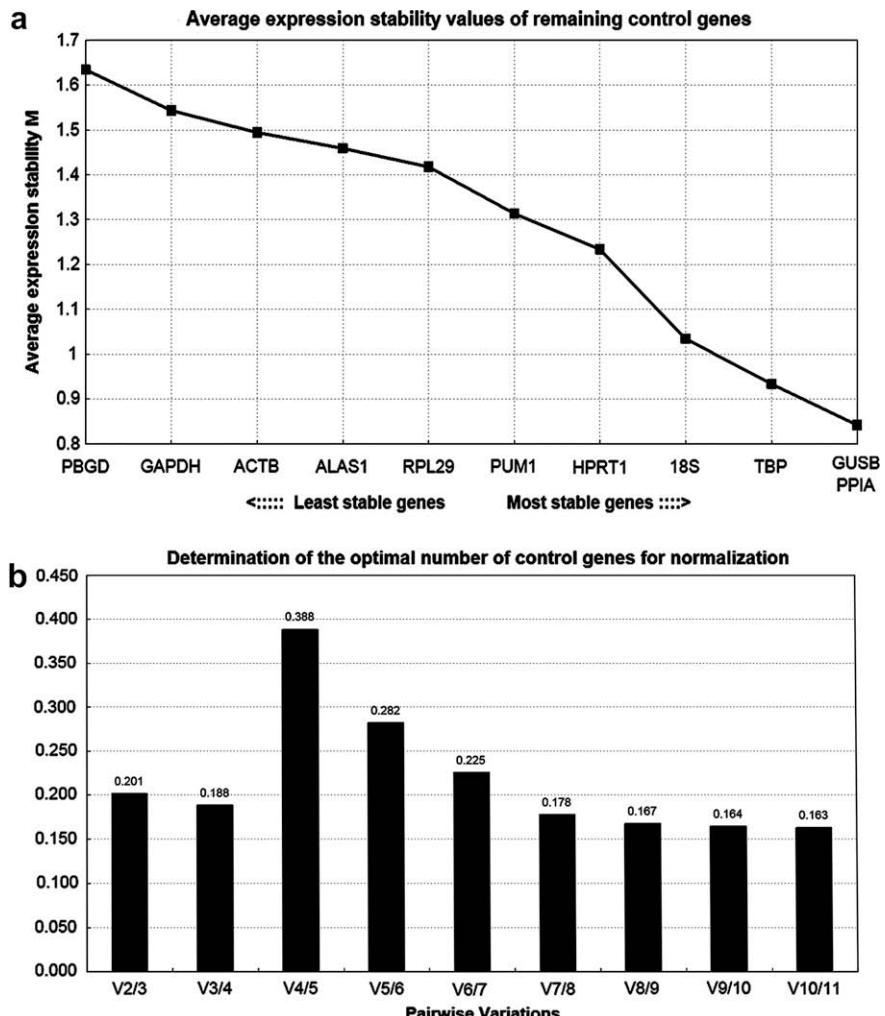


Fig. 2. GeNorm analysis of the candidate reference genes. Results are presented according to the output file of the geNorm program. (a) Stepwise exclusion of the least stable genes by calculating the average expression stability measure M . The value of M was calculated for each gene, and the least stable gene with the highest M value was automatically excluded for the next calculation round. The x-axis from left to right indicates the ranking of the reference genes according to expression stability and the y-axis indicates the stability measure, M . (b) Determination of the optimal number of reference genes for normalization.

Table 3

Candidate reference genes for normalization listed according to their expression stability calculated by the NormFinder program.

Raking order	Gene name	Stability value
1	PPIA	0.338
2	GUSB	0.345
3	18S	0.401
4	TBP	0.492
5	PUM1	0.633
6	ALAS1	0.795
7	ACTB	0.839
8	PBGD	0.906
9	RPL29	0.950
10	GAPDH	1.160
11	HPRT1	5.226

sion is unaffected by the experimental treatment. As far as we are aware, there are no published articles about optimal reference gene selection for ovarian cancer. We report herein the first systematic comparison of expression stability of candidate reference genes in ovarian carcinoma samples and benign counterparts.

