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(54) Title: DNAI - LIPOSOMES

(57) Abstract: The invention provides oligonucleotide compositions and methods for the treatment of cancer.

DNAi-LIPOSOMES

RELATED APPLICATION

[01] This application claims the benefit of the U.S. Provisional application No. 60/902,973, filed on, February 23, 2007, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[02] The present invention relates to methods and compositions for cancer therapy. In particular, the present invention provides liposomal oligonucleotides for treating cancer.

BACKGROUND

[03] Complement activation-related pseudoallergy (CARPA) can be induced by a number of agents, including liposomes. CARPA is characterized by acute non-IgE-mediated "pseudoallergic" reactions to liposomal formulations of intravenous drugs and imaging agents, infusion liquids containing micelle-forming amphiphilic lipids or synthetic block co-polymer emulsifiers and iodinated radiocontrast media with limited solubility, among others. Symptoms are typical manifestations of anaphylatoxin generation in blood, which is acute cardiopulmonary dysfunction (Szebeni, et al., "Liposome-induced pulmonary hypertension: properties and mechanism of a complement-mediated pseudoallergic reaction," Am. J. Physiol. Heart Circ. Physiol., 279:H1319-28, 2000; Szebeni, et al., "Complement activation-related cardiac anaphylaxis in pigs: role of C5a anaphylatoxin and adenosine in liposome-induced abnormalities in ECG and heart function," Am J. Physiol. Heart Circ. Physiol., 290:H1050-8, 2006, both of which are incorporated herein by reference).

[04] Complement can be activated by a classical pathway that involves C1q, Clr, Cls, C4, C2 or an alternative pathway involving C3, B, D, H, T and P. Activation is precisely regulated by interactions of C' proteins with each other, an activator and with cell membranes. There are three phases of activation: firstly, recognition-initiation via one of the pathways; secondly, C3 activation, binding and amplification; and thirdly, assembly of a C5b-9 membrane attack complex (MAC).

[05] Liposomes interact with the serum complement system. C' activation is an intrinsic property of charged phospholipid /cholesterol bilayers. Liposome induced C' activation and biological consequences show significant interspecies and inter-individual variation. Physical properties of vesicles, including particle size, polydispersity and net charge are

critical determinants of activation (Chanan-Khan, et al., "Complement activation following first exposure to peglyated liposomal doxorubicin (Doxil®): possible role in hypersensitivity reactions," Ann. Oncology 14:1430-37, 2003; Dritschilo, et al., "Phase I study of Liposome-Encapsulated *c-raf* Antisense Oligodeoxyrigonucleotide Infusion in combination with Radiation Therapy in Patients with Advanced Malignancies," Clin. Cancer Res., 12:1251-59, 2006, both of which are incorporated herein by reference).

SUMMARY

[06] Oligonucleotides can be encapsulated in lipids (*e.g.*, liposomes or micelles) to aid in delivery (See e.g., U.S. Patents 6,458,382, 6,429,200; U.S Patent Publications 2003/0099697, 2004/0120997, 2004/0131666, 2005/0164963, and International Publications WO 06/048329, WO 06/053646, each of which is herein incorporated by reference). Liposomes include, without limitation, cardiolipin based cationic liposomes (e.g., NeoPhectin, available from NeoPharm, Forest Lake, IL) and pH sensitive liposomes. These liposomes alone or in combination with oligonucleotides can activate complement, and in some cases induce CARPA. Complement activation can induce downstream effects, which may contribute to the efficacy of a liposomal oligonucleotide for treating cancer.

[07] In a first aspect, the invention provides a composition comprising an oligonucleotide and an agent that activates complement. In embodiments of this aspect the oligonucleotide is an oligonucleitode that hybridizes with SEQ ID NOs:1-15, 43, 71-74 or the complements thereof. In another embodiment, the oligonucleotide ranges from 15 to 50 nucleotides in length. In yet another embodiment, the oligonucleotide is selected from SEQ ID NOs:15, 16, 17, 19, 20, 23, 24, 25, 26, 27, 30, 34, 42 and the complements thereof. In still yet another embodiment, the oligonucleotide is selected from SEQ ID NOs: 44-70 and the complements thereof. In other embodiments, the oligonucleotide is SEQ ID NO:15, 16, 44, 45, 46, 47, 48 or 49. In other embodiments, the composition includes another oligonucleotide. The other oligonucleotide can include an oligonucleitode that hybridizes with SEQ ID NOs:1-15, 43, 71-74 or the complements thereof, or SEQ ID NOs: 15, 16, 17, 19, 20, 23, 24, 25, 26, 27, 30, 34, 42 or the complements thereof, or SEQ ID NOs: 44-70 or the complements thereof.

[08] In another embodiment of the first aspect, the agent comprises lipids. The lipids can be cardiolipins. In another embodiment, the lipids comprise CHEMS, POPC, MoChol and DOPE. In yet another embodiment, CHEMS, POPC, MoChol and DOPE are in the ratio of POPC/DOPE/MoChol/CHEMS of 6/24/47/23 mole %.

[09] In a second aspect, the invention provides a method for treating a proliferative disorder comprising administering to a subject or patient an effective dose of any of the compositions described above. In embodiments of the second aspect, the proliferative disorder is a neoplasm, a carcinoma, a sarcoma, a lymphoma, prostate cancer, non-Hodgkins lymphoma, melanoma, or breast cancer.

[10] In a third aspect the invention provides a method for treating prostate cancer, lymphoma or melanoma comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide of SEQ ID NO:15 or 16 and a lipid comprising a cardiolipin. In a fourth aspect, the invention provides a method for treating prostate cancer, lymphoma or melanoma comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide of SEQ ID NO:15 or 16 and a lipid mixture comprising POPC, DOPE, CHEMS and MoChol. In one embodiment, CHEMS, POPC, MoChol and DOPE are in the ratio of POPC/DOPE/MoChol/CHEMS of 6/24/47/23 mole %.

[11] In a fifth aspect, the invention provides a method for treating breast cancer, comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide selected from SEQ ID NOs:44-49 and a lipid comprising a cardiolipin. In a sixth aspect, the invention provides a method for treating breast cancer comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide selected from SEQ ID NOs:44-49 and a lipid mixture comprising POPC, DOPE, CHEMS and MoChol. In one embodiment, CHEMS, POPC, MoChol and DOPE are in the ratio of POPC/DOPE/MoChol/CHEMS of 6/24/47/23 mole %.

[12] In a seventh aspect, the invention provides a method of treating a proliferative disorder, as described above and further administering a chemotherapeutic agent. The chemotherapeutic agent can include, without limitation, rituximab, docetaxel, vincristine or R-CHOP.

BRIEF DESCRIPTION OF THE DRAWINGS

[13] Figure 1 is a graph showing the effect of SEQ ID NO:15 (PNT100) and SEQ ID NO:17 (PNT100R) formulated with NeoPhectin AT, on lymphoma xenografts.

[14] Figure 2 is a graph showing the effect of SEQ ID NO:15 (PNT100) and SEQ ID NO:17 (PNTC) formulated with NeoPhectin AT on PC-3 prostate carcinoma xenografts.

[15] Figure 3 shows graphs showing the effect of SEQ ID NO:15 formulated in liposomes on WSU-DLCL2 lymphoma xenografts.

[16] Figure 4 A, B and C are graphs showing the effect of SEQ ID NO:15 formulated in liposomes on Daudi (Burketts lymphoma) xenografts.

DETAILED DESCRIPTION

[17] Agents that activate complement or induce CARPA are often included in drug formulations. In particular, active agents, such as oligonucleotides are sequestered in liposomes as a method of delivering the oligonucleotide to a subject. The lipids and liposomes alone or in combination with oligonucleotides can active complement or, in acute cases, induce CARPA. Activation of complement may play a role in the effecaciousness of the active agent.

I. Agents that Activate Complement or induce CARPA

[18] As used herein, an agent that activates complement is one that activates all or part of the complement cascade. For example, without limitation, an agent that activates complement can be one that results in an increase in serum levels of C3a and/or C5a. An agent that activates complement can also result in a decrease in CH50.

[19] As used herein, an agent that induces CARPA is one that results in acute non-IgE-mediated "pseudoallergic" reactions.

[20] In general, lipids or liposomes are among agents that activate complement or induce CARPA. The lipids and liposomal formulations are described in International Publication No. WO 2007/064857 and WO 2007/065017, which are incorporated herein by reference in their entirety. The lipids include those used in liposomal formulations, including cholesterol, CHEMS, DOPE (dioleoylphosphatidylethanolamine), MoChol, POPC (palmitoyl-oleoylphosphatidylcholine) and cardiolipins, including neophectin.

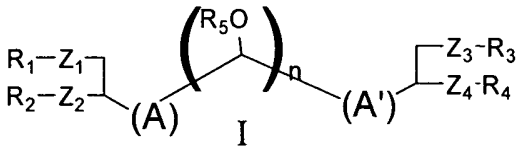
[21] In general, liposomes with a high cholesterol/phospholipids ratio are agents that activate complement more than liposomes that have a lower cholesterol/phospholipids ratio. The high cholesterol makes liposomes stick to endothelial cells and extracellular matrix (proteoglycan, heparin, elastin), better, which may, at least in part, result in complement activation. Cholesterol can exchange in membranes and make them more rigid, as cholesterol changes the fluidity of the membrane. Cholesterol sticking to the extracellular matrix or making membranes more rigid can cause complement to come in and bind.

[22] Other properties of liposomes that affect complement activation include particle size, polydispersity and net charge.

[23] Complement activation by an oligonucleotide-liposomal formulation could have a number of possible results, which include, without limitation, lysis of liposomes and release of the oligonucleotides, cytolysis of tumor cells, and/or vascular leak via inflammatory interactions with endothelial cells.

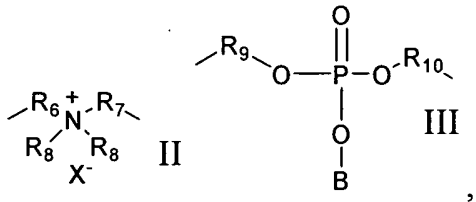
[24] In some embodiments of the present invention, NeoPhectin is utilized as the liposomal delivery vehicle. In some embodiments, the NeoPhectin is formulated with the oligonucleotide so as to reduce free NeoPhectin. In other embodiments, NeoPhectin is present at a charge ratio 6:1 or less (e.g., 5:1, and 4:1) of NeoPhectin to oligonucleotide. In other embodiments, amphoteric liposomes are used.

[25] Cardiolipins can also be used in the liposomal formulations. In one embodiment, the liposomal mixture comprises a zwitterionic colipid, a sterol, and a cationic cardiolipin analog with the general structure I.



I

[26] In structure I, n is 1 or 2; Z_1, Z_2, Z_3, and Z_4 are independently -O-C(O)-, -O-, -S-, or -NH-C(O)-; R_1, R_2, R_3, and R_4 are independently H, C_1 to C_{32} saturated or unsaturated aliphatic, optionally substituted with one or more hydroxy, amino, sulfinyl, alkoxy, cycloaliphatic, PEG, halo, or combinations thereof; R_5 is H, aliphatic, alkoxy, cycloaliphatic, alkanoyl, alkenoyl, or alkynoyl, each optionally substituted with one or more hydroxy, amino, sulfinyl, epoxy, cycloalkyl, PEG, halo, or combinations thereof; an amino acid, a peptide, a peptidomimetic moiety, a dipeptide, a polypeptide, a protein, an oligosaccharide or polysaccharide, a polyamine, a heterocyclic, a nucleoside or a polynucleotide; Each A and A' are independently selected from the structures of formulae II and III

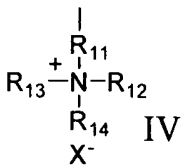


II

III

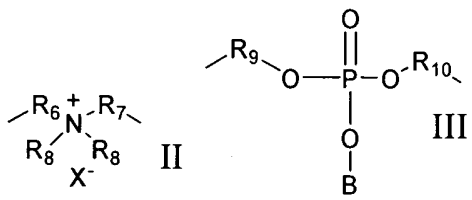
, wherein R_6 and R_7 are independently absent or comprise a linker comprising a C_1 to C_{32} alkyl, cycloalkyl, or alkyloxy group, each optionally substituted with one or more hydroxy, amino, thio, epoxy, cycloalkyl, PEG, halo, or combinations thereof; each R_8 is independently C_1 to C_{25} saturated or unsaturated aliphatic or alkoxy, each optionally substituted with one or more hydroxy, amino, sulfinyl, alkoxy, cycloaliphatic, PEG, halo, or combinations thereof; X is a non-toxic anion; R_9 and R_{10} are independently absent or comprise a linker comprising a C_1 to C_{32} aliphatic, cycloaliphatic, or

alkoxy, each optionally substituted with one or more hydroxy, amino, sulfinyl, alkoxy, cycloaliphatic, PEG, halo, or combinations thereof; and B is a non-toxic cation or a structure

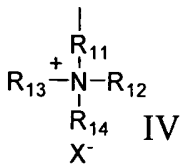


having the general formula IV , wherein R_{11} is a C_2 to C_{32} alkylene, alkenylene, or alkoxo, each optionally substituted with one or more hydroxy, amino, sulfinyl, alkoxy, cycloaliphatic, PEG, halo, or combinations thereof; R_{12}, R_{13} and R_{14} are independently H, aliphatic, alkoxy, cycloaliphatic, alkanoyl, alkenoyl, or alkynoyl, each optionally substituted with one or more hydroxy, amino, sulfinyl, alkoxy, cycloaliphatic, PEG, halo, or combinations thereof; an amino acid, a peptide, a peptidomimetic moiety, a dipeptide, a polypeptide, a protein, an oligosaccharide or polysaccharide, a polyamine, a heterocyclic, a nucleoside or a polynucleotide; and X is a non-toxic anion.

