US 20080160019A1

(19) **United States**

(12) **Patent Application Publication**

Wood et al.

(10) **Pub. No.: US 2008/0160019 A1**

(43) **Pub. Date: Jul. 3, 2008**

(54) **SEQUENTIAL COMBINATION THERAPY**

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(21) Appl. No.: **11/873,856**

(22) Filed: **Oct. 17, 2007**

Related U.S. Application Data

(60) Provisional application No. 60/852,263, filed on Oct. 17, 2006, provisional application No. 60/875,736, filed on Dec. 19, 2006.

Publication Classification

(51) **Int. Cl.**

A61K 39/395	(2006.01)
A61K 31/44	(2006.01)
A61P 35/00	(2006.01)

(52) **U.S. Cl.** **424/133.1**; 424/142.1; 514/350; 424/138.1

(57) **ABSTRACT**

Disclosed are new methods for treatment of angiogenesis-related disorders. Angiogenesis-related disorders are treated by administration of a Tie1 ectodomain-binding agent and a VEGF antagonist agent.

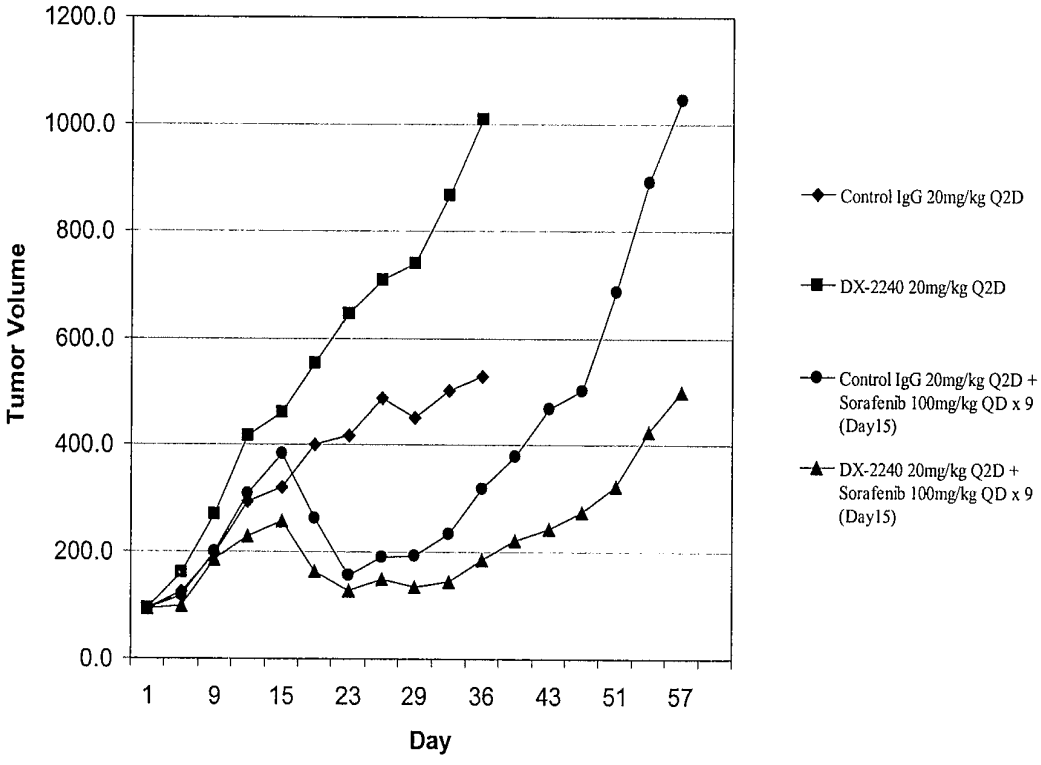

FIGURE 1

```
1    MVWRVPPFLL PILFLASHVG AAVDLTLLAN LRLTDPQRFF LTCVSGEAGA GRGSDAWGPP
61   LLLEKDDRIV RTPPGPPLRL ARNGSHQVTL RGFSKPSDLV GVFSCVGGAG ARRTRVIYVH
121  NSPGAHLLPD KVTHTVNKGD TAVLSARVHK EKQTDVIWKS NGSYFYTLDW HEAQDGRFLL
181  QLPNVQPPSS GIYSATYLEA SPLGSAFFRL IVRGCGAGRW GPGCTKECPG CLHGGVCHDH
241  DGECVCPPGF TGTRCEQACR EGRFGQSCQE QCPGISGCRG LTFCLPDPYG CSCGSGWRGS
301  QCQEACAPGH FGADCRLQCQ CQNGGTCDRF SGCVCPSGWH GVHCEKSDRI PQILNMASEL
361  EFNLETMPRI NCAAAGNPFP VRGSIELRKP DGTVLLSTKA IVEPEKTTAE FEVPRLVLAD
421  SGFWECRVST SGGQDSRRFK VNVKVPPVPL AAPRLLTKQS RQLVVSPLVS FSGDGPISTV
481  RLHYRPQDST MDWSTIVVDP SENVTLMNLR PKTGYSVRVQ LSRPGEGGEG AWGPPTLMTT
541  DCPEPLLQPW LEGWHVEGTD RLRVSWSLPL VPGPLVGDGF LLRLWDGTRG QERRENVSSP
601  QARTALLTGL TPGTHYQLDV QLYHCTLLGP ASPPAHVLLP PSGPPAPRHL HAQALSDSEI
661  QLTWKHPEAL PGPISKYVVE VQVAGGAGDP LWIDVDRPEE TSTIIRGLNA STRYLFRMRA
721  SIQGLGDWSN TVEESTLGNG LQAEGPVQES RAAEEGLDQQ LILAVVGSVS ATCLTILAAL
781  LTLVCIRRSC LHRRRTFTYQ SGSGEETILQ FSSGTLTLTR RPKLQPEPLS YPVLEWEDIT
841  FEDLIGEGNF GQVIRAMIKK DGLKMNAAIK MLKEYASEND HRDFAGELEV LCKLGHHPNI
901  INLLGACKNR GYLYIAIEYA PYGNLLDFLR KSRVLETDPA FAREHGTAST LSSRQLLRFA
961  SDAANGMQYL SEKQFIHRDL AARNVLVGEN LASKIADFGL SRGEEVYVKK TMGRLPVRWM
1021 AIESLNYSVY TTKSDVWSFG VLLWEIVSLG GTPYCGMTCA ELYEKLPQGY RMEQPRNCDD
1081 EVYELMRQCW RDRPYERPPF AQIALQLGRM LEARKAYVNM SLFENFTYAG IDATAEEA
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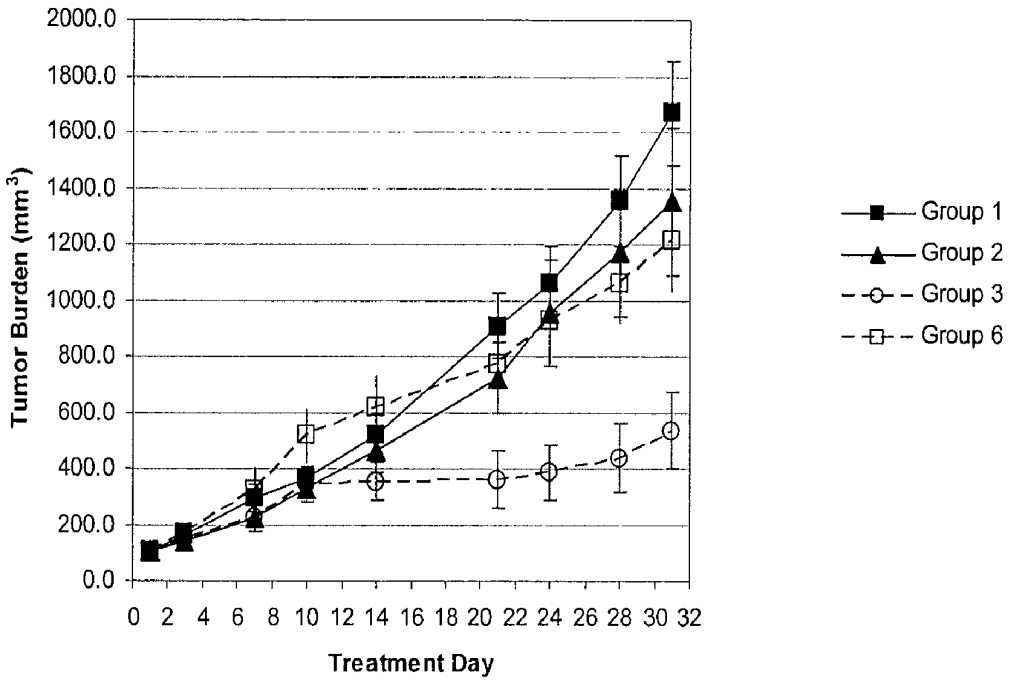
FIGURE 2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1800.0
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Tumor Burden (mm3)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Treatment Day
Group 1
Group 2
Group 3
Group 6

FIGURE 3

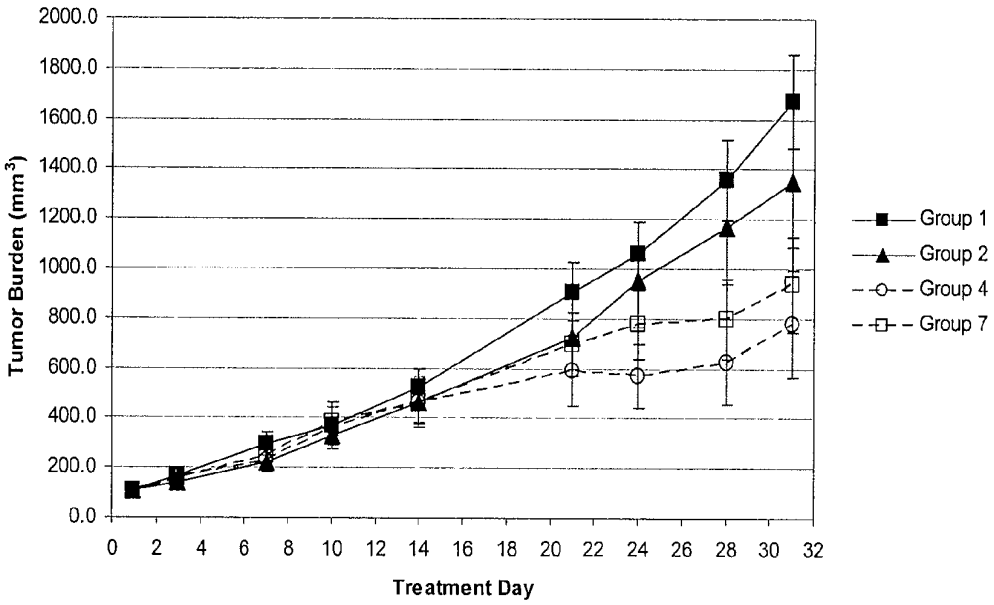

2000.0
1800.0
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Tumor Burden (mm3)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Treatment Day
Group 1
Group 2
Group 4
Group 7

FIGURE 4

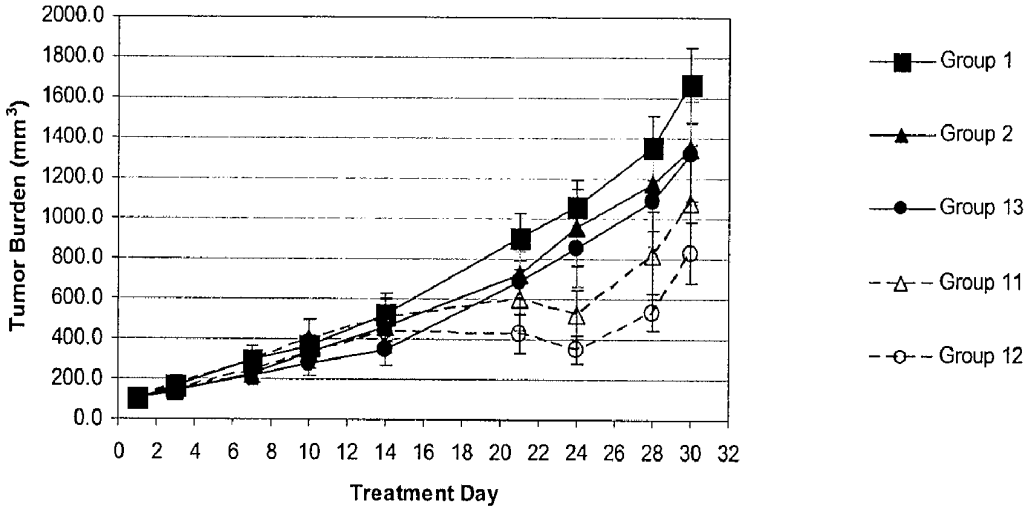

2000.0
1800.0
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Tumor Burden (mm3)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Treatment Day
Group 1
Group 2
Group 13
Group 11
Group 12

FIGURE 5

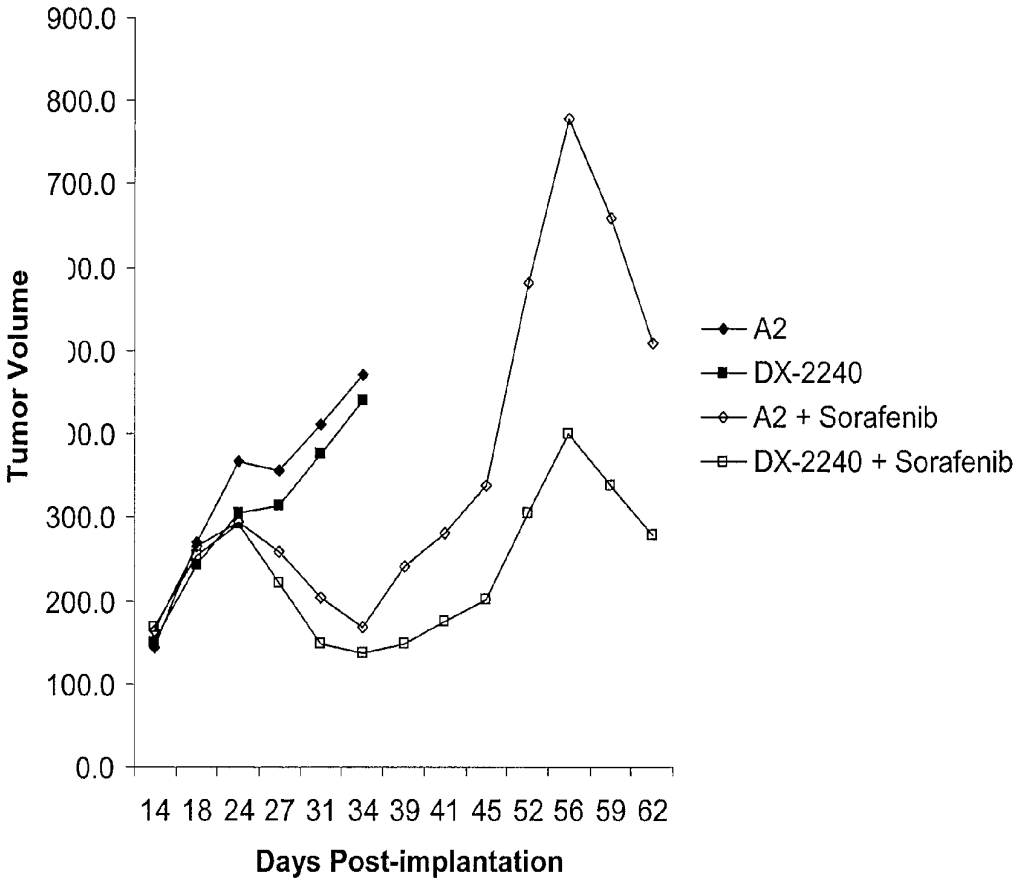

900.0
800.0
700.0
)0.0
)0.0
)0.0
300.0
200.0
100.0
0.0
Tumor Volume
14 18 24 27 31 34 39 41 45 52 56 59 62
Days Post-implantation
A2
DX-2240
A2 + Sorafenib
DX-2240 + Sorafenib

FIGURE 6

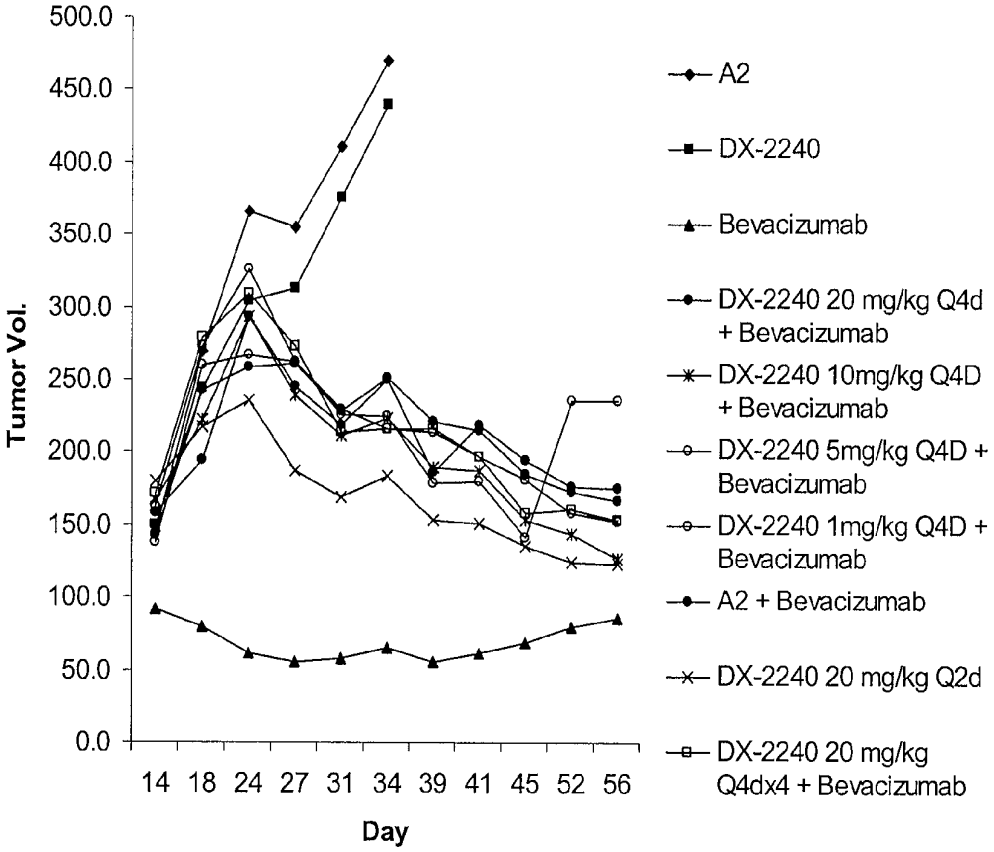

500.0
450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Tumor Vol.
14 18 24 27 31 34 39 41 45 52 56
Day
A2
DX-2240
Bevacizumab
DX-2240 20 mg/kg Q4d + Bevacizumab
DX-2240 10mg/kg Q4D + Bevacizumab
DX-2240 5mg/kg Q4D + Bevacizumab
DX-2240 1mg/kg Q4D + Bevacizumab
A2 + Bevacizumab
DX-2240 20 mg/kg Q2d
DX-2240 20 mg/kg Q4dx4 + Bevacizumab

FIGURE 7

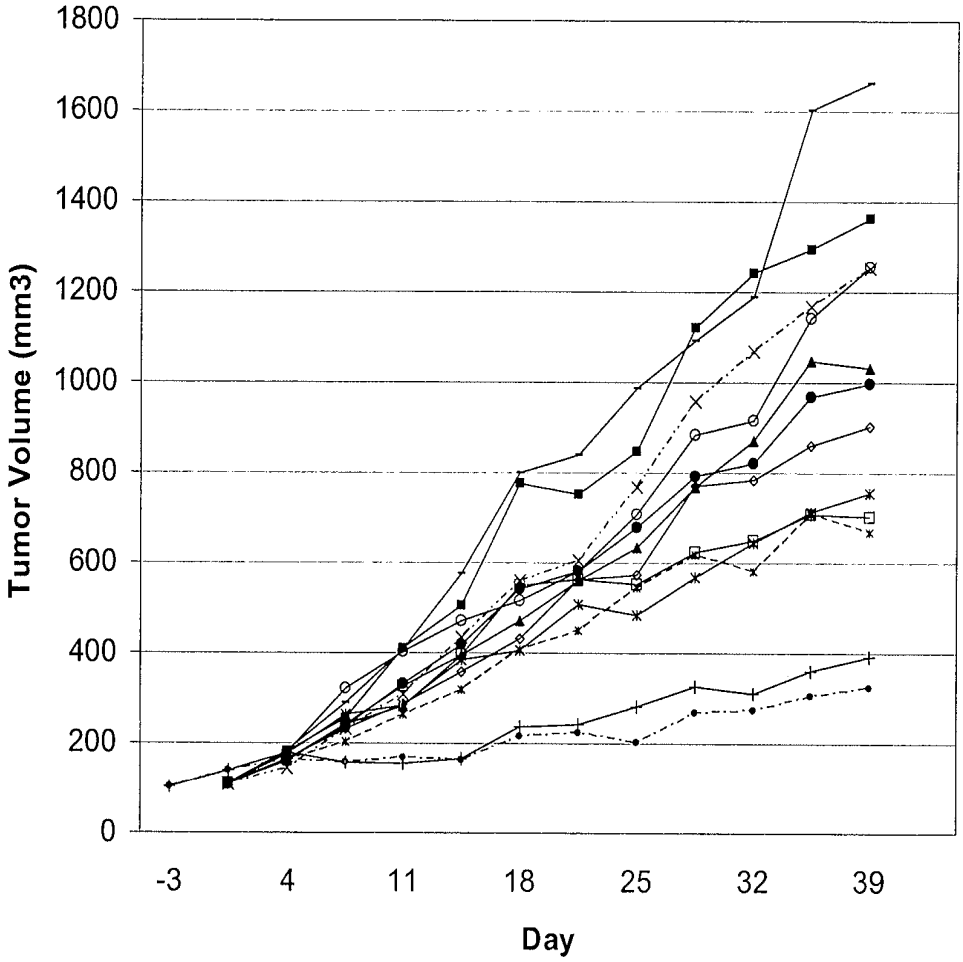

1800
1600
1400
1200
1000
800
600
400
200
0
Tumor Volume (mm3)
-3
4
11
18
25
32
39
Day
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day1)
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day1)
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day5)
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day5)
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day10)
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day10)
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day20)
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day20)
Control IgG 20mg/kg Q2D (Day1) + Rat anti-mouse VEGF/Bevacizumab 10mg/kg Q3D (Day20)
DX-2240 20mg/kg Q2D (Day1) + Rat anti-mouse VEGF/Bevacizumab 10mg/kg Q3D (Day20)
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day10) [cell implantation]
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day10) [cell implantation]

FIGURE 8

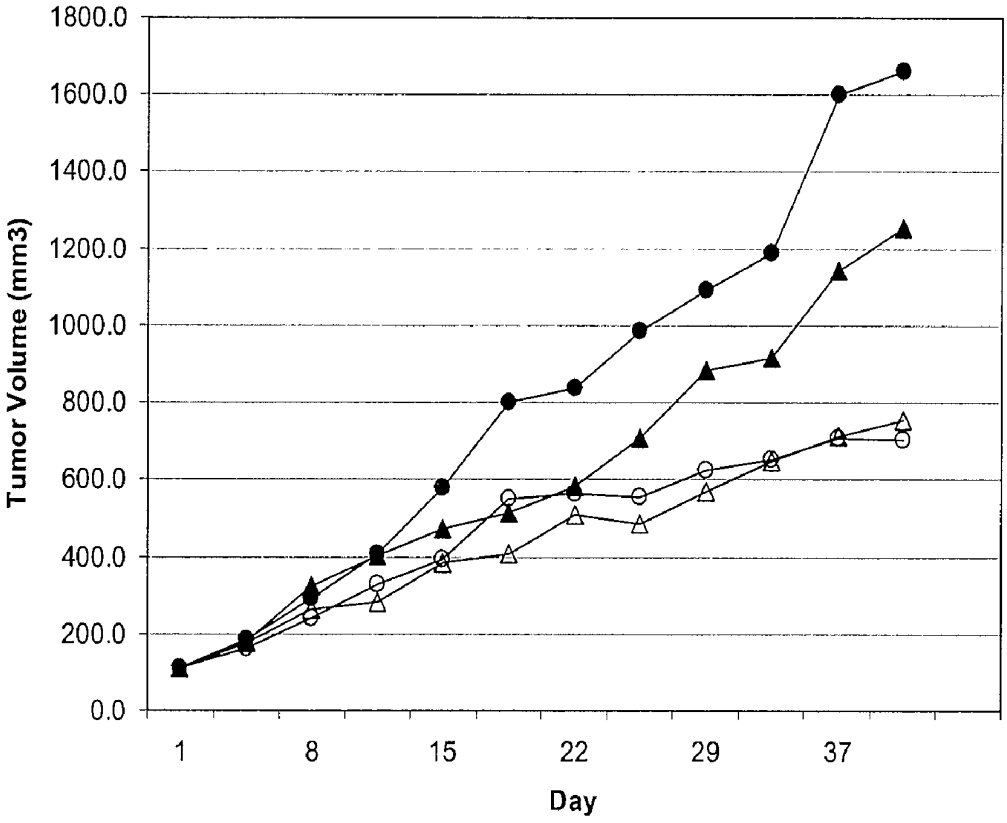

1800.0
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Tumor Volume (mm3)
1
8
15
22
29
37
Day
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day10)
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day10)
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day20)
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day20)

FIGURE 9

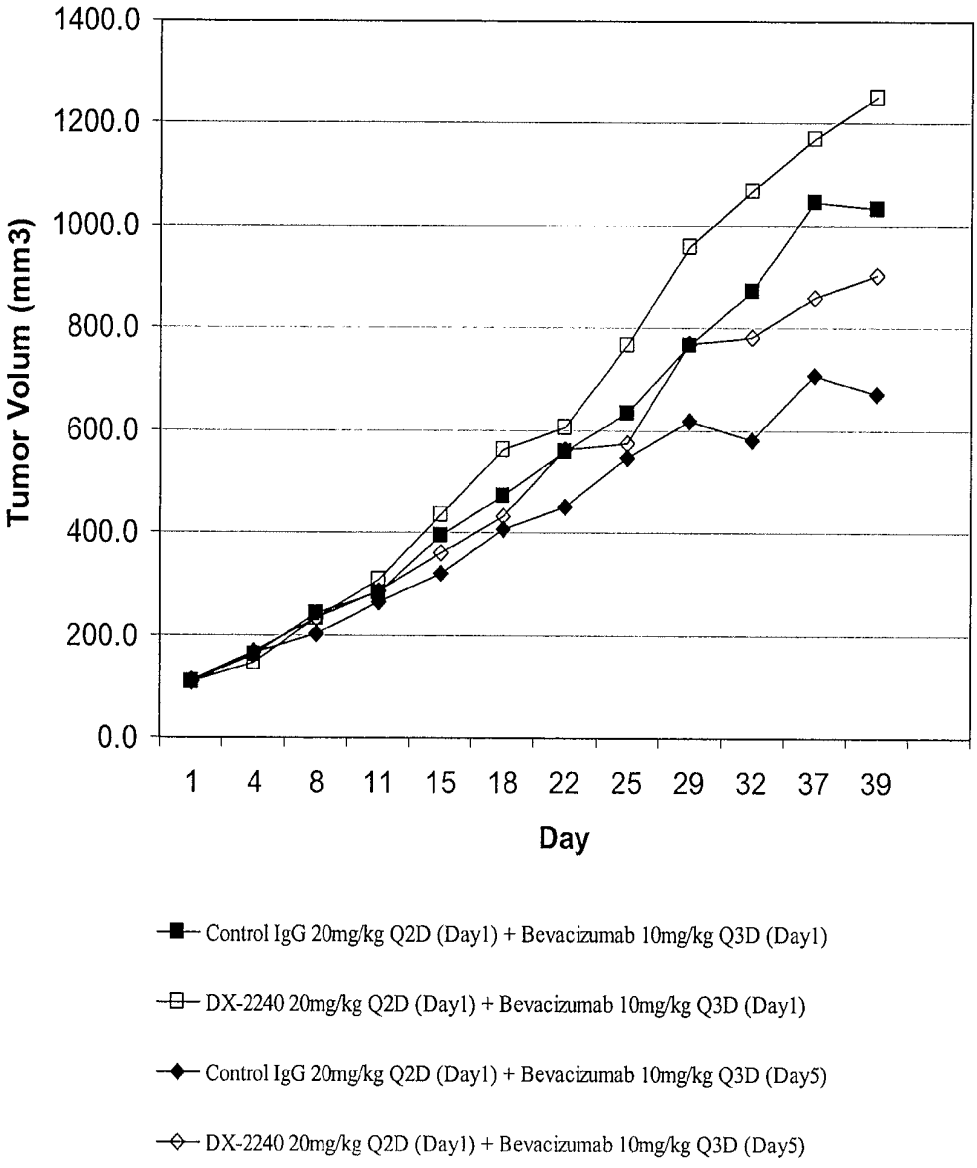