A group of widely used reference genes have been applied in recent ovarian cancer gene profiling studies, such as GAPDH, ACTB, TBP, 18S rRNA, RPL29, GUSB, and HPRT1, for RT-PCR [19–24]. In

the present study, we evaluated a panel of 11 candidate reference genes to determine the most reliable one for accurate normalization of gene expression.

To obtain reliable data, we carefully designed our study before RT-qPCR analysis based on the following features: (1) The majority of patients with ovarian cancer are diagnosed at late stages, when the whole ovary has been damaged and it is difficult to get tumor tissues and valid paratumor tissues in the same ovary. Thus, we obtained normal ovarian epithelial tissues from patients receiving surgery because of benign gynecological diseases, though it is known that the use of paired samples from the same patient may minimize the interindividual variation [3,8]. (2) We used strict RNA quality control, precise RNA concentration determination, and gene-specific primer selection, (3) simultaneous examination of a considerable number of 11 candidate genes from different function classes, and (4) employment of two received software, geNorm and NormFinder, combined with a *t*-test and a equivalence test to rank the candidate reference genes according to their stability.

The expression of candidate reference genes can be influenced not only by tissue types but also by physiological or pathological factors like age, tumor stage, and tumor grade. Thus, it should be decided whether the expression is correlated with the biological

conditions listed above. The results of our data proved that the expression of these genes in this cohort was independent on age, FIGO stage, and tumor grade. Additionally, because ovarian epithelial tissues obtained from postmenopausal women were used as normal control, we also evaluated the correlation between candidate gene expression and menopausal status. The results showed there was no association between the two variables and thus the use of a control group described above was warranted and feasible. However, due to the reduced size of the cohort of patients in this study, a definite correlation between gene expression and clinic pathologic features should be further determined in a larger sample.

In the current study, we calculated the best performing reference genes using two distinct statistical models, a pairwise comparison model, geNorm, and an ANOVA-based model, NormFinder. Finally, geNorm identified GUSB, PPIA, and TBP as the three most stably expressed reference genes while NormFinder indicated GUSB, PPIA, and 18S rRNA as the three genes with the best stability, followed by TBP. However, the previously performed *t*-test and equivalence test analyses of gene expression in malignant and non-malignant groups revealed that only three genes GUSB, PPIA, and TBP did not differ in their expression in the two groups. It could be concluded only the three genes fulfill the criterion of expression stability and could be considered as suitable normalizers for relative gene quantification in serous ovarian cancer samples. Thus, 18S RNA should be excluded and GUSB, PPIA, and TBP were finally indicated as the three most stable reference genes for studying target gene profiling in serous ovarian cancer.

However, it is recommended that normalization using a single reference gene should be replaced by normalization based on the several best performing candidate reference genes [15]. A normalization strategy applying multiple reference genes has the benefit of minimizing the influence of minor fluctuations and making accurate data normalization is also suggested by some other authors [25,26]. In the present study, we found GUSB, PPIA, and TBP yield a variation value of 0.188. As reported by Vandesompele et al. [16], though GeNorm proposes a pairwise variation of 0.15 as the cutoff under which the inclusion of an additional reference gene is unnecessary, the cutoff of 0.15 should not be considered in a strict sense, but rather as guidance to determine the optimal number of reference genes. Sometimes the observed trend can be equally informative, and using the three best reference genes is, in most cases, a valid normalization strategy. Therefore, GUSB, PPIA, and TBP is a reliable set of genes for normalizing data generated from RT-qPCR analysis in serous ovarian cancer. Additionally, GUSB and PPIA were also indicated as the best combination of two genes by NormFinder.

Nevertheless, as a limitation of our study, we should note that the present study is limited to the serous ovarian cancer subtype. However, it is the most frequent subtype of ovarian carcinoma. The applicability of the three recommended reference genes GUSB, PPIA, and TBP in other subtypes of ovarian cancer was not tested and further studies are needed to confirm their potential use.