[27] In one embodiment, n is 1, Each A and A' are independently selected from the structures of formulae II and III



. In formula II, R_6 and R_7 can be the same or different, and can independently be absent or comprise a linker comprising a C_1 to C_{32} alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl or an alkyloxy or substituted alkyloxy group such as a PEGylated ether containing from 1 to 500 PEG (polyethylene glycol units. The R_8 groups are the same or different and are independently C_1 to C_{25} saturated or unsaturated alkyl, alkyloxy, substituted alkyl or substituted alkyloxy. In formula III, R_9 and R_{10} are independently absent or comprise a linker comprising a C_1 to C_{32} aliphatic, cycloaliphatic, or alkoxy, each optionally substituted with one or more hydroxy, amino, sulfinyl, alkoxy, cycloaliphatic, PEG, halo, or combinations thereof. B is either a non-toxic cation or a structure of formula IV



. In formula IV, R_{11} is a C_2 to C_{32} alkylene, alkenylene, or alkoxo, each optionally substituted with one or more hydroxy, amino, sulfinyl, alkoxy, cycloaliphatic, PEG, halo, or combinations thereof. R_{12}, R_{13} and R_{14} are independently H, aliphatic, alkoxy, cycloaliphatic, alkanoyl, alkenoyl, or alkynoyl, each optionally substituted with one or more hydroxy, amino, sulfinyl, alkoxy, cycloaliphatic, PEG, halo, or combinations thereof; an amino acid, a

peptide, a peptidomimetic moiety, a dipeptide, a polypeptide, a protein, an oligosaccharide or polysaccharide, a polyamine, a heterocyclic, a nucleoside or a polynucleotide. X is a non-toxic anion.

[28] In another embodiment, n is 2, A and A' can independently be structures II and III. Z_1 to Z_4 can be the same or different and can be independently -O-C(O)-, -O-, -S- or -NH-C(O)-. R_1, R_2, R_3, and R_4 are the same or different and can be independently H, C_1 to C_{32} saturated or unsaturated alkyl, alkenyl, or alkynyl groups, optionally hydroxylated, aminenated, thiolated epoxylated, cyclolated, PEGylated, halogenated, or substituted with combinations thereof. R_5 can be H, aliphatic, alkoxy, cycloaliphatic, alkanoyl, alkenoyl, or alkynoyl, each optionally substituted with one or more hydroxy, amino, sulfinyl, epoxy, cycloalkyl, PEG, halo, or combinations thereof; an amino acid, a peptide, a peptidomimetic moiety, a dipeptide, a polypeptide, a protein, an oligosaccharide or polysaccharide, a polyamine, a heterocyclic, a nucleoside or a polynucleotide.

[29] Liposomes can be formulated in a number of ways. See e.g., PCT applications PCT/US06/45955 and PCT/US06/46928, which are herein incorporated by reference in their entireties. One lipid formulation includes Palmitoyl-oleoylphosphatidylcholine (POPC), 4-(2-Aminoethyl)-Morpholino-Cholesterolhemisuccinate (MoChol), Cholesterolhemisuccinate (CHEMS) and Dioleoylphosphatidylethanolamine (DOPE). Critical determinants for an optimal formulation for clinical use include oligonucleotide to lipid ratio, which can range from 2-4 mg/ml; a particle size of 90-120 ηm with a low polydispersity index, stability in a vial with an intravenous diluent for infusions, including, without limitation, 5% dextrose/water, and a maximum therapeutic margin *in vivo*.

[30] A number of other lipids can be used in liposomes. They include, without limitation the following lipids. Abbreviations for lipids refer primarily to standard use in the literature and are included here as a helpful reference.

[31] DMPC Dimyristoylphosphatidylcholine

[32] DPPC Dipalmitoylphosphatidylcholine

[33] DSPC Distearoylphosphatidylcholine

[34] POPC Palmitoyl-oleoylphosphatidylcholine

[35] OPPC 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine

[36] DOPC Dioleoylphosphatidylcholine

[37] DOPE Dioleoylphosphatidylethanolamine

[38] DMPE Dimyristoylphosphatidylethanolamine

[39] DPPE Dipalmitoylphosphatidylethanolamine

[40]	DOPG	Dioleoylphosphatidylglycerol
[41]	POPG	Palmitoyl-oleoylphosphatidylglycerol
[42]	DMPG	Dimyristoylphosphatidylglycerol
[43]	DPPG	Dipalmitoylphosphatidylglycerol
[44]	DLPG	Dilaurylphosphatidylglycerol
[45]	DSPG	Distearoylphosphatidylglycerol
[46]	DMPS	Dimyristoylphosphatidylserine
[47]	DPPS	Dipalmitoylphosphatidylserine
[48]	DOPS	Dioleoylphosphatidylserine
[49]	POPS	Palmitoyl-oleoylphosphatidylserine
[50]	DMPA	Dimyristoylphosphatidic acid
[51]	DPPA	Dipalmitoylphosphatidic acid
[52]	DSPA	Distearoylphosphatidic acid
[53]	DLPA	Dilaurylphosphatidic acid
[54]	DOPA	Dioleoylphosphatidic acid
[55]	POPA	Palmitoyl-oleoylphosphatidic acid
[56]	CHEMS	Cholesterolhemisuccinate
[57]	DC-Chol	3-β-[N-(N′,N′-dimethylethane) carbamoyl]cholesterol
[58]	Cet-P	Cetylphosphate
[59]	DODAP	(1,2)-dioleoyloxypropyl)-N,N-dimethylammonium chloride
[60]	DOEPC	1,2-dioleoyl-sn-glycero-3-ethylphosphocholine
[61]	DAC-Chol	3-β-[N-(N,N′-dimethylethane) carbamoyl]cholesterol
[62]	TC-Chol	3-β-[N-(N′,N′, N′-trimethylaminoethane) carbamoyl] cholesterol
[63]	DOTMA	(1,2-dioleyloxypropyl)-N,N,N-trimethylammoniumchloride) (Lipofectin®)
[64]	DOGS	((C18)2GlySper3+) N,N-dioctadecylamido-glycyl-spermine (Transfectam®)
[65]	CTAB	Cetyl-trimethylammoniumbromide
[66]	CPyC	Cetyl-pyridiniumchloride
[67]	DOTAP	(1,2-dioleoyloxypropyl)-N,N,N-trimethylammonium salt
[68]	DMTAP	(1,2-dimyristoyloxypropyl)-N,N,N-trimethylammonium salt
[69]	DPTAP	(1,2-dipalmitoyloxypropyl)-N,N,N-trimethylammonium salt
[70]	DOTMA	(1,2-dioleyloxypropyl)-N,N,N-trimethylammonium chloride)
[71]	DORIE	(1,2-dioleyloxypropyl)-3 dimethylhydroxyethyl ammoniumbromide)

[72] DDAB Dimethyldioctadecylammonium bromide

[73] DPIM 4-(2,3-bis-palmitoyloxy-propyl)-1-methyl-1H-imidazole

[74] CHIM Histaminyl-Cholesterolcarbamate

[75] MoChol 4-(2-Aminoethyl)-Morpholino-Cholesterolhemisuccinate

[76] HisChol Histaminyl-Cholesterolhemisuccinate

[77] HCChol Nα-Histidinyl-Cholesterolcarbamate

[78] HistChol Nα-Histidinyl-Cholesterol-hemisuccinate

[79] AC Acylcarnosine, Stearyl- & Palmitoylcarnosine

[80] HistDG 1,2—Dipalmitoylglycerol-hemisuccinat-N_-Histidinyl-hemisuccinate, & Distearoyl- ,Dimyristoyl, Dioleoyl or palmitoyl-oleoylderivatives

[81] IsoHistSuccDG 1,2-ipalmitoylglycerol-O_-Histidinyl-Nα-hemisuccinat, & Distearoyl-, Dimyristoyl, Dioleoyl or palmitoyl-oleoylderivatives

[82] DGSucc 1,2—Dipalmitoyglycerol-3-hemisuccinate & Distearoyl-, dimyristoyl-Dioleoyl or palmitoyl-oleoylderivatives

[83] EDTA-Chol cholesterol ester of ethylenediaminetetraacetic acid

[84] Hist-PS Nα-histidinyl-phosphatidylserine

[85] BGSC bisguanidinium-spermidine-cholesterol

[86] BGTC bisguanidinium-tren-cholesterol

[87] DOSPER (1.3-dioleoyloxy-2-(6-carboxy-spermyl)-propylarnide

[88] DOSC (1,2-dioleoyl-3-succinyl-sn-glyceryl choline ester)

[89] DOGSDO (1,2-dioleoyl-sn-glycero-3-succinyl-2-hydroxyethyl disulfide ornithine)

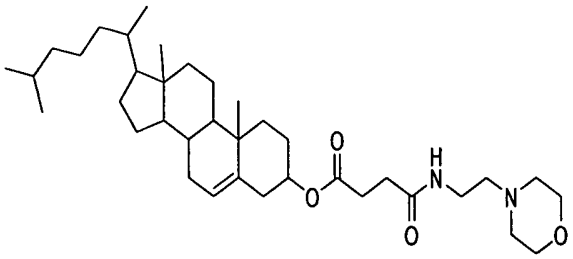
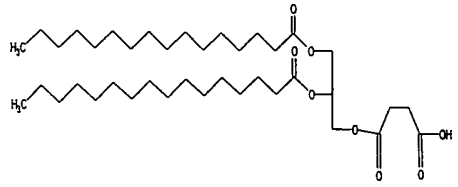
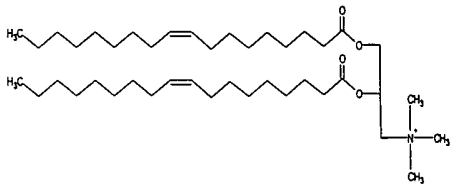
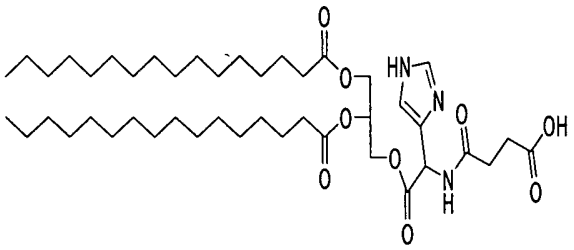
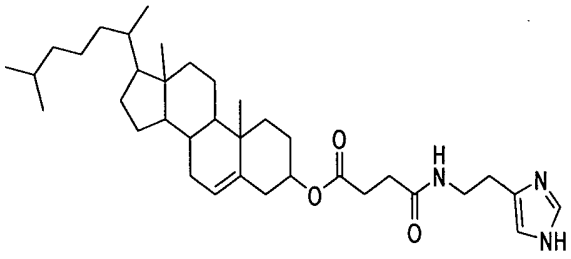
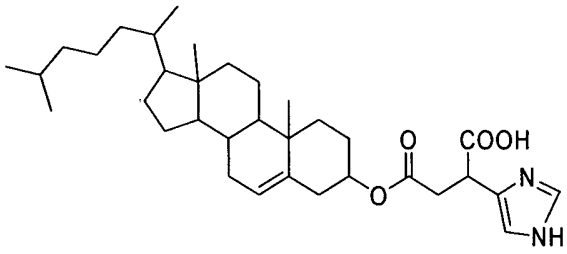
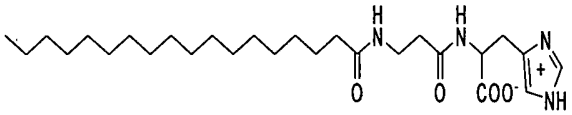
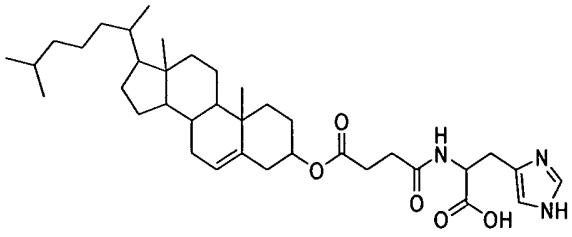
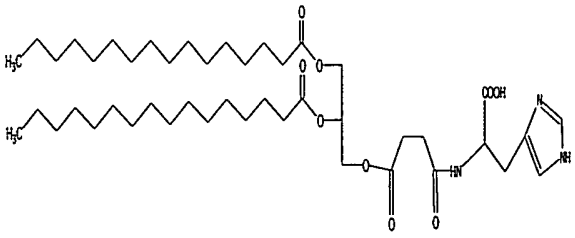
[90] DOGSucc 1,2-Dioleoylglycerol-3-hemisucinate

[91] POGSucc Palimtolyl-oleoylglycerol-oleoyl-3-hemisuccinate

[92] DMGSucc 1,2-Dimyristoylglycerol-3-hemisuccinate

[93] DPGSucc 1,2-Dipalmitoylglycerol-3-hemisuccinate

[94] The following table provides non-limiting examples of lipids that are suitable for use in the compositions in accordance with the present invention. The membrane anchors of the lipids are shown exemplarily and serve only to illustrate the lipids of the invention and are not intended to limit the same.