1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Tumor Volum (mm3)
1
4
8
11
15
18
22
25
29
32
37
39
Day
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day1)
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day1)
Control IgG 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day5)
DX-2240 20mg/kg Q2D (Day1) + Bevacizumab 10mg/kg Q3D (Day5)

FIGURE 10

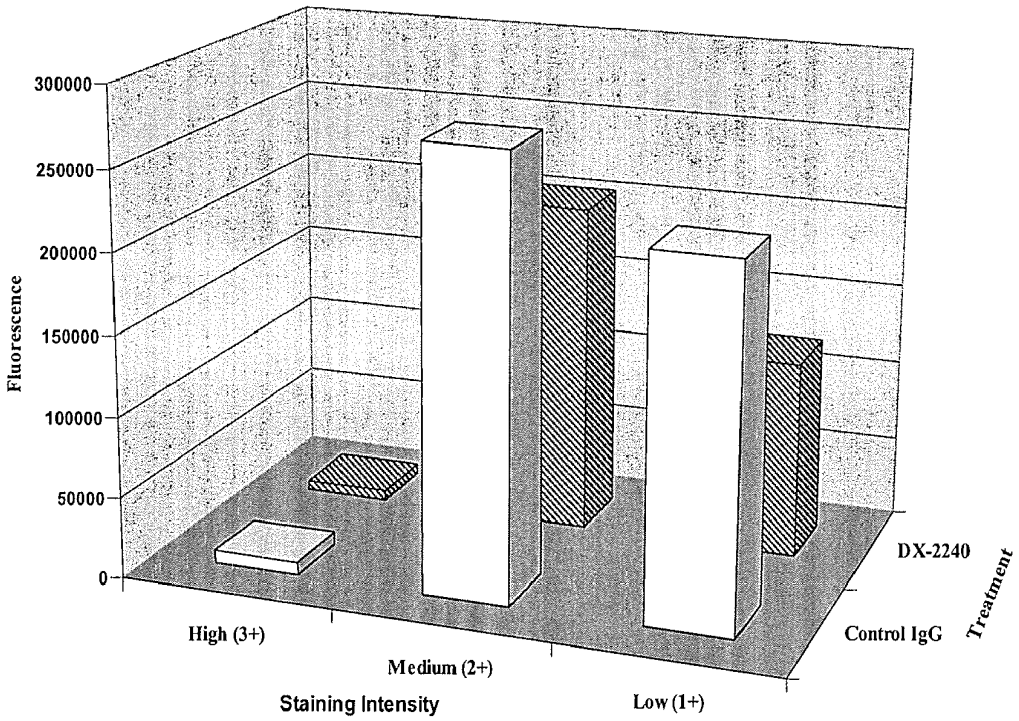

300000
250000
200000
150000
100000
50000
0
Fluorescence
High (3+)
Medium (2+)
Low (1+)
Staining Intensity
DX-2240
Control IgG
Treatment

FIGURE 11

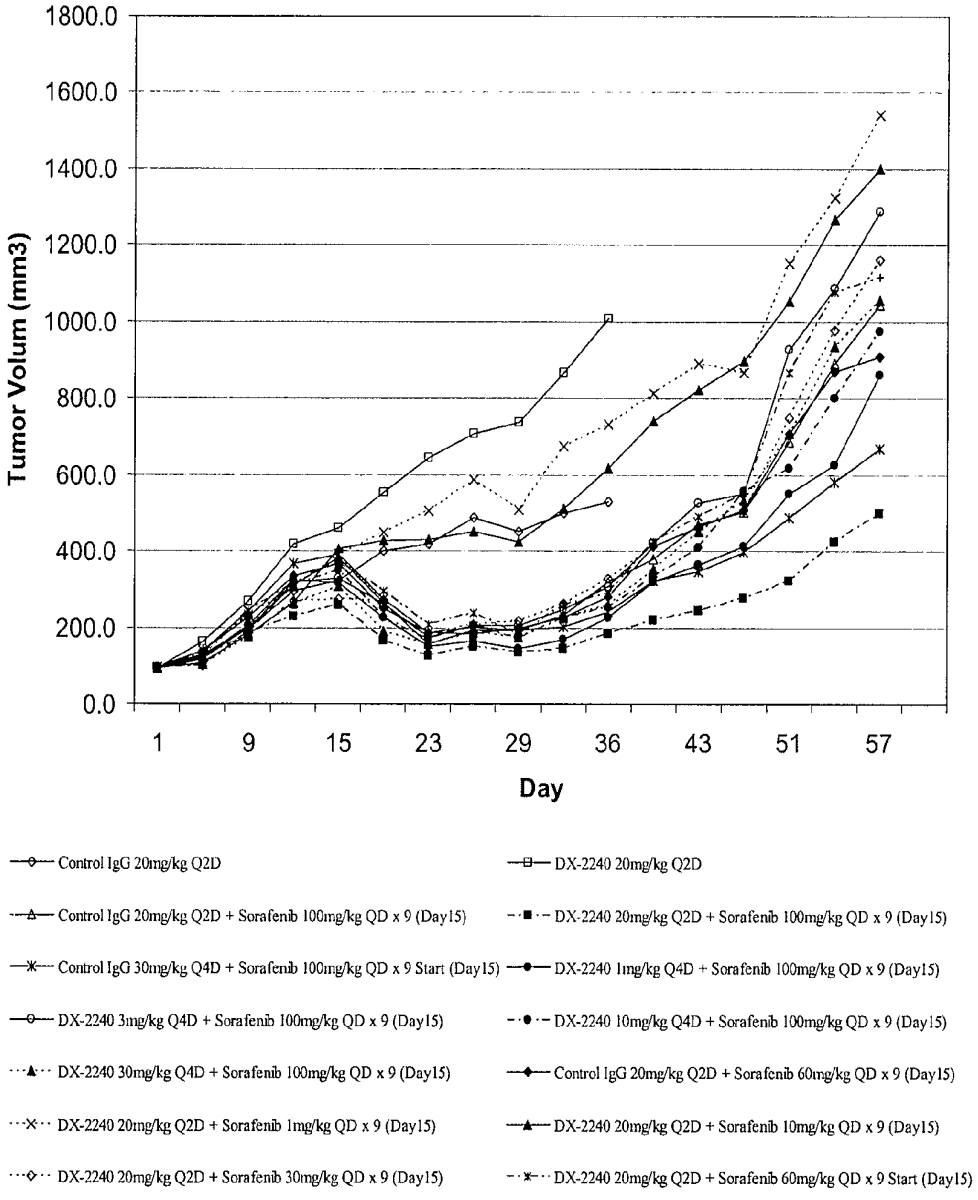
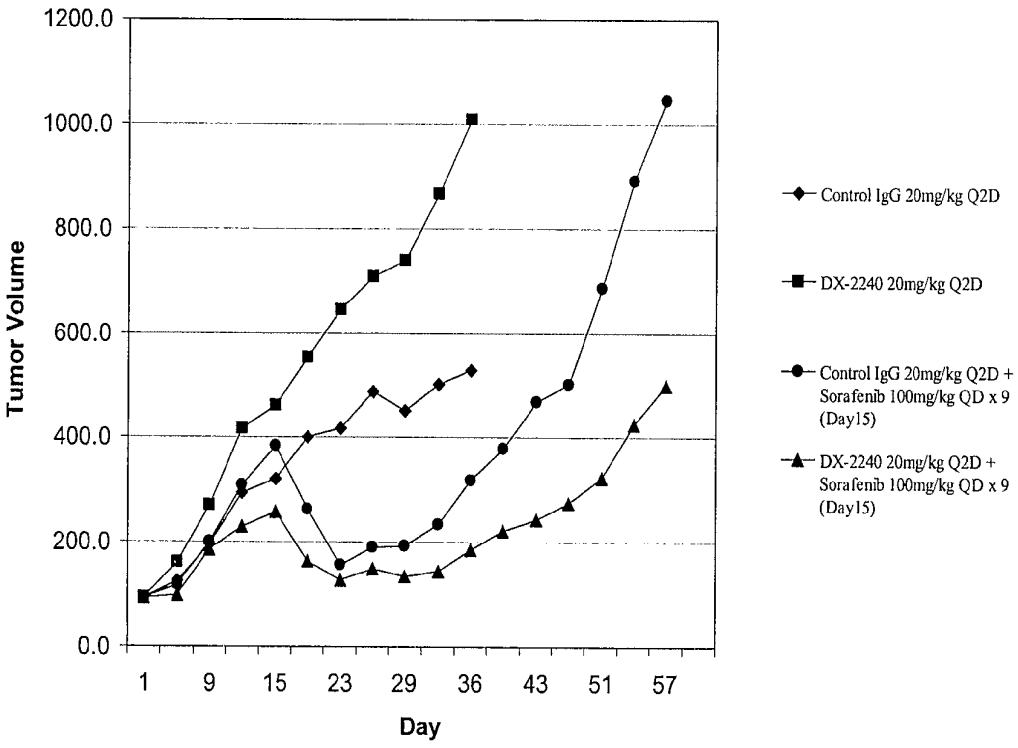

1800.0
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Tumor Volum (mm3)
1
9
15
23
29
36
43
51
57
Day
Control IgG 20mg/kg Q2D
DX-2240 20mg/kg Q2D
Control IgG 20mg/kg Q2D + Sorafenib 100mg/kg QD x 9 (Day15)
DX-2240 20mg/kg Q2D + Sorafenib 100mg/kg QD x 9 (Day15)
Control IgG 30mg/kg Q4D + Sorafenib 100mg/kg QD x 9 Start (Day15)
DX-2240 1mg/kg Q4D + Sorafenib 100mg/kg QD x 9 (Day15)
DX-2240 3mg/kg Q4D + Sorafenib 100mg/kg QD x 9 (Day15)
DX-2240 10mg/kg Q4D + Sorafenib 100mg/kg QD x 9 (Day15)
DX-2240 30mg/kg Q4D + Sorafenib 100mg/kg QD x 9 (Day15)
Control IgG 20mg/kg Q2D + Sorafenib 60mg/kg QD x 9 (Day15)
DX-2240 20mg/kg Q2D + Sorafenib 1mg/kg QD x 9 (Day15)
DX-2240 20mg/kg Q2D + Sorafenib 10mg/kg QD x 9 (Day15)
DX-2240 20mg/kg Q2D + Sorafenib 30mg/kg QD x 9 (Day15)
DX-2240 20mg/kg Q2D + Sorafenib 60mg/kg QD x 9 Start (Day15)

FIGURE 12


1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Tumor Volume
1
9
15
23
29
36
43
51
57
Day
Control IgG 20mg/kg Q2D
DX-2240 20mg/kg Q2D
Control IgG 20mg/kg Q2D + Sorafenib 100mg/kg QD x 9 (Day15)
DX-2240 20mg/kg Q2D + Sorafenib 100mg/kg QD x 9 (Day15)

SEQUENTIAL COMBINATION THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Ser. No. 60/852,263, filed on Oct. 17, 2006, and U.S. Provisional Application Ser. No. 60/875,736, filed on Dec. 19, 2006, the contents of which are incorporated by reference in their entirety.

BACKGROUND

[0002] Blood vessels are composed of an inner layer of endothelial cells and an outer layer of pericytes or smooth muscle cells. The first tubular structures are formed by endothelial cells that subsequently recruit pericytes and smooth muscle cells to ensheath them. The de novo formation of blood vessels from a dispersed population of mesodermally-derived endothelial precursor cells is termed vasculogenesis. This primitive network undergoes successive morphogenetic events including sprouting, splitting, and remodeling to generate the hierarchical vascular network from large to branched small vessels. These successive morphogenetic events are collectively called angiogenesis. Previous studies have identified a number of endothelial cell specific receptor tyrosine kinases (RTKs) and their cognate ligands, which mediate the vasculogenic and angiogenic development of blood vessels. Members of the vascular endothelial growth factor (VEGF) family and their receptors function during the formation of the initial embryonic vascular plexus, whereas angiopoietins (Angs) and their receptor, Tie2, as well as ephrins and their Eph receptors are implicated in the subsequent remodeling processes. See, e.g., Jones et al. (2001) *Nat. Rev. Molec. Cell Biol.* 2:257 for a review of receptors involved in angiogenic and lymphangiogenic responses.

[0003] Tie1 and Tie2 are RTKs that are expressed almost exclusively in endothelial cells and hematopoietic precursor cells. These two receptors are required for the normal development of vascular structures during embryogenesis. The two Tie receptors form a RTK subfamily since, unlike other RTK family members, they include extracellular EGF-homology domains. See, e.g., Partanen (1992) *Mol. Cell. Biol.* 12:1698 and WO 93/14124. Targeted disruption of the Tie1 gene in mice results in a lethal phenotype characterized by extensive hemorrhage and defective microvessel integrity. See, e.g., Puri et al. (1995) *EMBO J.* 14:5884. Tie2 null embryos have defects in vascular remodeling and maturation, resulting from improper recruitment of periendothelial supporting cells. Angiopoietins (Ang, e.g., Ang1, Ang2, Ang3, and Ang4) are proteins that interact with Tie2.

[0004] Vascular endothelial growth factor (VEGF) also plays a substantial role in the regulation of normal and abnormal angiogenesis. The loss of a single VEGF allele results in embryonic lethality, suggesting that this factor plays a key role in the development and differentiation of the vascular system (Ferrara et al. 1997, Endocr. Rev. 18:4-25). VEGF has also been shown to be a key mediator of neovascularization associated with tumors and intraocular disorders (Id.). VEGF mRNA is overexpressed by the majority of human tumors examined (Berkman et al., 1993, J Clin Invest 91:153-159; Brown et al., 1995, Human Pathol. 26:86-91; Brown et al., 1993, Cancer Res. 53:4727-35; Mattern et al., 1996, Brit. J. Cancer. 73:931-34; and Dvorak et al., 1995, Am J. Pathol. 146:1029-39). Anti-VEGF neutralizing antibodies suppress the growth of a variety of human tumor cell lines in nude mice (Kim et al., 1993, Nature 362:841-44; Warren et al., 1995, J. Clin. Invest. 95:1789-97; Borgstrom et al., 1996, Cancer Res. 56:4032-39; and Melnyk et al., 1996, Cancer Res. 56:921-24). More recently, a monoclonal antibody VEGF inhibitor, bevacizumab, has been approved for treatment of certain human cancers.