In conclusion, our current study demonstrated that the three most stable genes GUSB, PPIA, and TBP could be used as reference genes for normalization in gene profiling studies of serous ovarian cancer and the combination of two genes (GUSB and PPIA) or the all three genes should be recommended as a much more reliable normalization strategy.

Acknowledgments

We appreciate financial support from the National Natural Science Foundation of China (No. 30672230 and No. 30672229).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ab.2009.07.022.

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ALINHAMENTO

```
>SEQ1  
GGGAGCCGCTCATGAGGAAGTTG
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```
>SEQ2  
GGCAAGTCTCTCTCCCAGCGTCTC
```

```
>tomek-DR5.f  
GGGAGCCGCTCATGAGGAAGTTGG
```

```
>Tomek-DR5.r  
GGCAAGTCTCTCTCCCAGCGTCTC
```

```
>Kim-DR5F  
GGGAGCCGCTCATGAGGAAGTTGG
```

```
>Kim-DR5R  
GGCAAGTCTCTCTCCCAGCGTCTC
```

```
CLUSTAL O(1.2.4) multiple sequence alignment
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```
SEQ1          GGGAGCCGCTCATGAGGAAGTTG- 23  
tomek-DR5f    GGGAGCCGCTCATGAGGAAGTTGG 24  
*****  
#  
#  
#  Percent Identity Matrix - created by Clustal2.1  
#  
#  
1: SEQ1          100.00 100.00  
2: tomek-DR5f    100.00 100.00
```

```
CLUSTAL O(1.2.4) multiple sequence alignment
```

```
SEQ2          GGCAAGTCTCTCTCCCAGCGTCTC 24  
Tomek-DR5.r    GGCAAGTCTCTCTCCCAGCGTCTC 24  
*****  
#  
#  
#  Percent Identity Matrix - created by Clustal2.1  
#  
#  
1: SEQ2          100.00 100.00  
2: Tomek-DR5.r    100.00 100.00
```

```
CLUSTAL O(1.2.4) multiple sequence alignment
```

```
SEQ1          GGGAGCCGCTCATGAGGAAGTTG- 23  
Kim- DR5F      GGGAGCCGCTCATGAGGAAGTTGG 24  
*****
```

```
#  
#  
# Percent Identity Matrix - created by Clustal2.1  
#  
#  
1: SEQ1          100.00 100.00  
2: Kim- DR5F    100.00 100.00  
  
CLUSTAL O(1.2.4) multiple sequence alignment  
  
SEQ2          GGCAAGTCTCTCTCCCAGCGTCTC 24  
Kim-DR5R      GGCAAGTCTCTCTCCCAGCGTCTC 24  
*****  
#  
#  
# Percent Identity Matrix - created by Clustal2.1  
#  
#  
1: SEQ2          100.00 100.00  
2: Kim-DR5R    100.00 100.00
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Source: GENESEQ

Hit sequences: AED50828

Published: Oct 20, 2005

Patent Status: Dead

DWPI Patent Status: Dead

INPADOC Patent Status: Dead

Filed: Nov 3, 2004

Earliest priority: Mar 17, 1997

Assignee(s): [HUMAN GENOME SCIENCES INC](#)

Inventor(s): Ni J; Gentz RL; Yu G; Rosen CA

Authority United States

Title: New death domain containing receptor-5 (DR5) polypeptide, useful for treating diseases associated with reduced or increased levels of apoptosis, e.g. lung, breast, ovarian, colorectal or hematological cancer.

Citing Patents: EP2459233A1, EP2459233A4, EP2684896A1, EP3575323A1, US10702540B2, US10882913B2, US20020150985A1, US20030180296A1, US20030228309A1, US20040009552A1, US20040136951A1, US20040141952A1, US20050129616A1, US20050214209A1, US20060035334A1, US20060062786A1, US20060073570A1, US20060177456A1, US20060270837A1, US20080051375A1, US20080051380A1, US20090124587A1, US20100152426A1, US20100266542A1, US20110008354A1, US20110021470A1, US20110027239A1, US7314619B2, US7348003B2, US7361341B2, US7595046B2, US7749755B2, US7750118B2, US7807153B2, US7939631B2, US7947271B2, US8092799B2, US8642067B2, US9238064B2, WO2011014388A1, WO2014009358A1