MoChol 	DG-Succ 
DOTAP 	IsohistsuccDG 
HisChol 	HCChol 
AC 	Hist-Chol 
Hist-DG 	

[95] Other lipid formulations include POPC/DOPE/MoChol/CHEMS, POPC/DOTAP/CHEMS/Chol, POPC/MoChol/Cet-P/Chol, POPC/DOPE/MoChol/CHEMS, POPC/DOPE/MoChol/DMG-Succ and POPC/DOPE/MoChol/DMG-Succ. Ratios of the lipids can vary. Specific formulations include those in the following table.

POPC/DOPE/MoChol/CHEMS	15/45/20/20
POPC/DOTAP/CHEMS/Chol	30/10/20/40
POPC/MoChol/Cet-P/Chol	35/20/10/35
POPC/DOPE/MoChol/CHEMS	6/24/47/23
POPC/DOPE/MoChol/DMG-Succ	6/24/47/23
POPC/DOPE/MoChol/DMG-Succ	6/24/23/47

[96] Oligonucleotides and/or other agents, in combination with liposomes can also result in complement activation.

II. Oligonucleotides

[97] The DNAi oligonucleotides are DNA oligomers that are complementary to either the plus strand or minus strand of double stranded DNA, and can include any oligomer that hybridizes to regulatory regions of the *c-ki-ras*, *c-Ha-ras*, *c-myc*, *her-2*, *TGF-α*, or *bcl-2* gene. For the purposes of this invention, those regulatory regions are defined as SEQ ID NO:1 (for*bcl-2*), SEQ ID NO:43 (for *c-myc*), SEQ ID NO:71 (for *c-erb-2 or her-2*), SEQ ID NO:72 (for *c-ki-ras*), SEQ ID NO:73 (for *c-Ha-ras*) and SEQ ID NO:74 (for *TGF-α*). The DNA oligomers that hybridize to these regions can be 100%, 99%, 98%, 95%, 90%, 85%, 80%, 75%, 70% or 60% complementary to the sequences.

[98] In some embodiments, DNAi oligonucleotides are designed based on certain design criteria. Such DNAi oligonucleotides can then be tested for efficacy using the methods disclosed herein. For example, in some embodiments, the DNAi oligonucleotides are methylated on at least one, two or all of the CpG islands. In other embodiments, the DNAi oligonucleotides contain no methylation. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that DNAi oligonucleotides in some embodiments are those that have at least a 50% GC content and at least two GC dinucleotides. Also, in some embodiments, the DNAi oligonucleotides do not self hybridize. In further embodiments, DNAi oligonucleotides are designed with at least 1 A or T to minimize self hybridization. In yet further embodiments, commercially available computer

programs are used to survey DNAi oligonucleotides for the ability to self hybridize. In still other embodiments, DNAi oligonucleotides are at least 10, or 15 nucleotides and no more than 100 nucleotides in length. In further embodiments, DNAi oligonucleotides are 18-26 nucleotides in length. In some embodiments, DNAi oligonucleotides comprise the universal protein binding sequences CGCCC and CGCG or the complements thereof.

[99] In some embodiments, DNAi oligonucleotides hybridize to a regulatory region of a gene upstream from the TATA box of the promoter. In further embodiments, DNAi oligonucleotides are designed to hybridize to regulatory regions of oncogenes known to be bound by proteins (*e.g.*, transcription factors). In some embodiments, DNAi oligonucleotide compounds are not completely homologous to other regions of the human genome. The homology of the DNAi oligonucleotides to other regions of the genome can be determined using available search tools (*e.g.*, BLAST, available at the internet site of NCBI).

[100] The present invention is not limited to the DNAi oligonucleotides described herein. Other suitable DNAi oligonucleotides may be identified (*e.g.*, using the criteria described above or other criteria). Candidate DNAi oligonucleotides may be tested for efficacy using any suitable method. For example, candidate DNAi oligonucleotides can be evaluated for their ability to prevent cell proliferation at a variety of concentrations. In some embodiments, DNAi oligonucleotides inhibit gene expression or cell proliferation at a low concentration (*e.g.*, less that 20 μM, or 10 μM in *in vitro* assays.).

A. DNAi Oligomers

[101] The DNAi oligomers can include any oligomer that hybridizes to SEQ ID NOs:1, 2, 43, 71-74, or the complements thereof. In one aspect, the DNAi oligomer can be any oligomer that hybridizes to nucleotides 500-2026, nucleotides 500-1525, nucleotides 800-1225, nucleotides 900-1125, nucleotides 950-1075 or nucleotides 970-1045 of SEQ ID NO:1 or the complement thereof. The oligomers that hybridize to these regions include those that are 100%, 99%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, or 60% complementary to the above regions. Examples of DNAi oligomers include, without limitation, those oligomers listed in SEQ ID NOs 15-42, 44-70 and the complement thereof. In an embodiment of these aspects, the DNAi oligonucleotides are from 15-35 base pairs in length.

[102] In one embodiment the DNAi oligomer can be SEQ ID NO:15 or 16, or the complements thereof. In another embodiment, the DNAi oligomer can be SEQ ID NO:44, 45, 46, 47, 48, 49 or the complements thereof.

[103] In other aspects, the DNAi oligomers can include mixtures of DNAi oligonucleotides. For instance, the DNAi oligomer can include multiple DNAi oligonucleotides, each of which

hybridizes to different parts of SEQ ID NOs 1249 and 1254. DNAi oligomers can hybridize to overlapping regions on those sequences or the DNAi oligomers may hybridize to non-overlapping regions. In another embodiment, the mixture of DNAi oligomers comprises DNAi oligomers of at least 2 different sequences.

[104] In other embodiments, the oligomer can include a mixture of oligomers, each of which hybridizes to a regulatory region of different genes. For instance, the oligomer can include a first oligomer that hybridizes to SEQ ID NO:1 or 2 and second oligomer that hybridizes to a regulatory region of a second gene. In some embodiments, the second oligomer can be one that hybridizes to SEQ ID NO:43 or the complement thereof. In some embodiments, the second oligomer is selected from SEQ ID NOs:44-49.

B. Oligonucleotide Structure

i. Methylation

[105] In some embodiments, the present invention provides oligonucleotide therapeutics that are methylated at specific sites. The present invention is not limited to the use of methylated oligonucleotides. Indeed, the use of non-methylated oligonucleotides for the inhibition of gene expression is specifically contemplated by the present invention.

ii. Bases

[106] The oligonucleotides can be in a naturally occurring state, and can also contain modifications or substitutions in the nucleobases, the sugar moiety and/or in the internucleoside linkage.

[107] Nucleobases comprise naturally occurring nucleobases as well as non-naturally occurring nucleobases. Illustrative examples of such nucleobases include without limitation adenine, cytosine, 5-methylcytosine, isocytosine, pseudoisocytosine, guanine, thymine, uracil, 5-bromouracil, 5-propynyluracil, 5-propynylcytosine, 5-propyny-6-fluoroluracil, 5-methylthiazoleuracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 8-azaguanine, 8-azaadenine, 7-propyne-7-deazaadenine, 7-propyne-7-deazaguanine, 2-chloro-6-aminopurine, 4-acetylcytosine, 5-hydroxymethylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, 5-(carboxyhydroxyl-methyl) uracil, 5-fluorouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, N6-methyladenine, 7-methylguanine and other alkyl derivatives of adenine and guanine, 2-propyl adenine and other alkyl derivatives of adenine and guanine, 2-aminoadenine, 5-

methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 2-thiothymine, 5-halouracil, 5-halocytosine, 6-azo uracil, cytosine and thymine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, 8-halo, 8-amino, 8-thiol, 8-hydroxyl and other 8-substituted adenines and guanines, 5-trifluoromethyl uracil and cytosine, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, queosine, xanthine, hypoxanthine, 2-thiocytosine, 2,6-diaminopurine, 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

iii. Sugars and Sugar Substitutes

[108] Oligonucleotides can also have sugars other than ribose and deoxy ribose, including arabinofuranose (described in International Publication number WO 99/67378, which is herein incorporated by reference), xyloarabinofuranose (described in U.S. Patent Nos 6,316,612 and 6,489465, which are herein incorporated by reference), α-threofuranose (Schöning, et al. (2000) Science, 290, 1347-51, which is herein incorporated by reference) and L-ribofuranose. Sugar mimetics can replace the sugar in the nucleotides. They include cyclohexene (Wang et al.(2000) J. Am. Chem. Soc. 122, 8595-8602; Vebeure et al. Nucl. Acids Res. (2001) 29, 4941-4947, which are herein incorporated by reference), a tricyclo group (Steffens, et al. J. Am. Chem. Soc. (1997) 119, 11548-11549, which is herein incorporated by reference), a cyclobutyl group, a hexitol group (Maurinsh, et al. (1997) J. Org. Chem, 62, 2861-71; J. Am. Chem. Soc. (1998) 120, 5381-94, which are herein incorporated by reference), an altritol group (Allart, et al., Tetrahedron (1999) 6527-46, which is herein incorporated by reference), a pyrrolidine group (Scharer, et al., J. Am. Chem. Soc., 117, 6623-24, which is herein incorporated by reference), carbocyclic groups obtained by replacing the oxygen of the furnaose ring with a methylene group (Froehler and Ricca, J. Am. Chem. Soc. 114, 8230-32, which is herein incorporated by reference) or with an S to obtain 4'-thiofuranose (Hancock, et al., Nucl. Acids Res. 21, 3485-91, which is herein incorporated by reference), and/or morpholino group (Heasman, (2002) Dev. Biol., 243, 209-214, which is herein incorporated by reference) in place of the pentofuranosyl sugar. Morpholino oligonucleotides are commercially available from Gene Tools, LLC (Corvallis Oregon, USA).

[109] The nucleotide derivatives can include nucleotides containing one of the following at the 2' sugar position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or

O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl, $O[(CH_2)_nO]_mCH_2$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10, C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, $ON)_2$, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, Helv. Chim. Acta 78:486 [1995]) *i.e.*, an alkoxyalkoxy group, 2'-dimethylaminooxyethoxy (*i.e.*, an $O(CH_2)2ON(CH_3)_2$ group), also known as 2'-DMAOE, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O-CH_2-O-CH_2-$N(CH_2)_2$, 2'-methoxy (2'-O-CH_3), 2'-aminopropoxy(2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide.

iv. Internucleoside Linkages and Backbones

[110] In some embodiments, the oligonucleotides have non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

[111] Some modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphoroselenates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

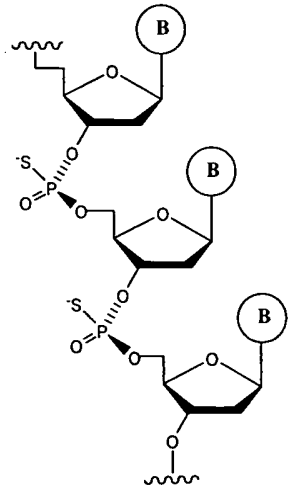
[112] Other modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those

having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[113] In yet other oligonucleotide mimetics, both the sugar and the internucleoside linkage (i.e., the backbone) of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos.: 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science 254:1497 (1991).

[114] In some embodiments, oligonucleotides of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular $-CH_2-$, $-NH-O-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ [known as a methylene (methylimino) or MMI backbone], $-CH_2-O-N(CH_3)-CH_2-$, $--CH_2-N(CH_3)-N(CH_3)-CH_2-$, and $-O-N(CH_3)-CH_2-CH_2-$ [wherein the native phosphodiester backbone is represented as $-O-P-O-CH_2-$] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Oligonucleotides can also have a morpholino backbone structure of the above-referenced U.S. Pat. No. 5,034,506.

[115] In some embodiments the oligonucleotides have a phosphorothioate backbone having the following general structure.



v. Other Modifications

[116] Another modification of the DNAi oligonucleotides of the present invention involves adding additional nucleotides to the 3' and/or 5' ends of the DNAi oligonucleotides. The 3' and 5' tails can comprise any nucleotide and can be as short as one nucleotide and as long as 20 nucleotides.

[117] Yet another modification of the oligonucleotides of the present invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, (e.g., hexyl-S-tritylthiol), a thiocholesterol, an aliphatic chain, (e.g., dodecandiol or undecyl residues), a phospholipid, (e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate), a polyamine or a polyethylene glycol chain or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

[118] Other modifications are described in International Patent Publication No. WO2007/064853, which is incorporated herein in its entirety.

[119] One skilled in the relevant art knows well how to generate oligonucleotides containing the above-described modifications. The present invention is not limited to the oligonucleotides described above. Any suitable modification or substitution may be utilized.

[120] It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes pharmaceutical compositions and formulations that include the antisense compounds of the present invention as described below.