SUMMARY

[0005] In general, the invention features administration regimens for administering a Tie1 ectodomain-binding agent and a VEGF antagonist to obtain a desirable therapeutic effect.

[0006] In one aspect, the invention provides methods of treating an angiogenesis-related disorder, the methods comprise administering to a subject a first agent comprising a Tie1 ectodomain-binding agent before administering to the subject a second agent comprising a VEGF antagonist.

[0007] In some embodiments, the first agent is administered about 1 day to 35 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30, or 35 days, or any day in between) before administration of the second agent. In other embodiments, the first agent is administered about 4 days (e.g., 3, 4, or 5 days), about 6 days (e.g., 5, 6, or 7 days), about 8 days (e.g., 7, 8, or 9 days), about 10 days (e.g., 8, 9, 10, 11, or 12 days), about 20 days (e.g., 19, 20, or 21 days), or about two weeks (e.g., 12, 13, 14, 15, or 16 days) before administration of the second agent.

[0008] In some embodiments, administration of the first agent is continued after administration of the second agent. In some embodiments, administration of the first agent is discontinued after administration of the second agent.

[0009] In some embodiments, the Tie1 ectodomain-binding agent is a Tie1 ectodomain-binding agent described herein. In some embodiments, the Tie1 ectodomain-binding agent is DX-2240, DX-2220 or combinations thereof.

[0010] In some embodiments, the VEGF antagonist is a VEGF antagonist described herein. In some embodiments, the VEGF antagonist is bevacizumab. In other embodiments, the VEGF antagonist is sorafenib.

[0011] In some embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount effective to individually treat the angiogenesis-related disorder. In other embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount less than an amount effective to individually treat the angiogenesis-related disorder. In some embodiments, the Tie1 ectodomain-binding agent and the VEGF antagonist are each administered in a synergistically effective amount to treat the angiogenesis-related disorder.

[0012] In some embodiments, the angiogenesis-related disorder is cancer or tumor, e.g., a cancer or tumor described herein. In some embodiments, the angiogenesis related disorder is colon cancer, lung cancer, breast cancer, kidney cancer, liver cancer, ovarian cancer, prostate cancer or pancreatic cancer. In some embodiments, the angiogenesis-related disorder is prostate cancer or pancreatic cancer.

[0013] In some embodiments, the method includes a step of monitoring the subject for a change in tumor vasculature, and the second agent is administered when tumor vasculature exhibits a change in tumor vasculature as compared to prior to the administration of the first agent. In some embodiments, the method includes a step of monitoring the subject for a change in tumor size.

[0014] In some embodiments, the method includes radiation therapy or chemotherapy. The radiation and/or chemotherapy can be prior to, during or after administration of the Tie1 ectodomain-binding agent and/or the VEGF antagonist.

[0015] In other aspects, the invention describes methods of providing a post-operative adjuvant therapy, the method comprises administering a first agent comprising a Tie1 ectodomain-binding agent for a period before administering a second agent comprising a VEGF antagonist, to a subject who has had surgery to remove a tumor.

[0016] In some embodiments, the first agent is administered about 1 day to 35 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30, or 35 days, or any in day between) before administration of the second agent. In other embodiments, the first agent is administered about 4 days (e.g., 3, 4, or 5 days), about 6 days (e.g., 5, 6, or 7 days), about 8 days (e.g., 7, 8, or 9 days), about 10 days (e.g., 8, 9, 10, 11, or 12 days), about 20 days (e.g., 19, 20, or 21 days), or about two weeks (e.g., 12, 13, 14, 15, or 16 days) before administration of the second agent.

[0017] In some embodiments, administration of the first agent is continued after administration of the second agent. In some embodiments, administration of the first agent is discontinued after administration of the second agent.

[0018] In some embodiments, the Tie1 ectodomain-binding agent is a Tie1 ectodomain-binding agent described herein. In some embodiments, the Tie1 ectodomain-binding agent is DX-2240, DX-2220 or combinations thereof. In some embodiments, the first agent is administered within about 5, 10, 15, 20, 24, 35, 40 or 48 hours of surgery.

[0019] In some embodiments, the VEGF antagonist is a VEGF antagonist described herein. In some embodiments, the VEGF antagonist is bevacizumab. In other embodiments, the VEGF antagonist is sorafenib.