Cited Patents: US6265556B1, US20020150985A1, US20020004227A1, US5530101A, US6461823B1, US20060073570A1, US20020072091A1, US20060115484A1, US6433147B1, US4411993A, US6342369B1, US5763223A, US6072047A, US20040141952A1, US5349053A, US5447851A, US6455040B1, US5565332A, US20020160446A1, US6342363B1, US20030004313A1, US6569642B1, US20040136951A1, US4946778A, US20030148455A1, US20040009552A1, US6635743B1, US20060251647A1, US20050282230A1, US7314619B2, US20020048785A1, US20060084147A1, US5910574A, US5807715A, US5478925A, US4002531A, US20030017161A1, US6689744B2, US20060035334A1, US6872568B1, US6252050B1, US6743625B2, US5643575A, US20020098550A1, US20030125540A1, US6313269B1, US6642358B1

DPCI Citing Patents: EP2459233A1, EP2459233A4, EP2684896A1, EP2877491B1, EP3575323A1, US10702540B2, US10882913B2, US20020150985A1, US20030180296A1, US20030228309A1, US20040009552A1, US20040136951A1, US20040141952A1, US20050129616A1, US20050214209A1, US20060035334A1, US20060062786A1, US20060073570A1, US20060177456A1, US20060270837A1, US20080051375A1, US20080051380A1, US20090124587A1, US20100152426A1, US20100266542A1, US20110008354A1, US20110021470A1, US20110027239A1, US7314619B2, US7348003B2, US7361341B2, US7595046B2, US7749755B2, US7750118B2, US7807153B2, US7939631B2, US7947271B2, US8092799B2, WO2011014388A1, WO2014009358A1

DPCI Cited Patents: US20020004227A1, US20020048785A1, US20020072091A1, US20020098550A1, US20020150985A1, US20020160446A1, US20030004313A1, US20030017161A1, US20030125540A1, US20030148455A1, US20040009552A1, US20040136951A1, US20040141952A1, US20050282230A1, US20060035334A1, US20060073570A1, US20060084147A1, US20060115484A1, US20060251647A1, US4002531A, US4411993A, US4946778A, US5349053A, US5447851A, US5478925A, US5530101A, US5565332A, US5643575A, US5763223A, US5807715A, US5910574A, US6072047A, US6252050B1, US6265556B1, US6313269B1, US6342363B1, US6342369B1, US6433147B1, US6455040B1, US6461823B1, US6569642B1, US6635743B1, US6642358B1, US6689744B2, US6743625B2, US6872568B1, US7314619B2

Claims:

1. An isolated polypeptide selected from the group consisting of: (a) the polypeptide of **SEQ ID NO:2**; and (b) a fragment of the polypeptide of (a).
2. The polypeptide of claim 1, wherein said fragment binds TRAIL.
3. The polypeptide of claim 1, wherein said fragment inhibits apoptosis.
4. The polypeptide of claim 1, wherein said fragment induces apoptosis.
5. The polypeptide of claim 1, wherein said polypeptide comprises amino acids n1 to 360 of **SEQ ID NO:2**, wherein n1 represents an integer from -50 to 355.

Sequence number	AED50828														
Description	Human death domain containing receptor 5 (DR5) encoding cDNA.														
Keywords	apoptosis; death domain containing receptor 5; DR5; cytostatic; cancer; gene; ss														
Comments	<p>The invention relates to an isolated death domain containing receptor 5 (DR5) polypeptide comprising the 411 amino acid sequence of AED50829, or its fragment. Also described: (1) a dimer, trimer, or tetramer of the DR5 polypeptide; (2) a composition comprising the DR5 polypeptide, and a carrier; (3) an isolated DR5 polypeptide encoded by the 1600 bp nucleotide sequence of AED50828; (4) an isolated DR5 polypeptide comprising amino acids n2 to 133 of AED50829, where n2 represents an integer from -51 to 128, and where the polypeptide binds TRAIL; (5) an isolated soluble polypeptide comprising an amino acid sequence at least 80 % identical to the sequence