C. Preparation and Formulation of DNAi Oligonucleotides

[121] Any of the known methods of oligonucleotide synthesis can be used to prepare the modified DNAi oligomers of the present invention. In some embodiments utilizing methylated oligonucleotides the nucleotide, dC is replaced by 5-methyl-dC where appropriate, as taught by the present invention. The modified or unmodified oligonucleotides of the present invention are most conveniently prepared by using any of the commercially available automated nucleic acid synthesizers. They can also be obtained from commercial sources that synthesize custom oligonucleotides pursuant to customer specifications.

III. Formulations, Administration and Uses

[122] DNAi- liposomes are useful for administering to animals, including humans, to treat cancer, such as by inhibiting or reducing tumor growth. The animal can be a human or a non-human animal, including, without limitation, vertebrates, such as rodents, non-human primates, ovines, bovines, ruminants, lagomorphs, porcines, caprines, horses, cats, dogs, birds or other animals. In one embodiment, the mixture is introduced to the animal at a dosage of between 0.01 mg to 100 mg/kg of body weight. In another embodiment, the amphoteric liposomes can be introduced to the animal one or more times per day or continuously.

[123] As used herein, the term "composition" refers to a mixture comprising one or more active agents, such as an oligonucleotide or a lipid, and one or more additional components used to administer the active agents to a subject or animal. For example, the additional component can be a pharmaceutically acceptable salt or other component as described below.

[124] As used herein, an effective amount is defined as the amount required to confer a therapeutic effect on the treated patient, subject or animal, and is typically determined based on age, surface area, weight and condition of the patient. The interrelationship of dosages for animals and humans (based on milligrams per meter squared of body surface) is described by Freireich et al., *Cancer Chemother. Rep.*, 50: 219 (1966). Body surface area can be approximately determined from height and weight of the patient. See, e.g., Scientific Tables, Geigy Pharmaceuticals, Ardsley, New York, 537 (1970).

[125] The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, intranasally, intraoccularly, buccally, vaginally, or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously. Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous

suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

[126] The mixture can be administered to the animal via different routes. Administration can be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary (*e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, *e.g.*, intrathecal or intraventricular, administration. Administration can also be via a medical device.

[127] For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[128] The pharmaceutically acceptable compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[129] Alternatively, the pharmaceutically acceptable compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by

mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[130] The pharmaceutically acceptable compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[131] Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

[132] For topical applications, the pharmaceutically acceptable compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutically acceptable compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[133] For ophthalmic use, the pharmaceutically acceptable compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutically acceptable compositions may be formulated in an ointment such as petrolatum.

[134] The pharmaceutically acceptable compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[135] In several embodiments, the pharmaceutically acceptable compositions of this invention are formulated for oral administration.

[136] The amount of the compounds of the present invention that may be combined with the carrier materials to produce a composition in a single dosage form will vary depending upon the host treated, the particular mode of administration. Preferably, the compositions should be formulated so that a dosage of between 0.01-100 mg/kg body weight/day of the modulator can be administered to a patient receiving these compositions.

[137] It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of a compound of the present invention in the composition will also depend upon the particular compound in the composition.

[138] The compositions described herein can be used to treat a number of different cellular proliferative disorders, including cancers, neoplasms and tumors. A "proliferative disorder" is any cellular disorder in which the cells proliferate more rapidly than normal tissue growth. Thus a "proliferating cell" is a cell that is proliferating more rapidly than normal cells. The proliferative disorder, includes but is not limited to neoplasms. A neoplasm is an abnormal tissue growth, generally forming a distinct mass that grows by cellular proliferation more rapidly than normal tissue growth. Neoplasms show partial or total lack of structural organization and functional coordination with normal tissue. These can be broadly classified into three major types. Malignant neoplasms arising from epithelial structures are called carcinomas, malignant neoplasms that originate from connective tissues such as muscle, cartilage, fat or bone are called sarcomas and malignant tumors affecting hematopoietic structures (structures pertaining to the formation of blood cells) including components of the immune system, are called leukemias and lymphomas. A tumor is the neoplastic growth of the disease cancer. As used herein, a "neoplasm", also referred to as a "tumor", is intended to encompass hematopoietic neoplasms as well as solid neoplasms. In some embodiments, the proliferative disorder can be selected from the group consisting of lung cancer, prostate cancer, colorectal cancer, thyroid cancer, renal cancer, adrenal cancer, liver cancer, pancreatic cancer, breast cancer, lymphomas, leukemias, and central and peripheral nervous system cancer. Other proliferative disorders include, but are not limited to neurofibromatosis.

[139] Depending upon the particular condition, or disease, to be treated or prevented, additional therapeutic agents, which are normally administered to treat or prevent that condition, may also be present in the compositions of this invention. As used herein,

additional therapeutic agents normally administered to treat or prevent a particular disease, or condition, are known as "appropriate for the disease, or condition, being treated."

[140] In additional embodiments, chemotherapeutic agents, including docetaxel and others can be combined with DNAi oligomers before or while sequestering in liposomes. The chemotherapeutic agents can also be administered to a subject or animal in a treatment regimen along with an oligonucleotide sequestered in a liposome. Chemotherapeutic agents and other therapies are described in International Publication No. WO2007/064945, which is incorporated herein by reference in its entirety.

EXAMPLES

[141] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLE 1: Complement Activation by an Oligonucleotide in Liposomes

[142] SEQ ID NO:15 formulated in liposomes comprising POPC/DOPE/MoChol/CHEMS in the ratios 6/24/47/23 was administered to cynomologus monkeys of 5, 25 and 65 mg of oligonucleotide per kg of body weight. Complement activation was initially assessed by measurement of the Bb split product, a marker for alternative pathway activation (typically seen with phosphorothioate oligos), and by CH50 (total hemolytic complement), which reflects the residual activity of the complement pathway (determined ex vivo, in terms of cell lysis potential, with a decrease indicating that complement activation had occurred *in vivo*). At 5 mg/kg, PNT 2254, containing 4 mg/mL oligonucleotide (2-hour infusion), there was no consistent increase in the alternative pathway split product Bb, but total hemolytic complement (CH50) was moderately decreased, indicating consumption of complement factors stemming from activation of some part of the pathway (most likely the classical side, since the alternative pathway was not activated). There was no clear effect on C3a and C5a at this dose, (except for a possible modest increase in C3a in the female). At 25 mg/kg PNT 2254, more pronounced complement activation was evident, i.e., Bb was modestly elevated (approximately 2-fold), CH50 was virtually completely exhausted (reduced to 1 unit/mL for the male and zero for the female), and C3a was elevated. However, no increase in C5a was observed. When the female was given ~68 mg/kg 2254, Bb was approximately doubled, CH50 was again reduced to zero, and C3a was markedly increased (but, again, no effect on C5a). The low CH50 readings of 1 or 0 for CH50 are indicative of extensive complement activation and consumption of the various factors at 25 or 68 mg/kg PNT 2254. These effects

were measured at the end of infusion, which is when complement activation is typically maximal (i.e., when it occurs in relation to blood concentration of the activating agent).

Table 1

Day	Formu-lation	PNT100 dose (mg/kg)	Lipid dose (mg/kg)	Time Point	Bb (μg/mL)		CH50 (Units/ mL)		C3a (ηg/mL)		C5a (ηg/mL)	
					M	F	M	F	M	F	M	F
1	PBS	0	0	Pre	0.60	0.54	198	202	173	229	9.0	9.0
				EOI	0.67	0.55	183	171	152	163	10.5	12.8
5	PNT2254	5	80	Pre	0.48	0.59	183	125	133	205	9.5	8.3
				EOI	0.56	0.9	136	53	185	365	8.6	9.9
12	PNT2254	25	400	Pre	0.47	0.46	104	173	104	206	----	14.4
				EOI	1.16	1.24	1	0	531	711	----	13.0
22	PNT2254	68	1080	Pre	-----	0.57	----	196	----	275	----	----
				EOI	-----	1.28	----	0	----	760	----	----
37	PNT2253	25	800	Pre	0.28	-----	111	----	5	----	----	----
				EOI	1.25	-----	57	----	1011	----	----	----
71	PNT2256	25 6 hr infusion	~1600	Pre	-----	-----	148	177	175	190	7.1	11.7
				EOI	-----	-----	88	101	676	802	8.2	7.5
138 to 142	PNT2255	5 (x 5 days	160 x 5	Day 1, Pre	-----	-----	116	137	51	115	---	---
				Day 1 EOI	-----	-----	96	76	510	746	---	---
				Day 5, Pre	-----	-----	81	83	409	430	---	---
				Day 5, EOI	-----	-----	0	0	796	566	---	---
Normal Range					0.017-1.09		135-426		0-368		6.4-10.5	

EOI—End of Infusion

[143] With administration of PNT 2253, containing 2 mg/mL of oligonucleotide, and thus, a higher concentration of lipid per oligonucleotide than PNT2254, at 25 mg/kg over 2 hours (male monkey), Bb was increased several-fold, CH50 was reduced to 57, and C3a was markedly increased. Comparison of the C3a data between the 25 mg/kg doses for PNT 2254 and PNT 2253 suggested that the activation was more pronounced with PNT 2253, although there was no clear difference in the CH50 response.

[144] When PNT 2256 (4 mg/mL oligonucleotide) was administered at a dose level of 25 mg/kg over 6 hours, a substantial degree of complement activation was still evident from CH50 and C3a data, but it seemed that the response may have been blunted a bit, especially with respect to CH50 depletion. Considering that the lipid dose associated with 2256 was

relatively high (see above table), it seems that the slower infusion may have afforded a larger benefit than was immediately apparent.

[145] With repeated dosing of PNT 2255 at 5 mg/kg/day for 5 days (2-hour infusions), there appeared to be a cumulative effect on complement activation. For both animals, there was a small decrease in CH50 (indicating modest consumption of complement factors) during the first infusion (Day 1, predose vs. EOI), and this was accompanied by a substantial (7- to 10-fold) increase in C3a. The absence of a more dramatic reduction in CH50 during the first dose suggests that the complement activation was not extensive. However, on Day 5, the predose CH50s were slightly depressed, and C3a was elevated, relative to Day 1 baseline. This suggests that the formulation may have a long half-life that prevents recovery of the complement system between doses. At the end of infusion on Day 5, the C3a was modestly further elevated, but the CH50 had dropped to zero, indicating more extensive complement activation. It is unusual to see an increase in a labile split product like C3a that persists between dosing days.

[146] Data supports involvement of the classical C' pathway more than the alternative complement pathway. This is consistent with findings for negatively charged liposome/drug products.

[147] Lower dosage levels of the oligonucleotide SEQ ID NO:15 sequestered in liposomes comprising POPC/DOPE/MoChol/CHEMS in the ratios 6/24/47/23 were tested for their effect on complement activation in cynomolgus monkeys. The animals were treated as shown in Table 2. All animals received doses via 2-hour iv infusions of the infusion control on days 1-5, followed approximately 3 days later by the vehicle control on days 8-12. After an approximate 10-day washout period, the animals received PNT2258 on days 22-26. (3 mg/kg PNT100 (SEQ ID NO:15) and days 36-40 (15 mg/kg PNT100 (SEQ ID NO:15).

[148] Accumulation of the complement C3a split product was observed, but was not associated with physiologic consequences. Plasma concentration of complement C3a split product was increased by treatment with the vehicle (empty liposomes) and by the complete formulation of PNT2258 at the 15 mg/kg/day dose level, but not convincingly at the 3 mg/kg/day dose level, which indicates an effect of one or more vehicle constitutents in eliciting complement activation to an extent that resulted in C3a accumulation. (See Table 3.) However, there was no increase in the biological active split product C5a in this study. C5a is believed to mediate most of the adverse sequelae stemming from complement activation, but it is also highly labile and, hence does not accumulate unless there is a burst of complement activation. Thus, it appears that the complement activation induced by the

vehicle material developed slowly during and following infusion, leading to accumulation of C3a, but without any accumulation of the much more potent C5a split product.

Table 2

N	Dosing Days	Dose Level Oligo (mg/kg)	Dose Level Lipids (mg.kg)	2-hour Infusion Rate	Oligo Concentration (mg/mL)
4	1, 2, 3, 4, 5	0 (infusion control)	0	3.75	0
	8, 9, 10, 11, 12	0 (vehicle control)	0	3.75	0
	22, 23, 24, 25, 26	3	90	0.75	2
	36, 37, 38, 39, 40	15	450	3.75	2

Table 3

Day	Formu-lation	PNT100 dose (mg/kg/ day)	Lipid dose (mg/kg/ day)	C3a (ηg/mL)			C5a (ηg/mL)		
				Pre	EOI	22 EOI*	Pre	EOI	22 EOI*
1	Infusion Control	0	0	124	137	144	9.2	8.3	8.2
5	Infusion Control	0	0	122	200	239	8.0	9.8	9.5
8	Vehicle Control	0	90	205	585	333	13.1	11.3	8.9
12	Vehicle Control	68	450	562	911	496	10.8	10.9	13.2
22	PNT2258	3	90	451	655	542	8.6	10.8	9.2
26	PNT2258	3	90	450	664	496	9.8	8.0	10.1
36	PNT2258	15	450	768	1303	1073	10.5	12.1	8.2
40	PNT2258	15	450	935	1926	770	11.2	10	10.8

*22 hours post EOI

[149] A third study utilizing a larger number of animals was performed to test the effect of PNT2258 and the vehicle on complement activation. Animals were assigned to the groups in Table 4.