[0020] In some embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount effective to individually treat the tumor regrowth. In other embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount less than an amount effective to individually treat the tumor regrowth. In some embodiments, the Tie1 ectodomain-binding agent and the VEGF antagonist are each administered in a synergistically effective amount to treat the tumor regrowth.

[0021] In some embodiments, the tumor is a tumor described herein. In some embodiments, the tumor is a colon tumor, a lung tumor, a breast tumor, a kidney tumor, a liver tumor, an ovarian tumor, a prostate tumor or a pancreatic tumor. In some embodiments, the tumor is a prostate tumor or a pancreatic tumor.

[0022] In some embodiments, the method includes radiation therapy or chemotherapy. The radiation and/or chemotherapy can be prior to, during or after administration of the Tie1 ectodomain-binding agent and/or the VEGF antagonist.

[0023] In other aspects, the invention provides methods of treating an angiogenesis-related disorder, the method comprises administering to a subject a first agent comprising a VEGF antagonist for a period before administering to the subject a second agent comprising a Tie1 ectodomain-binding agent.

[0024] In some embodiments, the first agent is administered about 1 to 35 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30 or 35 days, or any day in between) before the second agent is administered. In other embodiments, the first agent is administered about 4 days (e.g., 3, 4, or 5 days), about 6 days (e.g., 5, 6, or 7 days), about 8 days (e.g., 7, 8, or 9 days), about 10 days (e.g., 8, 9, 10, 11, or 12 days), about 20 days (e.g., 19, 20, or 21 days), or about two weeks (e.g., 12, 13, 14, 15, or 16 days) before administration of the second agent.

[0025] In some embodiments, administration of the first agent is continued after administration of the second agent. In some embodiments, administration of the first agent is discontinued after administration of the second agent.

[0026] In some embodiments, the Tie1 ectodomain-binding agent is a Tie1 ectodomain-binding agent described herein. In some embodiments, the Tie1 ectodomain-binding agent is DX-2240, DX-2220 or combinations thereof.

[0027] In some embodiments, the VEGF antagonist is a VEGF antagonist described herein. In some embodiments, the VEGF antagonist is bevacizumab. In some embodiments, the VEGF antagonist is sorafenib.

[0028] In some embodiments, the methods include a step of monitoring the subject for a change in tumor vasculature, and the second agent is administered when tumor vasculature exhibits a change as compared to prior to the administration of the first agent. In some embodiments, the methods include the step of monitoring for tumor growth, and the second agent is administered when tumor growth is exhibited.

[0029] In some embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount effective to individually treat the angiogenesis-related disorder. In other embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount less than an amount effective to individually treat the angiogenesis-related disorder. In some embodiments, the Tie1 ectodomain-binding agent and the VEGF antagonist are each administered in a synergistically effective amount to treat the angiogenesis-related disorder.

[0030] In some embodiments, the angiogenesis-related disorder is cancer or tumor, e.g., a cancer or tumor described herein. In some embodiments, the angiogenesis related disorder is colon cancer, lung cancer, breast cancer, kidney cancer, liver cancer, ovarian cancer, prostate cancer or pancreatic cancer. In some embodiments, the angiogenesis-related disorder is prostate cancer or pancreatic cancer.

[0031] In some embodiments, the method includes radiation therapy or chemotherapy. The radiation and/or chemotherapy can be prior to, during or after administration of the Tie1 ectodomain-binding agent and/or the VEGF antagonist.

[0032] In other aspects, the invention describes methods of providing a post-operative adjuvant therapy, the method comprises administering a first agent comprising a VEGF antagonist for a period before administering a second agent comprising a Tie1 ectodomain-binding agent, to a subject who has had surgery to remove a tumor.

[0033] In some embodiments, the first agent is administered about 1 day to 35 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30, or 35 days, or any in day between) before administration of the second agent. In other embodiments, the first agent is administered about 4 days (e.g., 3, 4, or 5 days), about 6 days (e.g., 5, 6, or 7 days), about 8 days (e.g., 7, 8, or 9 days), about 10 days (e.g., 8, 9, 10, 11, or 12 days), about 20 days (e.g., 19, 20, or 21 days), or about two weeks (e.g., 12, 13, 14, 15, or 16 days) before administration of the second agent.