of amino acids 1-133 of AED50829; (6) a multimer comprising the polypeptide, or at least two of the polypeptides described above; (7) an isolated polynucleotide encoding the polypeptide; (8) a composition comprising the multimer, dimer, trimer or tetramer as described above, and a carrier; and (9) an isolated antibody that specifically binds the polypeptide. The DR5 polypeptide and compositions are useful for treating diseases associated with reduced or increased levels of apoptosis, e.g. lung, breast, ovarian, colorectal or hematological cancer. The present sequence encodes the human death domain containing receptor 5 (DR5), which is used in the exemplification of the present invention.</p>														
Location	Claim 35; SEQ ID NO 1; 140pp; English.														
Sequence key	SBNN00004TYJ														
Feature table	<table> <thead> <tr> <th>Key</th> <th>Location</th> <th>Feature</th> </tr> </thead> <tbody> <tr> <td>CDS</td> <td>130..1365</td> <td>*tag: b product: "death domain containing receptor 5 (DR5)"</td> </tr> <tr> <td>sig_peptide</td> <td>130..282</td> <td>*tag: a</td> </tr> <tr> <td>mat_peptide</td> <td>283..1362</td> <td>*tag: c product: "death domain containing receptor 5 (DR5) mature protein"</td> </tr> </tbody> </table>			Key	Location	Feature	CDS	130..1365	*tag: b product: "death domain containing receptor 5 (DR5)"	sig_peptide	130..282	*tag: a	mat_peptide	283..1362	*tag: c product: "death domain containing receptor 5 (DR5) mature protein"
Key	Location	Feature													
CDS	130..1365	*tag: b product: "death domain containing receptor 5 (DR5)"													
sig_peptide	130..282	*tag: a													
mat_peptide	283..1362	*tag: c product: "death domain containing receptor 5 (DR5) mature protein"													
Search	TNFRSF10B [MSS: SWN]														
Score	115.0 120.0														
Expect	N/A for SWN search N/A for SWN search														
Query coverage	100.0% 100.0%														
Identities (query % / align %):	23 (100.00% / 100.00%) 24 (100.00% / 100.00%)														
Positives %	23 (100.00%) 24 (100.00%)														
Subject Identity (%)	1.44% 1.50%														
Subject Coverage	1.44% 1.50%														
Mismatches															

Matches/Alignments	Alignment Length	Score	Identities	Gaps	Query
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Alignment					
Query: 1 GGGAGCCGCTCATGAGGAAGTTG 23 Sbjct: 1103 GGGAGCCGCTCATGAGGAAGTTG 1125					
Reverse Alignment					
Query: 23 GTTGAAGGAGTACTCGCCGAGGG 1 Sbjct: 1125 GTTGAAGGAGTACTCGCCGAGGG 1103					
Alignment Length	Score	Identities	Gaps	Query	
24	120	24	0	Q2	
Alignment					
Query: 24 GAGACGCTGGGAGAGAGACTTGCC 1 Sbjct: 1261 GAGACGCTGGGAGAGAGACTTGCC 1284					
Reverse Alignment					
Query: 1 CCGTTAGAGAGAGGGTCGCAGAG 24 Sbjct: 1284 CCGTTAGAGAGAGGGTCGCAGAG 1261					
Combined Alignment					
[-----Q1-----] Queries: 1 GGGAGCCGCTCATGAGGAAGTTG 23 Subject: 1093 TTGACTCCTGGAGCCCTCATGAGGAAGTTGGCCTATGGACAATGAGATAAAGGTGGCTAAAGCTGAGGCAGCGGGCACAGGGACACCTTGACAC GATG [-----Q2-----] Queries: 24 GAGACGCTGGGAGAGAGACTTGCC 1 Subject: CTGATAAAAGTGGGTCAACAAAACGGGGAGATGCCCTGTCCACACCCCTGCTGGATGCCCTGGAGACGCTGGAGAGAGACTTGCACAGCAGAAGA 1294					

Source: GENESEQ

Hit sequences: AEE48562

Published: Dec 1, 2005

Patent Status: Dead

DWPI Patent Status: Dead

INPADOC Patent Status: Dead

Filed: Apr 21, 2005

Earliest priority: Apr 23, 2004

Assignee(s): US; SHEARER GENE; HERBEUVAL JEAN-PHILIPPE

Inventor(s): Shearer G; Herbeuval J