Table 4

Group Assignments				
Group	Treatment and Dose Level[a] (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals	
			M	F
1	Negative	8	5	5

	Control(PBS)			
2	Vehicle Control	8	2	2
3	PNT2258: 1	0.5	3	3
4	PNT2258: 4	2	3	3
5	PNT2258: 16	8	5	5
6	PNT2258cy: 16	8	3	3
[a]Doses administered via a 2-hour infusion on Days 1 to 5 and 20 to 24.				

[150] Complement factors C3a, C5a and CH50 were measured after dosing. Results are shown in Tables 5, 6 and 7.

[151] CH50 reflects the residual capacity of the entire system to form membrane attack complexes when triggered *ex vivo*, and the occurrence of a complement activation event *in vivo* will therefore result in a decrease in CH50 (i.e., due to consumption of complement factors). Therefore, it is not a direct measure of the potential for adverse sequelae, as consumption of complement factors can occur gradually over a period of time in a manner that does not result in formation of biologically-active split products. The mean values for CH50 are presented in Table 5.

Table 5

Serum CH50							
		Group #					
		Females					
Day	Timepoint	1	2	3	4	5	6
1	0 hour	147	164	177	127	122	111
	0 hour*	150	153	136	132	108	99
	EOI†	154	116	114	67	69	64
	24 hour	155	109	111	135	105	100
5	0 hour	158	53	136	48	0	0
	EOI	155	65	81	0	0	0
24	0 hour	197	172	185	74	79	62
	EOI	201	225	175	17	5	18
	24 hour	201	256	169	62	66	49
		Males					
1	0 hour	164	188	228	172	195	234
	EOI	144	105	209	144	94	154
	24 hour	155	193	223	168	184	220
5	0 hour	207	114	208	99	10	3
	EOI	204	84	63	24	0	0
24	0 hour	178	137	171	104	129	115
	EOI	185	143	68	35	30	9
	24 hour	178	143	150	98	98	91

* Extra 0-hour time point on Day 1 was drawn only for females. †EOI=end of 2-hour infusion.

[152] The data for CH50 (and C3a, below) reveal a clear effect of the vehicle components (i.e., the lipid constituents of the liposomal formulation) on the complement system. The somewhat larger magnitude of the changes in CH50 described below in the high-dose PNT2258 groups, relative to the vehicle control group, are most likely due to the greater stability of the liposomes containing the oligonucleotide active ingredient (PNT100), rather than an effect of the unmodified oligonucleotide.

[153] At the end of the first infusion (Day 1), mean CH50 was modestly reduced in the vehicle (empty liposome) control group and low-dose (1 mg/kg/day) PNT2258 group (Groups 2 and 3, respectively), and was more substantially reduced in the groups treated with 4 and 16 mg/kg/day PNT2258 and 16 mg/kg/day PNT2258cy (Groups 3-5). The magnitude of the effect in the latter groups was similar among these groups for females and also did exhibit a clear dose-response for males. This decrease in CH50 reflects in vivo consumption of complement factors during infusion, albeit minimally at the lowest dose level. By 24 hours after the first dose (prior to the second dose), mean CH50 values had largely recovered, reflecting re-synthesis of complement proteins, although full recovery had not occurred in the high-dose female and vehicle groups, which is likely due to the long circulation time of PNT2258 and continuing effect on the complement system after the end of infusion at a high dose level. This protracted period of complement factor consumption resulted in a progressive decrease through the sampling immediately prior to the last day of the first dosing period (predose on Day 5), at which time mean CH50 was more profoundly decreased in the vehicle control and higher dose groups, but was not clearly affected at the low-dose level (Group 3; 1 mg/kg/day). At the high-dose level (16 mg/kg/day of both PNT2258 and PNT2258cy.[SEQ ID NO:20]), extensive consumption of complement factors was evident from the very low or zero CH50 values at the Day 5 predose time point. As would be expected from the Day 1 data, the Day 5 dosing produced a further decline in mean CH50 (i.e., at the low- and middle-dose levels; the high-dose values were already at zero). On Day 24 (the last dosing day), predose CH50 values were not as low as seen prior to dosing on Day 5, although the time points are identical with respect the animals having received 4 previous doses in each of the dosing periods. This difference may reflect some induction of the rate of re-synthesis of complement proteins. The Day 24 dose produced a characteristic further decline in mean CH50 (i.e., more consumption of complement factors), with partial recovery

at 24 hours after the last dose. On this last dosing day, there was no clear effect on CH50 at the low (1 mg/kg) dose level for females and only a transient effect in males.

[154] The mean values for plasma C3a concentrations are shown in Table 6.

Table 6

Serum C3a							
		Group #					
		Females					
Day	Timepoint	1	2	3	4	5	6
1	0 hour	159	397	201	173	191	163
	0 hour*	162	198	338	155	150	152
	EOI†	153	658	222	350	573	616
	24 hour	144	259	174	175	312	197
5	0 hour	150	709	193	299	355	319
	EOI	147	1226	431	328	364	407
24	0 hour	250	963	300	497	808	636
	EOI	219	1085	679	587	920	946
	24 hour	232	880	277	385	556	549
		Males					
1	0 hour	340	269	195	189	155	159
	EOI	281	939	326	348	839	853
	24 hour	284	375	162	193	303	251
5	0 hour	358	600	186	292	507	422
	EOI	293	1039	451	329	554	607
24	0 hour	194	662	225	240	550	562
	EOI	212	1038	626	549	984	952
	24 hour	208	590	152	202	438	357

* Extra 0-hour time point on Day 1 was drawn only for females. †EOI=end of 2-hour infusion.

[155] The time course of complement activation that was evident from the CH50 values was also reflected by plasma C3a concentration. C3a is a split product formed at an early step in the common complement pathway cascade. At the end of the first infusion (Day 1), mean C3a concentrations were increased in the vehicle control and, dose dependently, in the PNT2258 groups (as well as in the PNT2258cy-treated group), with little of no effect in low-dose (1 mg/kg) females. Mean C3a had largely recovered by 24 hours after the first dose, except for a small persistent increase in the vehicle control and high-dose (PNT2258 and PNT2258cy) groups, as was seen with CH50. Mean C3a concentration was elevated in the latter groups, and, marginally, in the 4 mg/kg/day group prior to dosing on Day 5, with more pronounced increases occurring at the end of the Day 5 infusion (including an effect at the 1 mg/kg dose level). However, the relative difference in mean C3a between the predose and end-of-infusion values on Day 5 was generally not as large as on Day 1. On Day 24 (last

dosing day), a similar pattern was observed as on Day 5, with dose-dependent elevations in C3a prior to dosing, more pronounced increases in C3a at the end of infusion, and at least partial recovery by 24 hours after the last dose. Overall, some of the largest increases in C3a occurred in the vehicle-treated animals, indicating the absence of a role for the active ingredient, PNT100, and the increases observed in PNT2258-treated groups were generally dose-dependent. The C3a increases were similar for the 16 mg/kg/day PNT2258 and PNT2258cy groups, as would be expected from the absence of any influence of the oligonucleotide component.

[156] The mean values for plasma C5a concentration are shown in Table 7.

Table 7

Serum C5a							
		Group #					
		Females					
Day	Timepoint	1	2	3	4	5	6
1	0 hour	7.9	11.7	13.8	14.6	12.1	14.8
	0 hour*	12.2	11.4	12.5	14.4	13.3	19.9
	EOI†	9.3	7.2	7.9	8.9	10.0	10.3
	24 hour	6.8	10.6	6.3	10.4	8.1	8.5
5	0 hour	7.4	12.5	7.9	12.5	12.3	19.8
	EOI	7.3	11.5	10.1	10.2	13.1	18.3
24	0 hour	9.8	11.1	13.4	10.1	10.8	11.1
	EOI	8.5	13.8	13.7	9.5	11.4	13.6
	24 hour	8.5	10.3	8.7	7.9	9.5	13.8
		Males					
1	0 hour	9.5	11.3	10.5	10.3	9.2	8.0
	EOI	8.2	8.5	8.2	21.9	12.2	9.2
	24 hour	8.0	12.6	8.1	9.2	18.7	8.7
5	0 hour	8.0	16.6	7.7	10.4	11.4	11.7
	EOI	8.6	12.6	7.8	9.2	16.2	17.7
24	0 hour	8.0	10.9	9.1	7.0	8.2	9.5
	EOI	8.6	10.1	15.6	8.0	13.8	11.1
	24 hour	8.3	11.6	8.0	8.1	8.6	12.7
* Extra 0-hour time point on Day 1 was drawn only for females. †EOI=end of 2-hour infusion.							

[157] C5a is a common pathway split product that is formed downstream from C3a. It is also known to be much more highly potent than C3a, such that accumulation of C5a is generally viewed as indicative of the biological significance of a complement activation event. It is more labile than C3a, as would be expected based on its potency in mediating the downstream sequelae of complement activation, such that it does not accumulate unless there is a burst of complement activation. Hence, a modest level of activation of the complement

system can occur that results in consumption of complement factors (reflected by CH50) and accumulation of some split products (i.e., those with less instability and less biological activity, like C3a), but is not associated with downstream sequelae because of the absence of significant accumulation of C5a. This appears to be applicable to PNT2258 (i.e., the vehicle-mediated effects on the complement system), as there were only small increases in C5a in a few animals. In fact, very few of the C5a values in PNT2258-treated, PNT2258cy-treated or vehicle-treated animals exceeded the range of values observed in the negative control group or the range of values for the predose time point on Day 1. Where sporadic small increases occurred, the incidence was not dose-dependent, and it was not possible to discern a clear difference in the incidence of marginally higher C5a values between the test article, vehicle control and negative control groups, particularly when viewed against the Day 1 predose values. Overall, no clear increase in plasma C5a concentration was evident, which suggests that the complement activation induced by the vehicle components would probably not be associated with downstream adverse sequelae mediated by C5a. Thus, it appears that the complement activation induced by the liposomal lipids develops slowly during infusion (and apparently also following infusion), leading to consumption of complement factors (decreased CH50) and accumulation of C3a but without any accumulation of the much more potent C5a split product.

[158] The above results show a diffuse activation of the complement system by PNT2258 or the vehicle alone, that is not associated with accumulation of the primary biologically active split product (C5a). Thus, a more acute result, CARPA, is not induced at the dosages used in these studies.

[159] Liposomes sequestering oligonucleotides comprising SEQ ID NO:15 have been shown to reduce tumor growth in xenograft mouse models (See e.g., PCT International Publication No. WO 05/118824, PCT applications PCT/US06/45955, PCT/US06/46928, PCT/US06/46111, and PCT/US06/45946, as well as U.S. Publication Nos 2006/0198828, 2005/0287667, 2006/0073596, 2006/0135455, and 2006/0229267; and U.S. Application Nos. 10/858,094 and 11/233,312, all of which are incorporated herein by reference in their entirety.) The above studies suggest a possible role for complement activation by SEQ ID NO:15 in liposomes as an additional mechanism of action in reducing tumor growth, perhaps by an immunostimulatory mechanism.

[160] The vehicle contains 47mol % MoChol, which hydrolyzes to cholesterol and 23 mol% CHEMS, which contains cholesterol. Most other liposomes have about 23 mol% cholesterol.

The high concentration of cholesterol in the liposomal vehicle may be responsible, at least in part, for complement activation.

EXAMPLE 2: Effect of Liposomal SEQ ID NO:1 on the Growth of WSU-DLCL2 Xenografts

[161] The effect of liposomal SEQ ID NO:1 on the Growth of WSU-$DLCL_2$ xenografts was measured in CB-17 SCID mice. Xenografts were generated in C.B-17 SCID mice by sub-cutaneous injection of 2 X 10^6 WSU-$DLCL_2$ cells. PNT100 (SEQ ID NO:15) and PNT-100R (SEQ ID NO:17) were formulated with NeoPhectin-AT, comprising cationic lipids) as follows. A 25 ml liposome delivery vehicle (LDV) consisting of NeoPhectin-AT (NeoPharm, IL) bottle was placed at room temperature for 15 min. The bottle was gently swirled for 30 seconds to mix. 1000 µl LDV was transferred to 50 ml sterile polypropylene tubes labeled: Day # PNT100. The PNT100 stock tube was vortexed and quickly centrifuged. 75 µl PNT100 (Stock) was transferred to the Day # PNT100 tube and the mixture was vortexed vigorously for 2 minutes. 5000 µl dH_2O was mixed with 5000 µl 20% sucrose in a sterile 50 ml tube. 2150 µl of the diluted sucrose was added to the PNT100/Neophectin-AT solution and mixed. An appropriate drug injection volume was transferred to a 1.5 ml polypropylene tube. The LDV control was generated by mixing 75 µl RNAse/DNAse free water instead of PNT100 with 1000 µl LDV, 2150 10% sucrose was added and the mixture was injected.

[162] The mice were treated as described in Table 8.