[0034] In some embodiments, administration of the first agent is continued after administration of the second agent. In some embodiments, administration of the first agent is discontinued after administration of the second agent.

[0035] In some embodiments, the VEGF antagonist is a VEGF antagonist described herein. In some embodiments, the VEGF antagonist is bevacizumab. In other embodiments, the VEGF antagonist is sorafenib. In some embodiments, the first agent is administered within about 5, 10, 15, 20, 24, 35, 40 or 48 hours of surgery.

[0036] In some embodiments, the Tie1 ectodomain-binding agent is a Tie1 ectodomain-binding agent described herein. In some embodiments, the Tie1 ectodomain-binding agent is DX-2240, DX-2220 or combinations thereof.

[0037] In some embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount effective to individually treat the tumor regrowth. In other embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount less than an amount effective to individually treat the tumor regrowth. In some embodiments, the Tie1 ectodomain-binding agent and the VEGF antagonist are each administered in a synergistically effective amount to treat the tumor regrowth.

[0038] In some embodiments, the tumor is a tumor described herein. In some embodiments, the tumor is present in the colon, lung, breast, kidney, liver, ovary, prostate or pancreas. In some embodiments, the tumor is a prostate tumor or a pancreatic tumor.

[0039] In some embodiments, the method includes radiation therapy or chemotherapy. The radiation and/or chemotherapy can be prior to, during or after administration of the Tie1 ectodomain-binding agent and/or the VEGF antagonist.

[0040] In other aspects, the invention provides methods of sensitizing tumor vasculature to a decrease of VEGF, the method comprises administering to a subject having an angiogenesis-related disorder a first agent comprising a Tie1 ectodomain-binding agent before administering to the subject a second agent comprising a VEGF antagonist.

[0041] In some embodiments, the first agent is administered about 1 day to 35 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30, or 35 days, or any in day between) before administration of the second agent. In other embodiments, the first agent is administered about 4 days (e.g., 3, 4, or 5 days), about 6 days (e.g., 5, 6, or 7 days), about 8 days (e.g., 7, 8, or 9 days), about 10 days (e.g., 8, 9, 10, 11, or 12 days), about 20 days (e.g., 19, 20, or 21 days), or about two weeks (e.g., 12, 13, 14, 15, or 16 days) before administration of the second agent.

[0042] In some embodiments, administration of the first agent is continued after administration of the second agent. In some embodiments, administration of the first agent is discontinued after administration of the second agent.

[0043] In some embodiments, the Tie1 ectodomain-binding agent is a Tie1 ectodomain-binding agent described herein. In some embodiments, the Tie1 ectodomain binding agent is DX-2240, DX-2220, or combinations thereof.

[0044] In some embodiments, the VEGF antagonist is a VEGF antagonist described herein. In some embodiments, the VEGF antagonist is bevacizumab.

[0045] In some embodiments, the methods include a step of monitoring the subject for a change in tumor vasculature, and the second agent is administered when tumor vasculature exhibits a change as compared to prior to the administration of the first agent.

[0046] In some embodiments, the angiogenesis related disorder is a cancer or tumor, e.g., a cancer or tumor described herein. In some embodiments, the cancer is colon cancer, lung cancer, breast cancer, kidney cancer, liver cancer, ovarian cancer, prostate cancer or pancreatic cancer. In some embodiments, the cancer is a prostate cancer or a pancreatic cancer.

[0047] In some embodiments, the method includes radiation therapy or chemotherapy. The radiation and/or chemotherapy can be prior to, during or after administration of the Tie1 ectodomain-binding agent and/or the VEGF antagonist.

[0048] In some aspects, the invention provides methods of decreasing the rate of tumor regrowth in a subject having cancer that is being administered a VEGF antagonist, the method comprising administering to the subject a first agent comprising a Tie1 ectodomain-binding agent and a second agent comprising the VEGF antagonist.

[0049] In some embodiments, the first agent is administered about 1 day to 35 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 20, 21, 28, 30, or 35 days, or any in day between) before administration of the second agent. In other embodiments, the first agent is administered about 4 days (e.g., 3, 4, or 5 days), about 6 days (e.g., 5, 6, or 7 days), about 8 days (e.g., 7, 8, or 9 days), about 10 days (e.g., 8, 9, 10, 11, or 12 days), about 20 days (e.g., 19, 20, or 21 days), or about two weeks (e.g., 12, 13, 14, 15, or 16 days) before administration of the second agent.

[0050] In some embodiments, administration of the first agent is continued after administration of the second agent. In some embodiments, administration of the first agent is discontinued after administration of the second agent.