Authority WIPO

Title: Determining whether a mammal suffers from a condition involving immune system activation by assaying for the presence or concentration of a TNF-Related Apoptosis-Inducing Ligand (TRAIL) compound in a biological fluid of the mammal.

Citing Patents: EP2102370A2, US8173128B2, US9120855B2

Cited Patents: WO2000066156A1, WO1999009165A1, WO2001022987A1

DPCI Citing Patents: EP2102370A2

DPCI Cited Patents: WO1999009165A1, WO2000066156A1, WO2001022987A1

Claims:

What Is Claimed Is:

1. Claim 1. A method for determining whether a mammal suffers from a disease or condition involving immune system activation, wherein said method comprises assaying for the presence or concentration of a TRAIL Compound in a biological fluid of said mammal.
2. Claim 2. The method of claim 1, wherein said mammal is selected from the group consisting of a human, simian, feline, bovine, equine, canine, ovine or porcine mammal.
3. Claim 3. The method of claim 1, wherein said TRAIL compound is sTRAIL.
4. Claim 4. The method of claim 1, wherein said method comprises an immunoassay that determines the presence or concentration of said TRAIL Compound, said immunoassay comprising the steps of: (a) contacting a sample of said biological fluid with an antibody specific for said TRAIL Compound, said contacting being under conditions sufficient to permit said TRAIL Compound if present in said sample to bind to said antibody and form a TRAIL Compound - antibody complex; (b) contacting said formed TRAIL Compound - antibody complex with a molecule capable of specific binding to said complex, said contacting being under conditions sufficient to permit said molecule to bind to said complex and form an extended complex; and (c) determining the presence or concentration of said TRAIL Compound in said biological fluid by determining the presence or concentration of said formed extended complex in said sample.

Sequence number	AEE48562		
Description	Human TNF-Related Apoptosis-Inducing Ligand (TRAIL) DR5 receptor DNA.		
Keywords	diagnosis; immunoassay; acquired immune deficiency syndrome; HIV infection; cancer; atherosclerosis; systemic lupus erythematosus; Alzheimers disease; inflammation; asthma; Crohns disease; graves disease; autoimmune disease; multiple sclerosis; parkinsons disease; transplant rejection; graft versus host disease; neoplasm; immune disorder; metabolic disorder; degeneration; neurological disease; TNF-related apoptosis-inducing ligand DR5 receptor; TRAIL DR5 receptor; ds; gene		
Comments	The invention relates to: determining whether a mammal suffers from a disease or condition involving immune system activation by assaying for the presence or concentration of a TNF-Related Apoptosis-Inducing Ligand (TRAIL) compound (defined in the specification as including TRAIL, TRAIL DR5 receptor, p53, CD69, HLA-DR alpha, interferon alpha and beta and the nucleic acids that encode them) in a biological fluid of the mammal; an immunoassay that determines and a kit for measuring the presence or concentration of a TRAIL compound in a biological fluid of a mammal. The methods may be used for monitoring the progression of AIDS and other diseases whose progression involves immune system activation, including cancer, atherosclerosis, Alzheimers disease, inflammation, autoimmune disorder, allergic asthma, Crohn's disease, Grave's disease, lupus, multiple sclerosis, Parkinson's disease, allograft transplant rejection or graft versus host disease. This sequence is DNA encoding human TNF-Related Apoptosis-Inducing Ligand (TRAIL) DR5 receptor. Revised record issued on 18-OCT-2007 : Enhanced with precomputed information from BOND.		