Table 8

Group	Description	Dose mg/kg	Schedule	Route	n
1	Sucrose Control	0.00	qd x 5	I.V. bolus	6
2	Neophectin-AT only	0.33	qd x 5	I.V. bolus	6
3	PNT100/Neophectin-AT	0.50	qd x 5	I.V. bolus	6
4	PNT100R/Neophectin-AT	0.50	qd x 5	I.V. bolus	6

qd=once daily, sc=subcutaneous, I.V. =intravenous

[163] Each animal was checked three times weekly for tumor growth by caliper measurements and tumor volume was calculated by the formula ($Width^2$x Length) x ½. Results in Figure 1 show an anti-tumor effect of Neophectin-AT alone as well as an anti-tumor effect from PNT100 formulated with NeoPhectin AT. The results suggest that the anti-tumor effect may be due, at least in part to complement activation by NeoPhectin AT and/or complement activation by SEQ ID NO:15 sequestered in NeoPhectin AT liposomes.

EXAMPLE 3: Effect of Liposomal SEQ ID NO:1 on the Growth of PC-3 Xenografts

[164] A similar effect was seen with NeoPhectin-AT (Neopharm) on tumor growth in PC-3 Xenografts, a prostate carcinoma subcutaneous model. (See e.g., Yang et al., Cancer Research 59, 781–786, [1999]; Glinskii et al., Cancer Research 63, 4239–4243, [2003]; and Kalikin et al., Cancer Biology and Therapy 2:6, 17-21 [2003].)

[165] PC-3 cells were first transduced with the green fluorescent protein (GFP) gene. A GFP expression vector, pLEIN, was purchased from Clontech (Palo Alto, CA). The vector expresses enhanced GFP and the neomycin resistance gene on the same bicistronic message that contains an internal ribosome entry site. To produce packaged GFP viral particles, PT67, an NIH3T3 derived packaging cell line, expressing the 10 AI viral envelopes (Clontech) was used. PT67 cells were cultured in DMEM supplemented with 10% fetal bovine serum. PT67 cells, at 70% confluence, were incubated with a precipitated mixture of N- [1-(2 ,3-dioleoyloxyl) propyl]-N, N,-trimethylammoniummethyl sulfate reagent and saturating amounts of pLEIN plasmid for 18 h. For selection, the cells were cultured in the presence of 200-1000 μg/ml G418 for 7 days. For GFP gene transduction, 20% confluent PC-3 cells (ATCC, CRL 1435) were incubated with a 1:1 precipitated mixture of retroviral supernatants of PT67 cells and Ham's F-12 K containing 7% fetal bovine serum for 72 h. Fresh medium was replenished at this time. PC-3 cells were harvested 72 h posttransduction and subcultured at a ratio of 1:15 into selective medium that contained G-418. The brightest PC-3 cell clones expressing GFP were selected, combined, and then amplified and transferred by conventional culture methods.

[166] Tumor stocks were prepared by subcutaneously injecting PC-3-GFP cells at a concentration of 5 x 10^6 cells /200 μL into the flank of nude mice (male athymic NCr nude mice between 5 and 6 weeks of age (Taconic Quality Laboratory Animals and Services for Research (Germantown, NY)). Strong GFP expression of tumors grown in the subcutis of mice was certified before harvest. The tumor tissues harvested from subcutaneous growth in nude mice were inspected and any grossly necrotic or suspected necrotic or non GFP tumor tissues were removed. Tumor tissues were subsequently cut into small fragments of approximately 2 mm^3. A tumor stock of the prostate cancer PC-3 GFP was established by subcutaneously injecting PC-3 GFP cells to the flank of nude mice. The tumor was maintained in nude mice subcutaneously as tumor stock prior to use. Before implantation, strong GFP expression of the PC-3 GFP tumor tissue was confirmed by fluorescent light. On the day of implantation, the tumor was harvested from the subcutaneous site and placed in RPMI-1640 medium. Necrotic tissues were removed and viable tissues were cut into 2 mm^3

pieces. The tissue fragments were then implanted subcutaneously to right flank of the nude nice. Tumor size was measured by caliper monitoring. Approximate tumor volume was calculated by the formula (Width x Length) x 1/2.

[167] PNT100 (SEQ ID NO:15) and PNT-1 (SEQ ID NO:18) were formulated with NeoPhectin-AT as follows. A 25 mL liposome delivery vehicle (LDV) consisting of NeoPhectin-AT (NeoPharm, IL) bottle was placed at room temperature for 15 min. The bottle was gently swirled for 30 seconds to mix. 1000 µL LDV was transferred to 50 mL sterile polypropylene tubes labeled: Day # PNT100 (SEQ ID NO:15). The PNT100 (SEQ ID NO:15) stock tube was vortexed and quickly centrifuged. 75 µL PNT100 [SEQ ID NO:15] (Stock) was transferred to the Day # PNT100 (SEQ ID NO:15) tube and the mixture was vortexed vigorously for 2 minutes. 5000 µL dH_2O was mixed with 5000 µL 20% sucrose in a sterile 50 mL tube. 2150 µL of the diluted sucrose was added to the PNT100/Neophectin-AT solution and mixed. An appropriate drug injection volume was transferred to a 1.5 ml polypropylene tube. The LDV control was generated by mixing 75 µL RNAse/DNAse free water instead of PNT100 with 1000 µL LDV, 2150 10% sucrose was added and the mixture was injected.

[168] Mice bearing 50-100 mm^3 estimated tumor volume were injected subcutaneously into the tumor with NeoPhectin-AT-PNT-100 (SEQ ID NO:15) or PNT-1 (SEQ ID NO:18) at a dose of 2.5-5.0 mg/kg daily for five days. A second group of mice received 5-10 mg/kg of Taxotere™ intravenously on days 2 and 5. A third group of mice received 5 mg/kg of NeoPhectin-AT-PNT-100 (SEQ ID NO:15) injected subcutaneously into the tumor daily for five days and 5-10 mg/kg of Taxotere™ injected intravenously on days 2 and 5.

[169] The study design is shown in Table 9.

Table 9

Sub-group ID	Description	Dose (mg/kg)	Schedule	Route	N
A	PBS Control	200 µl	qd X 5	s.c	10
B	PNT-C (5'-NNNNNNNNNNNNNNNNNNNNNNN-3' [SEQ ID NO:18]+ LDV	5	qd X 5	s.c.	10
C	PNT-100 [SEQ ID NO:15] + LDV	2.5	qd X 5	s.c.	10
D	PNT-100X (CAXGCAXGXGCATCCCXGCCXGTG, X = Methyl C; [SEQ ID NO:16]) + LDV	5	qd X 5	s.c.	10

E	TAXOTERE™	10 and 5	Day 2 and 5	i.v.	10
F	TAXOTERE™ + PNT-100 [SEQ ID NO:15]/LDV	10 and 5 + 5	Day 2 and 5 + qd X 5	i.v. + s.c.	10

[170] Tumor growth was monitored for 40 days. Twelve days after implantation, whole body optical imaging of GFP-expressing tumors was performed once per week using a fluorescence microscope. The final tumor weights were taken after animals were sacrificed at the forty-sixth day of the study.

[171] Results in Figure 2 show mean tumor volume of tumors in the PC-3 GFP prostate carcinoma subcutaneous model following treatment with PNT-100 and/or TAXOTERE™. The results indicate that NeoPhectin-AT containing a scrambled oligomer slows tumor growth, although not as much as NeoPhectin-AT containing PNT-100.

[172] The above examples show the effect of a cationic liposomal formulation on tumor growth. The effect of an amphoteric liposomal formulation comprising POPC, DOPE, MoChol and CHEMS on tumor growth was also examined.

EXAMPLE 4: Effect of an Alternate Liposomal SEQ ID NO:15 Formulation on the Growth of WSU-DLCL2 Xenografts

[173] SEQ ID NO:15 formulated in liposomes comprising POPC/DOPE/MoChol/CHEMS in the ratios 6/24/47/23 was tested in WSU-DLCL2 xenograft bearing mice. The concentration of SEQ ID NO:15 oligonucleotide was 4 mg/ml (PNT2254) and 2 mg/ml (PNT2253). C.B.-17 SCID mice between 6-8 weeks old were supplied by Taconic (Hudson, NY). When the tumors reached approximately 100 mm^3 volume, treatment with PNT2253 or PNT2254 was initiated. The mice received 0, 0.3, 3, 10, or 20 mg/kg of PNT2254 daily for five days, 30 mg/kg of PNT2254 daily for 2 days, 60 mg/kg of PNT2254 once, 0.3, 3, or 10 mg/kg of PNT2253 daily for 5 days, 20 mg/kg of PNT2253 daily for 2 days, or 30 mg/kg of PNT2253 once via an iv bolus injection. (n=7 (PNT2254) or 8 (PNT2253). The animals were checked at least three times weekly for tumor growth by caliper measurements, and the animals were weighed at least three times weekly. Tumor volumes of all treatment groups were analyzed using GraphPad™ statistical software.

[174] A maximum tolerated dose of 20 mg/kg/day of PNT2254 and 10 mg/kg/day of PNT2253 was established. (See Figure 3.) Toxicity was achieved at 30 mg/kg/day for PNT2254 and at 20 mg/kg/day for PNT2253, and dosing was stopped after two days due to

animal efficacy. A steep dose response was seen with strong anti-tumor efficacy for an extended time period after one dosing cycle.

[175] A mathematical measure of each dose was calculated that determined the drug response in delaying tumor growth rate to 750 mg size in PNT2254 and PNT2253 drugged vs. control non-drugged tumors (**Tables 10 and 11**).

Table 10. Antitumor Activity of PNT2254 in WSU-DLCL$_2$-Bearing SCID Mice

Agent	No. of Animals	T/C(%)	T-C	Log_{10} kill gross
PBS control daily for 5 days	7	100	0.0	0.0
0.3 mg/kg PNT2254 daily for 5 days	7	100	0.0	0.0
3 mg/kg PNT2254 daily for 5 days	7	75	3	0.45
10 mg/kg PNT2254 daily for 5 days	7	34	10	1.5
20 mg/kg PNT2254 daily for 5 days	7	32	10	1.5
30 mg/kg PNT2254 daily for 2 days	5 (5/7 mice survived)	27	11	1.65

Table 11. Antitumor Activity of PNT2253 in WSU-DLCL$_2$-Bearing SCID Mice

Agent	No. of Animals	T/C(%)	T-C	Log_{10} kill gross
PBS control daily for 5 days	8	100	0.0	0.0
0.3 mg/kg PNT2253 daily for 5 days	8	92	0.0	0.0
3 mg/kg PNT2253 daily for 5 days	8	90	2	0.3
10 mg/kg PNT2253 daily for 5 days	8	38	9	1.4
20 mg/kg PNT2253 daily for 2 days	6 (6/8 mice survived	28	12	1.8
30 mg/kg PNT2253 daily for 1 day	8 (8/8 dead)	--	--	--

[176] T and C are the median times in days for the treatment group (T) and the control group (C) tumors to reach a predetermined weight (750 mg). T-C is a measure of tumor growth delay and is the difference in the median days to 750 mg between the treated (T) and the control (C) group. Log_{10} kill Gross = T-C value in days/3.32 X T_d. T_d is the mean tumor doubling time (days) estimated from a log-linear growth plot of the control tumors growing in exponential phase. The higher the Log_{10} kill Gross value, the more efficacious the drug, and a value over 2.8 is considered highly efficacious (Corbett, T.H. et al., "Transplantable Syngeneic Rodent Tumors", Tumor Models in Cancer Research. Ed. Teicher B.A. Totowa, NJ: Humana Press Inc., 2002. 41-71). Volume and weight were calculated according to the formula described by Cammisuli, S., et al., Int. J. Cancer, 65, 351-9, 1996.

[177] PNT2253 treatment resulted in increased toxicity compared to PNT2254. The most efficacious dose was 10 mg/kg/day for both PNT2253 and PNT2254, and the maximum tolerated dose is 20 mg/kg/day for PNT2254 and 10 mg/kg/day of PNT2253.

[178] The lipid load of the PNT2254 formulation was lower than the lipid load of PNT2253, and there is a difference in efficacy and toxicity between the two formulations. The lipid dose ranges for PNT2253 and PNT2254 are 9.6-640 mg/kg and 4.8-480 mg/kg, respectively.

[179] A similar study was performed with formulations PNT2255 and PNT2256, which are similar to PNT2253 and PNT2254, respectively, except that they contain sucrose. Results are shown in Table 12 below.

TABLE 12

	TTE	TGD	TFS	CR	PR
PBS	17.7				
PNT2255 qd X 5	40.8	23.1	2/8	4/8	5/8
PBS	10.1				
PNT2256 qd X 5	47.7	37.6	1/7	4/7	5/7

[180] TTE = $[\log_{10}(750) - b]/m$; TTE = time to tumor endpoint, b = y intercept; m = slope of the line calculated from a linear regression of log-transformed tumor growth data for each tumor calculated from a minimum of three time points preceding 750 mm^3 and a minimum of one that surpassed the time point for 750 mm^3.

[181] TGD = T – C; TGD = tumor growth delay; T = median TTE for drug treated tumors; C = median for control tumors.

[182] As with PNT2253 and PNT 2254, the formulation with a higher oligonucleotide to lipid ratio had a higher efficacy against tumor growth as measured by tumor growth delay.