[0051] In some embodiments, the Tie1 ectodomain-binding agent is a Tie1 ectodomain-binding agent described herein. In some embodiments, the Tie1 ectodomain-binding agent is DX-2240, DX-2220 or combinations thereof.

[0052] In some embodiments, the VEGF antagonist is a VEGF antagonist described herein. In some embodiments, the VEGF antagonist is bevacizumab or sorafenib.

[0053] In some embodiments, the methods include a step of monitoring the subject for tumor growth and administering the second agent upon an indication of growth.

[0054] In some embodiments, the cancer is a cancer described herein. In some embodiments, the cancer is colon cancer, lung cancer, breast cancer, kidney cancer, liver cancer, ovarian cancer, prostate cancer or pancreatic cancer. In some embodiments, the cancer is a prostate cancer or a pancreatic cancer.

[0055] In some embodiments, the method includes radiation therapy or chemotherapy. The radiation and/or chemotherapy can be prior to, during or after administration of the Tie1 ectodomain-binding agent and/or the VEGF antagonist.

[0056] In some aspects, the invention provides methods of decreasing frequency of administration of a VEGF antagonist to a subject, by administering to the subject a first agent comprising a Tie1 ectodomain-binding agent prior to the administration of a second agent comprising a VEGF antagonist.

[0057] In some embodiments, the Tie1 ectodomain-binding agent is a Tie1 ectodomain-binding agent described herein. In some embodiments, the tie1 ectodomain-binding agent is DX-2240, DX-2220, or combinations thereof.

[0058] In some embodiments, the VEGF antagonist is a VEGF antagonist described herein. In some embodiments, the VEGF antagonist is sorafenib

[0059] In other embodiments, the methods include a step of monitoring the subject for tumor growth.

[0060] In some embodiments, the cancer is a cancer described herein. In some embodiments, the cancer is colon cancer, lung cancer, breast cancer, kidney cancer, liver cancer,

ovarian cancer, prostate cancer or pancreatic cancer. In some embodiments, the cancer is a prostate cancer or a pancreatic cancer.

[0061] In some embodiments, the method includes radiation therapy or chemotherapy. The radiation and/or chemotherapy can be prior to, during or after administration of the Tie1 ectodomain-binding agent and/or the VEGF antagonist.

[0062] In another aspect, the invention includes a Tie1 ectodomain-binding agent and a VEGF antagonist agent for the manufacture of a medicament for treating an angiogenesis-related disorder in accordance with the methods disclosed herein.

[0063] In other aspects, the invention provides a method described herein in which a Tie2 ectodomain-binding agent is used instead of or in conjunction with a Tie1 ectodomain-binding agent.

[0064] In other aspects, the invention provides a kit that includes a first agent comprising a Tie1 ectodomain-binding agent, a second agent comprising a VEGF antagonist, and instructions for use in accordance with a method described herein.

[0065] In the aspects described herein, angiogenesis-related disorders include, but are not limited to, neoplastic disease (e.g., solid tumors, tumor metastases, and benign tumors, particularly neoplastic disease requiring a blood supply or angiogenesis); inflammatory disorders (e.g., rheumatoid arthritis, lupus, restenosis, psoriasis, graft v. host response, or multiple sclerosis); ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration (e.g., wet and/or dry forms of age-related macular degeneration), corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis; Osler-Webber Syndrome; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; and wound granulation.

[0066] Benign tumors include, but are not limited to hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas. Solid tumors include, but are not limited to malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, gastrointestinal (e.g., colon), and genitourinary tract (e.g., renal, urothelial cells), pharynx, as well as adenocarcinomas which include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, and pancreatic cancer. Still further examples of solid tumors that can be treated include: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastrointestinal system carcinomas, colon carcinoma, pancreatic cancer, breast cancer, genitourinary system carcinomas, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, endocrine system carcinomas, testicular tumor, lung carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

[0067] In some aspects described herein, the angiogenesis-related disorder is an inflammatory disorder, e.g., rheumatoid arthritis, psoriasis, rheumatoid or rheumatic inflammatory disease, or other chronic inflammatory disorders, such as chronic asthma, arterial or post-transplantational atherosclerosis, and endometriosis. Other angiogenesis-related disorders that can be treated include those that have deregulated or undesired angiogenesis, such as ocular neovascularization, e.g., retinopathies (including diabetic retinopathy and age-related macular degeneration) hemangioblastoma, hemangioma, and arteriosclerosis.