Location	Disclosure; SEQ ID NO 12; 123pp; English.		
Sequence key	SBNN00004QLI		
Feature table	Key CDS	Location 1..1236	Feature *tag: a product: "Human TNF-Related Apoptosis-Inducing Ligand (TRAIL) DR5 receptor"
Search	TNFRSF10B [MSS: SWN]		
Score	115.0 120.0		
Expect	N/A for SWN search N/A for SWN search		
Query coverage	100.0% 100.0%		
Identities (query % / align %):	23 (100.00% / 100.00%) 24 (100.00% / 100.00%)		
Positives %	23 (100.00%) 24 (100.00%)		
Subject Identity (%)	1.86% 1.94%		
Subject Coverage	1.86% 1.94%		
Mismatches			

Matches/Alignments	Alignment Length	Score	Identities	Gaps	Query
	23	115	23	0	Q1
Alignment					
Query: 1 GGGAGCCGCTCATGAGGAAGTTG 23 Sbjct: 974 GGGAGCCGCTCATGAGGAAGTTG 996					
Reverse Alignment					
Query: 23 GTTGAAGGAGTACTCGCCGAGGG 1 Sbjct: 996 GTTGAAGGAGTACTCGCCGAGGG 974					
Alignment Length	Score	Identities	Gaps	Gaps	Query
24	120	24	0	0	Q2
Alignment					
Query: 24 GAGACGCTGGGAGAGAGACTTGC 1 Sbjct: 1132 GAGACGCTGGGAGAGAGACTTGC 1155					
Reverse Alignment					
Query: 1 CCGTTAGAGAGAGGGTCGAGAG 24 Sbjct: 1155 CCGTTAGAGAGAGGGTCGAGAG 1132					
Combined Alignment					
[-----Q1-----] Queries: 1 GGGAGCCGCTCATGAGGAAGTTG 23 Subject: 964 TTTGACTCCTGGAGCCGCTCATGAGGAAGTTGGCCTCATGGACAATGAGATAAAGGTGGCTAAAGCTGAGGCAGCGGCCACAGGGACACCTTGACACG ATGC [-----Q2-----] Queries: 24 GAGACGCTGGGAGAGAGACTTGC 1 Subject: TGATAAAAGTGGTCAACAAAACCAGGGCAGATGCCCTGTGCCACACCCCTGCTGGATGCCCTGGAGACGCTGGAGAGAGACTTGCCAAGCAGAAGA 1165					

Source: GENESEQ

Hit sequences: AZF38069

Published: Feb 17, 2011

Patent Status: Dead

DWPI Patent Status: Dead

INPADOC Patent Status: Dead

Filed: Apr 2, 2009

Earliest priority: Feb 24, 2005

Assignee(s): [JOLLA INST ALLERGY IMMUNOLOG...US](#)

Inventor(s): Schoenberger SP; Green DR; Janssen EM; Droin NM

Authority United States

Title: Promoting or inducing apoptosis or death of T cells, comprises contacting T cells with activator of tumor necrosis factor related apoptosis-inducing ligand receptor (death receptor (DR) 4 or DR5) expression or activity.

Citing Patents: AU2015250039B2, CN106573049A, EP3145530B1, US10046059B2, US10722468B2, US11007251B2, US9901620B2, WO2015164217A1

DPCI Citing Patents: AU2015250039B2, CN106573049A, EP3145530B1, US10046059B2, US10722468B2, US11007251B2, WO2015164217A1

Claims:

1-34. (canceled)

35. A method of promoting or inducing apoptosis or death of T cells, comprising contacting T cells with an amount of an activator of TRAIL receptor (DR4 or DR5) expression or activity sufficient to promote or induce apoptosis or death of T cells.

36. A method of treating a physiological condition, disorder, illness, disease or symptom of a subject that is ameliorated by promoting or inducing T cell apoptosis or death, comprising administering an amount of an activator of TRAIL receptor (DR4 or DR5) expression or activity effective to promote or induce T cell apoptosis or death, thereby ameliorating the physiological condition, disorder, illness, disease or symptom.

37. The method of claim 36, wherein activated CD8+ T cells contribute to, stimulate, enhance or mediate the physiological disorder or disease.