EXAMPLE 5: Effect of Liposomal SEQ ID NO:1 on the Growth of Daudi Xenografts

[183] Complement activation by the oligonucleotide-liposomal formulations may also be a mechanism involved in synergy between rituximab and a PNT-100 liposomal formulation in Daudi Xenografts. Daudi cells are a model of Burkett's lymphoma. Xenografts with Daudi cells were generated in mice as described in the previous examples. Liposomal PNT-100 was formulated as in the previous example, has similar properties and a concentration of 2.4 mg PNT-100 per ml. The mice were divided into 10 groups and treated as in Table 13.

Table 13

Group ID	Description	Dose (mg/kg)	Schedule	Route	N
1	PBS Control	200 µl	qd X 5	i.v.	10
2	Rituximab	20 mg/kg	Schedule 2	i.v.	10
3	Liposomal PNT-100 [SEQ ID NO:15]	30 mg/kg	Schedule 1	i.v.	10
4	Liposomal PNT-100 [SEQ ID NO:15]	20 mg/kg	Schedule 1	i.v.	10
5	Liposomal PNT-100 [SEQ ID NO:15]	13.3 mg/kg	Schedule 1	i.v.	10
6	Liposomal PNT-100 [SEQ ID NO:15]	8.89 mg/kg	Schedule 1	i.v.	10
7	Liposomal PNT-100 [SEQ ID NO:15]	5.92 mg/kg	Schedule 1	i.v.	10
8	Rituximab + Liposomal PNT-100 [SEQ ID NO:15]	20 mg/kg RTX, 20 mg/kg PNT-100	Schedule 1-rituximab, Schedule 2-PNT-100	i.v.	10
9	Rituximab + Liposomal PNT-100	20 mg/kg RTX, 13.3 mg/kg PNT-100	Schedule 1-rituximab, Schedule 2-PNT-100	i.v.	10

Schedule 1 is 5 daily doses, 2 days off and then 5 daily doses, 2 days off, then 3 daily doses.
Schedule 2 is i.v. delivery of rituximab biweekly for 2.5 weeks for a total of 5 injections.

[184] Tumor volume was caliper measured. Studies were concluded when control animal xenografts reached 2000 mm^3. Results are shown in Figure 4, A-C which show mean tumor volume up to 50 days (A), a Kaplan-Meyer plot (B), showing the percent of mice whose tumors have not yet reached 2000 mm^3 each day and a figure showing the change in body weight of the mice in each group (C). The results show little effect with either rituximab or PNT-100 alone, but a dramatic effect, when PNT-100 and rituximab are given together. Indeed, in Daudi xenografts, the tumors shrink and disappear when the mice bearing them are treated with PNT-100 and rituximab.

[185] The synergy observed with rituximab and PNT2255 could involve complement activation mechanisms, for example, C' enhanced ADCC and apoptosis induction.

[186] PNT225X has also been shown to reduce tumor growth in nude mice bearing human A375 melanoma.

[187] Taken together, the above results suggest that complement activation by the oligonucleotide-liposomal formulations could represent an additional mechanism of action of this drug product, particularly the PNT-100-liposomal formulations.

OTHER EMBODIMENTS

[188] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages and modifications are within the scope of the following claims.

[189] All references cited herein, are incorporated herein by reference in their entirety.

What is claimed is

1. A composition comprising an oligonucleotide and an agent that activates complement.

2. The composition of claim 1, wherein the oligonucleotide is selected from an oligonucleitode that hybridizes with SEQ ID NOs:1-15. 43, 71-74 and the complements of SEQ ID NOs:1-15. 43, 71-74.

3. The composition of claim 2, wherein the oligonucleotide ranges from 15 to 50 nucleotides in length.

4. The composition of claim 2, wherein the oligonucleotide hybridizes with SEQ ID NO:1 or the complement of SEQ ID NO:1.

5. The composition of claim 1, wherein the oligonucleotide is selected from SEQ ID NOs:15, 16, 17, 19, 20, 23, 24, 25, 26, 27, 30, 34, 42 and the complements of SEQ ID NOs:15, 16, 17, 19, 20, 23, 24, 25, 26, 27, 30, 34, 42.

6. The composition of claim 1, wherein the oligonucleotide is SEQ ID NO:15.

7. The composition of claim 1, wherein the oligonucleotide is SEQ ID NO:16.

8. The composition of claim 2, wherein the oligonucleotide hybridizes with SEQ ID NO:43 or the complement of SEQ ID NO:43.

9. The composition of claim 1, wherein the oligonucleotide is selected from SEQ ID NOs: 44-70 and the complements of SEQ ID NOs 44-70.

10. The composition of claim 1, wherein the oligonucleotide is selected from SEQ ID NOs: 44-49 and the complements of SEQ ID NOs: 44-49.

11. The composition of claim 1, wherein the oligonucleotide is SEQ ID NO:46.

12. The composition of claim 1, wherein the oligonucleotide is SEQ ID NO:48.

13. The composition of claim 1, wherein the agent comprises lipids.

14. The composition of claim 13, wherein the lipids comprise cardiolipins.

15. The composition of claim 13, wherein the lipids comprise CHEMS, POPC, MoChol and DOPE.

16. The composition of claim 15, wherein CHEMS, POPC, MoChol and DOPE are in the ratio of POPC/DOPE/MoChol/CHEMS of 6/24/47/23 mole%.

17. A method for treating a proliferative disorder comprising administering to a subject or patient an effective dose of the composition of any of claims 1-16.

18. The method of claim 17, wherein the proliferative disorder is a neoplasm.

19. The method of claim 18, wherein the neoplasm is a carcinoma.

20. The method of claim 18, wherein the neoplasm is a sarcoma.

21. The method of claim 18, wherein the neoplasm is a lymphoma.

22. The method of claim 17, wherein the proliferative disorder is prostate cancer.

23. The method of claim 17, wherein the proliferative disorder is non-Hodgkins lymphoma.

24. The method of claim 17, wherein the proliferative disorder is melanoma.

25. The method of claim 17, wherein the proliferative disorder is breast cancer.

26. A method for treating prostate cancer comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide of SEQ ID NO:15 or 16 and a lipid comprising a cardiolipin.

27. A method for treating prostate cancer comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide of SEQ ID NO:15 or 16 and a lipid mixture comprising POPC, DOPE, CHEMS and MoChol.

28. The method of claim 27, wherein CHEMS, POPC, MoChol and DOPE are in the ratio of POPC/DOPE/MoChol/CHEMS of 6/24/47/23 mole %.

29. A method for treating lymphoma comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide of SEQ ID NO:15 or 16 and a lipid comprising a cardiolipin.

30. A method for treating lymphoma comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide of SEQ ID NO:15 or 16 and a lipid mixture comprising POPC, DOPE, CHEMS and MoChol.

31. The method of claim 30, wherein CHEMS, POPC, MoChol and DOPE are in the ratio of POPC/DOPE/MoChol/CHEMS of 6/24/47/23 mole %.

33. A method for treating melanoma, comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide of SEQ ID NO:15 or 16 and a lipid comprising a cardiolipin.

34. A method for treating melanoma, comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide of SEQ ID NO:15 or 16 and a lipid mixture comprising POPC, DOPE, CHEMS and MoChol.

35. The method of claim 34, wherein CHEMS, POPC, MoChol and DOPE are in the ratio of POPC/DOPE/MoChol/CHEMS 6/24/47/23 mole %.

36. A method for treating breast cancer, comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide selected from SEQ ID NOs:44-49 and a lipid comprising a cardiolipin.

37. A method for treating breast cancer comprising administering to a subject or patient an effective dose of a composition comprising an oligonucleotide selected from SEQ ID NOs:44-49 and a lipid mixture comprising POPC, DOPE, CHEMS and MoChol.

38. The method of claim 30, wherein CHEMS, POPC, MoChol and DOPE are in the ratio of POPC/DOPE/MoChol/CHEMS of 6/24/47/23 mole %.

39. The method of any of claims 17-38, further comprising administering a chemotherapeutic agent.

40. The method of claim 39, wherein the chemotherapeutic agent is selected from rituximab, docetaxel, vincristine or R-CHOP.

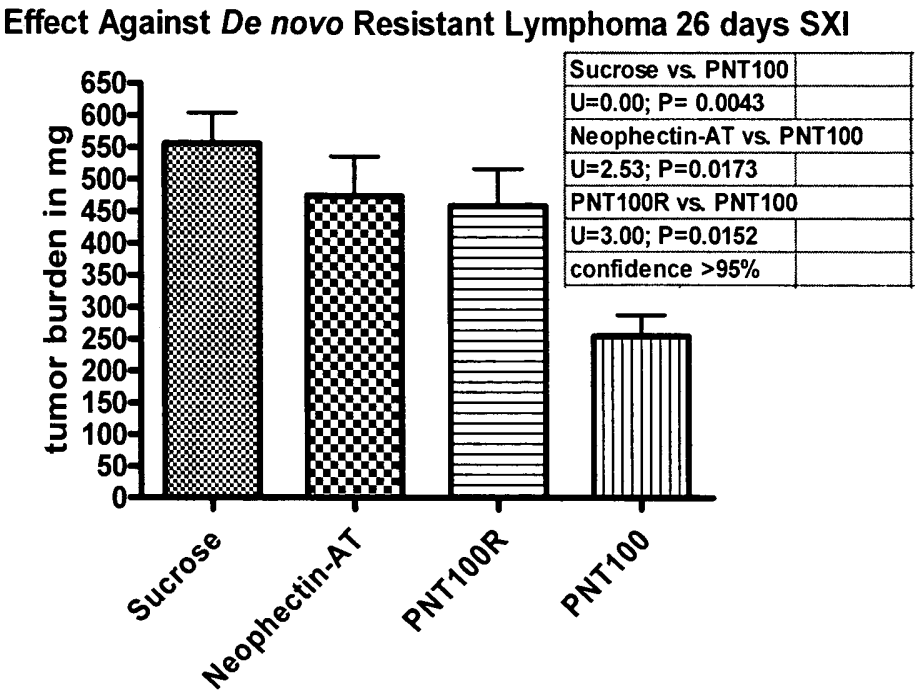

Effect Against De novo Resistant Lymphoma 26 days SXI
tumor burden in mg
650
600
550
500
450
400
350
300
250
200
150
100
50
0
Sucrose
Neophectin-AT
PNT100R
PNT100
Sucrose vs. PNT100
U=0.00; P= 0.0043
Neophectin-AT vs. PNT100
U=2.53; P=0.0173
PNT100R vs. PNT100
U=3.00; P=0.0152
confidence >95%

FIGURE 1

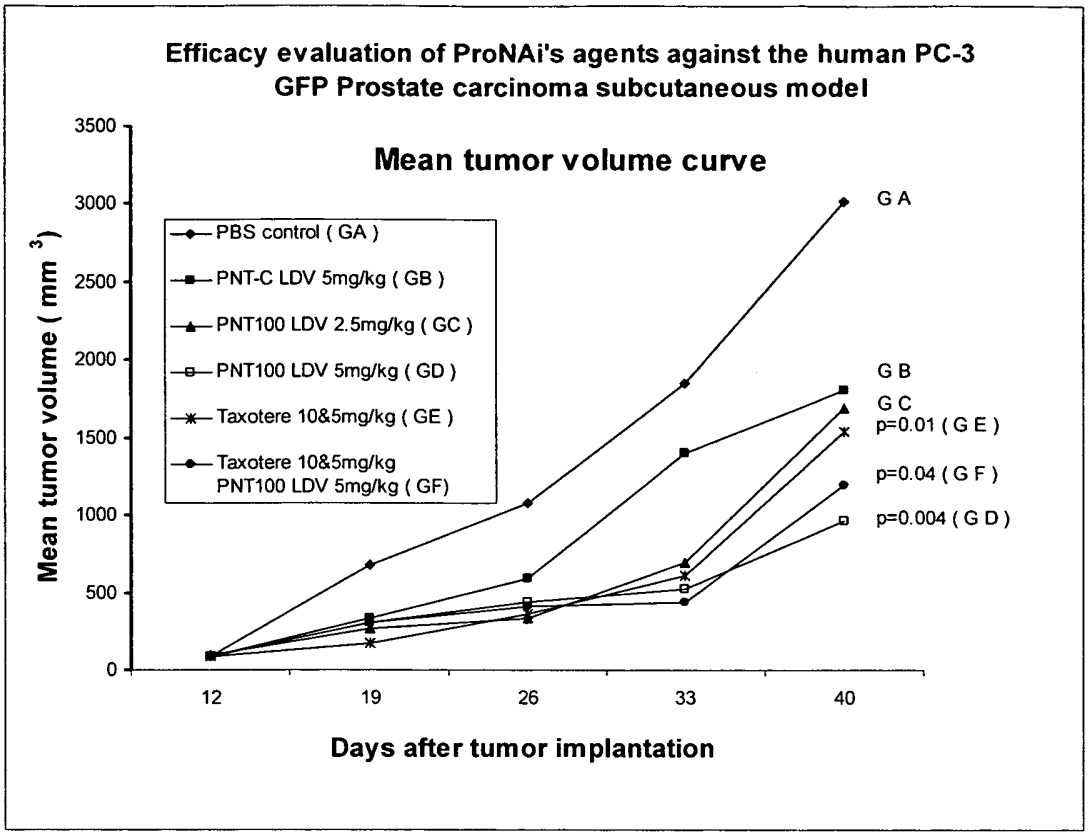

Efficacy evaluation of ProNAi's agents against the human PC-3 GFP Prostate carcinoma subcutaneous model
Mean tumor volume curve
Mean tumor volume (mm 3)
3500
3000
2500
2000
1500
1000
500
0
12
19
26
33
40
Days after tumor implantation
PBS control (GA)
PNT-C LDV 5mg/kg (GB)
PNT100 LDV 2.5mg/kg (GC)
PNT100 LDV 5mg/kg (GD)
Taxotere 10&5mg/kg (GE)
Taxotere 10&5mg/kg PNT100 LDV 5mg/kg (GF)
G A
G B
G C
p=0.01 (G E)
p=0.04 (G F)
p=0.004 (G D)