[0068] In some aspects described herein, the subject is in need of reduced angiogenesis, or identified as such. For example, the subject has a neoplastic disorder, e.g., a metastatic cancer. For example, the subject has an angiogenesis-dependent cancer or tumor. The tumor can be a solid tumor, e.g., a tumor at least 1, 2, 3, 5, 8 or 10 mm in diameter. In one embodiment, the solid tumor has a hypoxic core. The method can include, prior to administering the antagonist, evaluating the subject and detecting a solid tumor in the subject.

[0069] In some aspects described herein, the Tie1 ectodomain-binding agent increases Tie complex formation. In some aspects described herein, the Tie1 ectodomain-binding agent increases tyrosine phosphorylation of Tie1. In some aspects described herein, the Tie1 ectodomain-binding agent induces down modulation of Tie1 from the surface of the cell.

[0070] In some aspects described herein, the Tie1 ectodomain-binding agent is an antibody that includes at least one complementarity determining region (CDR, e.g., HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and/or LC CDR3) from E3 (DX-2240), E3b (DX-2220), M0044-A06; M0044-A11; M0044-B04; M0044-B05; M0044-B08; M0044-B09; M0044-B10; M0044-B12; M0044-C07; M0044-D01; M0044-E03; M0044-F03; M0044-F06; M0044-F09; M0044-G06; M0044-G07; M0044-G11; M0044-H03; M0044-H05; M0044-H07; M0044-H09; M0045-A02; M0045-A04; M0045-B01; M0045-B03; M0045-B11; M0045-C02; M0045-C11; M0045-C12; M0045-D01; M0045-D07; M0045-G01; M0045-G10; M0046-A11; M0046-B06; M0046-B10; M0046-G12; M0046-H03; M0046-H10; M0046-H11; M0047-B03; M0047-D01; M0047-D03; M0047-E10; M0047-G09; M0053-A02; M0053-A03; M0053-A05; M0053-A09; M0053-B09; M0053-B11; M0053-D03; M0053-D06; M0053-D12; M0053-E03; M0053-E04; M0053-E08; M0053-F04; M0053-F05; M0053-F06; M0053-F08; M0053-G04; M0053-G05; M0054-A08; M0054-B06; M0054-B08; M0054-C03; M0054-C07; M0054-E04; M0054-G01; M0054-G05; M0054-H10; M0055-A09; M0055-B11; M0055-B12; M0055-C05; M0055-C07; M0055-D03; M0055-D06; M0055-D12; M0055-E04; M0055-E06; M0055-E10; M0055-E12; M0055-F10; M0055-G02; M0055-G03; M0055-H04; M0056-A01; M0056-A06; M0056-B08; M0056-B09; M0056-C03; M0056-C04; M0056-E08; M0056-F01; M0056-F02; M0056-F10; M0056-F11; M0056-G03; M0056-G04; M0056-G08; M0056-G12; M0056-H04; M0056-H12; M0057-B05; M0057-H07; M0058-A09; M0058-D04; M0058-E09; M0058-F03; M0058-G03; M0058-H01; M0059-A02; M0059-A06; M0060-B02; M0060-H01;

M0061-A03; M0061-C05; M0061-C06; M0061-F07; M0061-G12; M0061-H09; M0062-A12; M0062-B05; M0062-B07; M0062-C08; M0062-D04; M0062-E02; M0062-E03; M0062-E11; M0062-F10; M0062-G06; or M0062-H01.

[0071] The VEGF antagonist agent is one that targets or negatively regulates the VEGF signaling pathway. Examples of this latter class include VEGF inhibitors (e.g., agents that directly inhibit VEGF (e.g., VEGF-A, -B, -C, or -D), such as by binding VEGF (e.g., anti-VEGF antibodies such as bevacizumab (AVASTIN®) or ranibizumab (LUCENTIS®), or other inhibitors such as pegaptanib, NEOVASTAT®, AE-941, VEGF Trap, and PI-88)), modulators of VEGF expression (e.g., INGN-241, oral tetrathiomolybdate, 2-methoxyestradiol, 2-methoxyestradiol nanocrystal dispersion, bevasiranib sodium, PTC-299, Veglin), inhibitors of a VEGF receptor (e.g., KDR or VEGF receptor III (Flt4), for example anti-KDR antibodies, VEGFR2 antibodies such as CDP-791, IMC-1121B, VEGFR2 blockers such as CT-322), modulators of VEGFR expression (e.g., VEGFR1 expression modulator Sirna-027) or inhibitors of VEGF receptor downstream signaling. In some aspects described herein, the VEGF antagonist agent is bevacizumab, pegaptanib, ranibizumab, sorafenib, sunitinib, NEOVASTAT®, AE-941, VEGF Trap, pazopanib, vandetanib, vatalanib, cediranib, fenretinide, squalamine, INGN-241, oral tetrathiomolybdate, tetrathiomolybdate, Panzem NCD, 2-methoxyestradiol, AEE-788, AG-013958, bevasiranib sodium, AMG-706, axitinib, BIBF-1120, CDP-791, CP-547632, PI-88, SU-14813, SU-6668, XL-647, XL-999, IMC-1121B, ABT-869, BAY-57-9352, BAY-73-4506, BMS-582664, CEP-7055, CHIR-265, CT-322, CX-3542, E-7080, ENMD-1198, OSI-930, PTC-299, Sirna-027, TKI-258, Veglin, XL-184, or ZK-304709.

[0072] In some aspects described herein, the administration of a Tie1 ectodomain-binding agent and a VEGF antagonist agent is used as an adjuvant therapy. The adjuvant therapy can be a post-operative therapy that is administered to the subject after the subject has undergone surgery to remove all or part of a tumor (e.g., after surgery to treat glioblastoma or colorectal, breast, or lung cancer). In some embodiments, administration in accordance with the invention is initiated within 6, 12, 24, 48, or 100 hours of surgery.

[0073] In some aspects described herein, the method includes an additional therapeutic modality. For example, the additional therapeutic modality is radiation therapy or a cytotoxic chemotherapy agent, such as an anti-metabolite (e.g., 5-FU, with leucovorin), irinotecan, (or other topoisomerase inhibitor), doxorubicin, or any combination all of these agents, including administration of all of these agents.