38. The method of claim 36, wherein the physiological condition, disorder, illness, disease or symptom comprises an autoimmune disorder or disease.

Sequence number	AZF38069
Description	Human TNF-related apoptosis-inducing ligand receptor-2 (DR5) cDNA, SEQ 6.
Keywords	TNFRSF10B gene; TRAIL-2 receptor; antianemic; antidiabetic; antiinflammatory; aplastic anemia; apoptosis stimulation; autoimmune disease; autoimmune hepatitis; cytostatic; gastrointestinal-gen.; graft versus host disease; hematological-gen; hepatotropic; immunosuppressive; insulin dependent diabetes; metabolic-gen.; multiple sclerosis; muscular-gen; myelodysplastic syndrome; neuroprotective; polymyositis; primary biliary cirrhosis; ss; therapeutic; transplant rejection
Comments	The present invention relates to a method for promoting or inducing apoptosis or death of T cells. The method comprises contacting T cells with an amount of an activator of TNF-related apoptosis-inducing ligand (TRAIL (Apo-2L)) receptor (death receptor DR4 or DR5) expression or activity sufficient to promote or induce apoptosis or death of T cells. The invention also includes a method of treating a physiological condition, disorder, illness, disease or symptom of a subject that is ameliorated by promoting or inducing T cell apoptosis or death. The method of the invention is useful for promoting or inducing apoptosis or death of T cells and treating a physiological condition, disorder, illness, disease or symptom including autoimmune disorder or disease, multiple sclerosis, autoimmune diabetes, autoimmune hepatitis, primary biliary cirrhosis, myelodysplastic syndrome, aplastic anemia, polymyositis, transplant rejection and graft versus-host disease. The present sequence is a human TNF-related apoptosis-inducing ligand receptor-1 (death receptor (DR4)) cDNA., TNFRSF10B. An antisense and RNAi can be produced based upon the TRAIL (Apo-2L) or TRAIL receptor (DR4 or DR5) sequences which is useful promoting or inducing apoptosis or death of T cells as described in the invention.Revised record issued on 23-MAR-2011 : Enhanced with precomputed information from BOND.
Location	Disclosure; SEQ ID NO 6; 70pp; English.
Sequence key	SBNN00020RV5
Search	TNFRSF10B [MSS: SWN]
Score	115.0 120.0
Expect	N/A for SWN search N/A for SWN search
Query coverage	100.0% 100.0%
Identities (query % / align %):	23 (100.00% / 100.00%) 24 (100.00% / 100.00%)
Positives %	23 (100.00%) 24 (100.00%)
Subject Identity (%)	1.86% 1.94%
Subject Coverage	1.86% 1.94%
Mismatches	

Matches/Alignments	Alignment Length	Score	Identities	Gaps	Query
	23	115	23	0	Q1
Alignment					
Query: 1 GGGAGCCGCTCATGAGGAAGTTG 23 Sbjct: 974 GGGAGCCGCTCATGAGGAAGTTG 996					
Reverse Alignment					
Query: 23 GTTGAAGGAGTACTCGCCGAGGG 1 Sbjct: 996 GTTGAAGGAGTACTCGCCGAGGG 974					
Alignment Length	Score	Identities	Gaps	Query	
24	120	24	0	Q2	
Alignment					
Query: 24 GAGACGCTGGGAGAGAGACTTGCC 1 Sbjct: 1132 GAGACGCTGGGAGAGAGACTTGCC 1155					
Reverse Alignment					
Query: 1 CCGTTAGAGAGAGGGTCGCAGAG 24 Sbjct: 1155 CCGTTAGAGAGAGGGTCGCAGAG 1132					
Combined Alignment					
[-----Q1-----] Queries: 1 GGGAGCCGCTCATGAGGAAGTTG 23 Subject: 964 TTTGACTCCTGGAGCCGCTCATGAGGAAGTTGGCCTCATGGACAATGAGATAAAGGTGGCTAAAGCTGAGGCAGCGGGCACAGGGACACCTTGACACG ATGC					
[-----Q2-----] Queries: 24 GAGACGCTGGGAGAGAGACTTGCC 1 Subject: TGATAAAAGTGGTCAACAAAACCAGGGGAGATGCCCTGTCACACCTGCTGGATGCCCTGGAGACGCTGGGAGAGAGACTTGCCAAGCAGAAGA 1165					