FIGURE 2

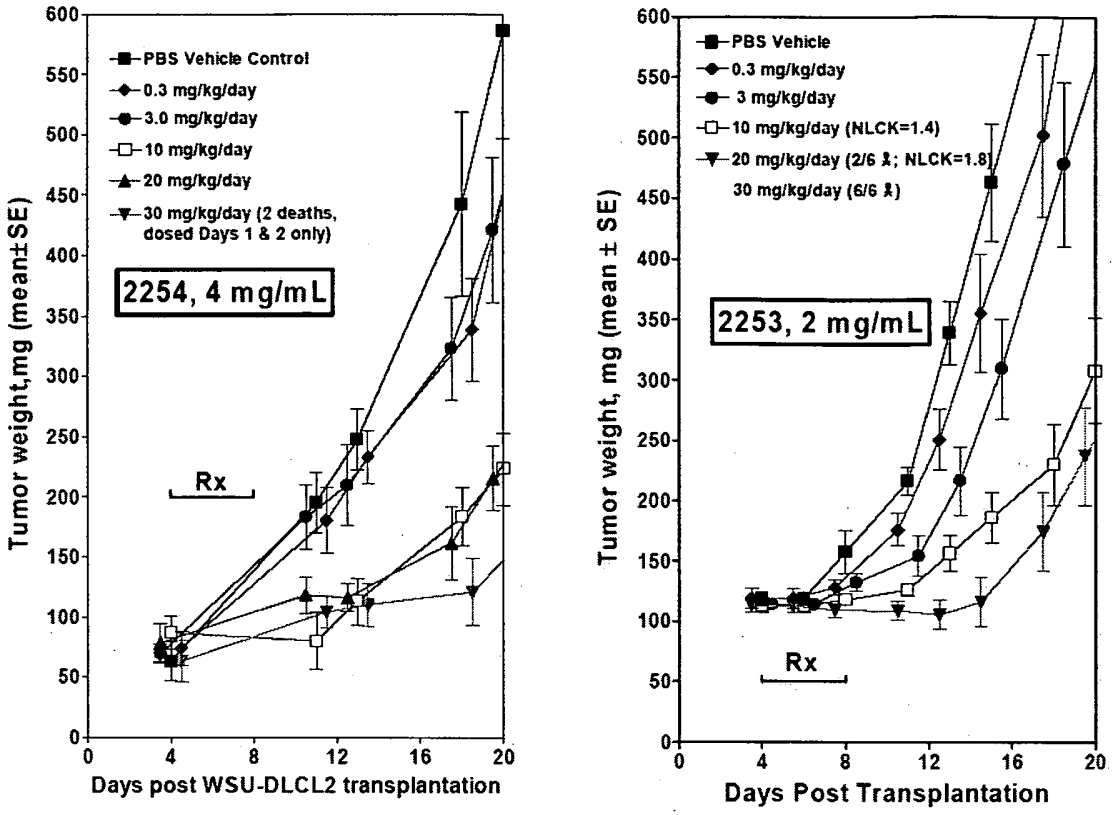

PBS Vehicle Control
0.3 mg/kg/day
3.0 mg/kg/day
10 mg/kg/day
20 mg/kg/day
30 mg/kg/day (2 deaths, dosed Days 1 & 2 only)
2254, 4 mg/mL
Rx
Tumor weight,mg (mean±SE)
Days post WSU-DLCL2 transplantation
PBS Vehicle
0.3 mg/kg/day
3 mg/kg/day
10 mg/kg/day (NLCK=1.4)
20 mg/kg/day (2/6 ♗; NLCK=1.8)
30 mg/kg/day (6/6 ♗)
2253, 2 mg/mL
Rx
Tumor weight, mg (mean ± SE)
Days Post Transplantation

FIGURE 3

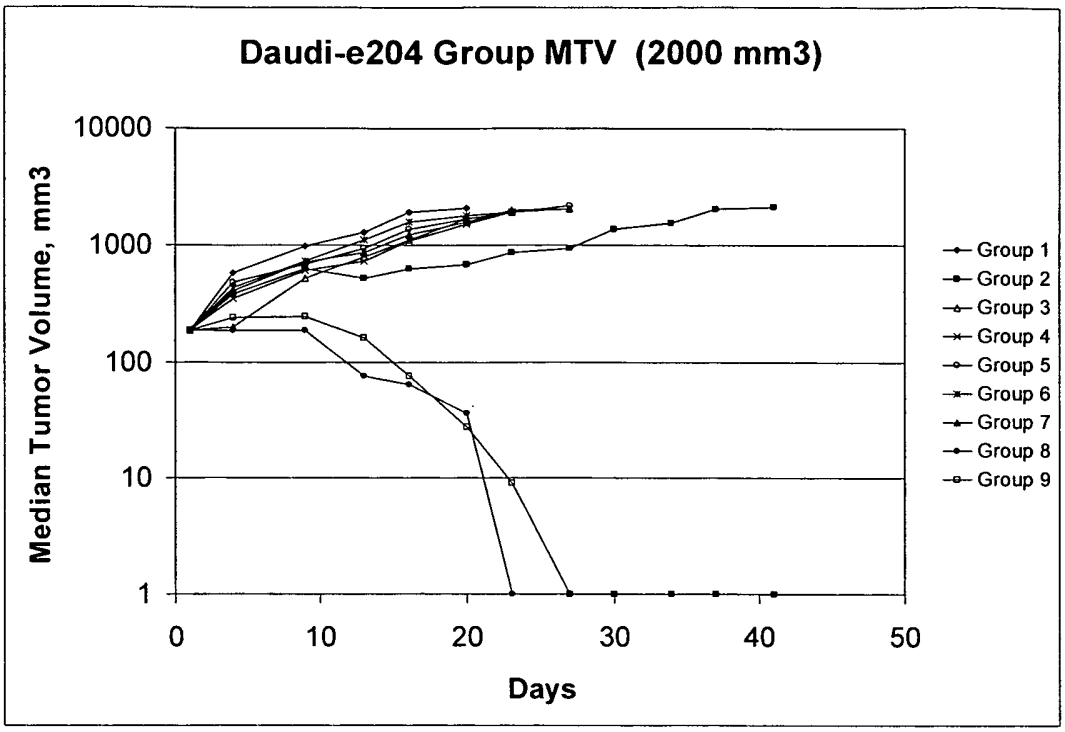

Daudi-e204 Group MTV (2000 mm3)
Median Tumor Volume, mm3
10000
1000
100
10
1
0
10
20
30
40
50
Days
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
Group 9

FIGURE 4A

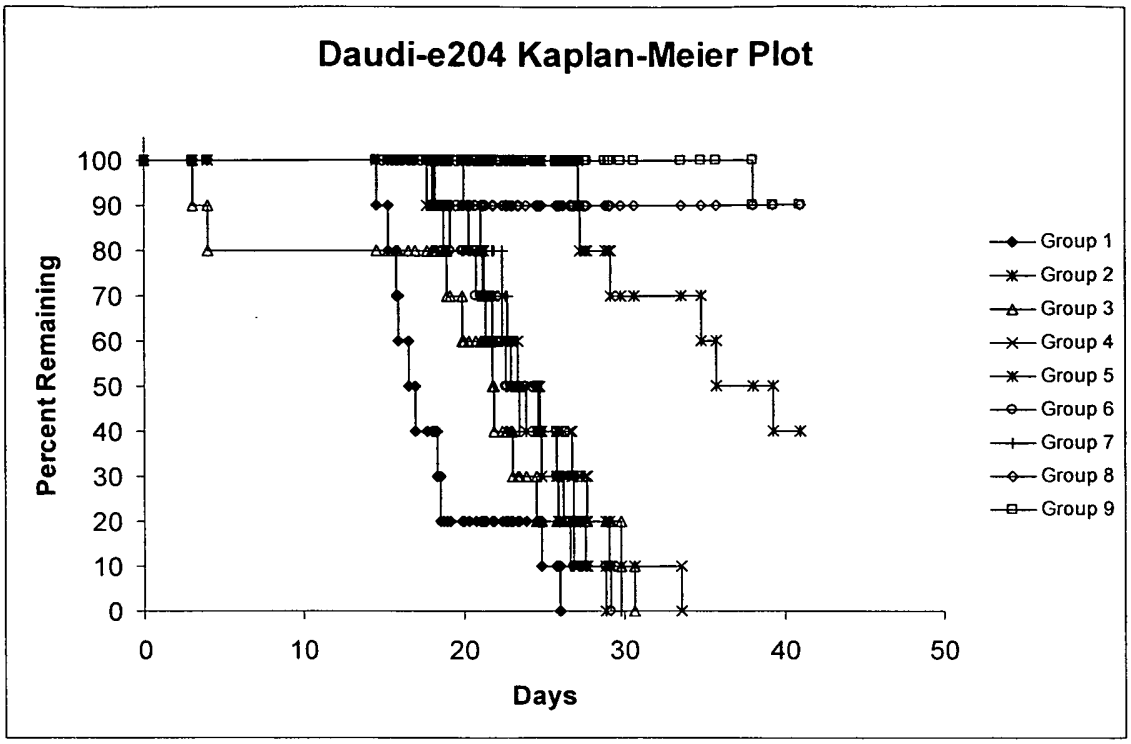

Daudi-e204 Kaplan-Meier Plot
Percent Remaining
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Days
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
Group 9

FIGURE 4B

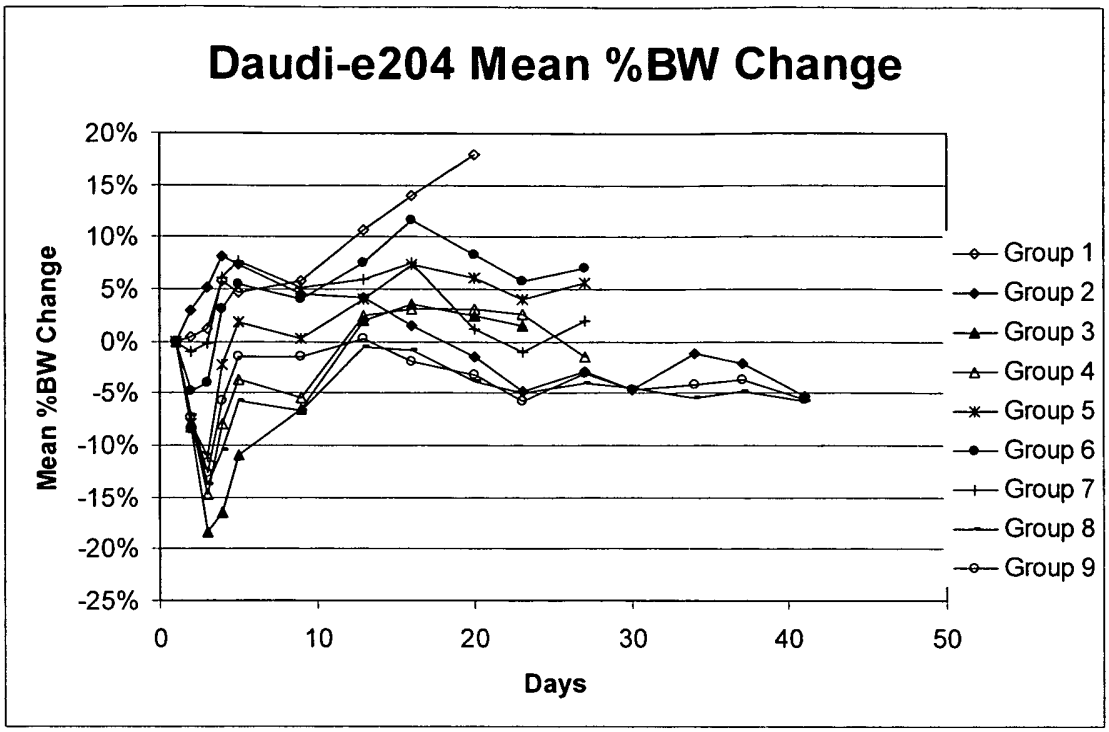

Daudi-e204 Mean %BW Change
Mean %BW Change
20%
15%
10%
5%
0%
-5%
-10%
-15%
-20%
-25%
0
10
20
30
40
50
Days
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
Group 9

FIGURE 4C