[0074] The methods can further include the step of monitoring the subject, e.g., for a reduction in one or more of: a reduction in tumor size; reduction in cancer markers, e.g., levels of cancer specific antigen; reduction in the appearance of new lesions, e.g., in a bone scan; a reduction in the appearance of new disease-related symptoms; or decreased or stabilization of size of soft tissue mass; or any parameter related to improvement in clinical outcome. The subject can be monitored in one or more of the following periods: prior to beginning of treatment; during the treatment; or after one or more elements of the treatment have been administered. Monitoring can be used to evaluate the need for further treatment with the same Tie1-binding protein and/or VEGF antagonist agent or for additional treatment with additional agents. Generally, a decrease in or stabilization of one or more of the parameters described above is indicative of the improved condition of the subject. Information about the monitoring can be recorded, e.g., in electronic or digital form.

[0075] The subject can be a mammal, e.g., a primate, preferably a higher primate, e.g., a human.

[0076] Other features and advantages of the instant invention will become more apparent from the following detailed description and claims. Embodiments of the invention can include any combination of features described herein. In no case does the term "embodiment" necessarily exclude one or more other features disclosed herein, e.g., in another embodiment. The contents of all references, patent applications and patents, cited throughout this application are hereby expressly incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

[0077] FIG. **1** shows the amino acid sequence of human Tie1.

[0078] FIG. **2** shows a graph depicting the administration of DX-2240 followed by administration of bevacizumab 10 days later in a pancreatic tumor model.

[0079] FIG. **3** shows a graph depicting administration of DX-2240 followed by administration of bevacizumab 20 days later in a pancreatic tumor model.

[0080] FIG. **4** shows a graph depicting administration of DX-2240 followed by administration of sorafenib 15 days later in a pancreatic tumor model.

[0081] FIG. **5** shows a graph depicting administration of DX-2240 followed by administration of sorafenib in a prostate tumor model.

[0082] FIG. **6** shows a graph depicting administration of DX-2240 followed by administration of bevacizumab in a prostate tumor model.

[0083] FIG. **7** shows a graph depicting administration of DX-2240 followed by bevacizumab on various schedules in a pancreatic tumor model.

[0084] FIG. **8** shows a graph depicting administration of DX-2240 followed by bevacizumab administered 10 or 20 days later in a pancreatic tumor model.

[0085] FIG. **9** shows a graph depicting administration of DX-2240 with bevacizumab administered on the same day or 5 days later in a pancreatic tumor model.

[0086] FIG. **10** shows lectin staining of tumors from DX-2240 treated mice.

[0087] FIG. **11** shows a graph depicting administration of DX-2240 followed by various doses of bevacizumab 15 days later in a prostate tumor model.

[0088] FIG. **12** shows a graph depicting administration of DX-2240 followed by bevacizumab 15 days later in a prostate tumor model.

DETAILED DESCRIPTION

[0089] The inventors have surprisingly found that administration of a Tie1 ectodomain-binding agent and a VEGF antagonist agent results in a synergistic effect when one agent is administered for a sufficient period of time before the administration of the second agent. For example, a synergistic effect occurs when a Tie1 ectodomain-binding agent is administered before the administration of a VEGF antagonist agent. Accordingly, provided herein are methods of treating (e.g., ameliorating at least one symptom of) an angiogenesis-related disorders by administration of a Tie1 ectodomain-binding agent and a VEGF antagonist agent to a subject at

different times. For example, in one aspect, methods of treating (e.g., ameliorating at least one symptom of) an angiogenesis-related disorder in a subject includes first administering a Tie1 ectodomain-binding agent followed by administration of a VEGF antagonist.

[0090] Administration of Tie1 ectodomain-binding agents (e.g., DX-2240 or DX-2220) results in the down-modulation of Tie1/Tie2 heterodimers from the surface of cells expressing Tie1 and Tie2, e.g., endothelial cells. Accordingly, the invention also provides methods for ameliorating at least one symptom of an angiogenesis-related disorders by first administering a Tie1 ectodomain-binding agent, followed by administration of a VEGF antagonist agent.

[0091] The term "treat" or "treatment" refers to the application or administration of an agent, alone or in combination with one or more other agents (e.g., a second agent) to a subject, e.g., a patient, e.g., a patient who has a disorder (e.g., a disorder as described herein), a symptom of a disorder or a predisposition for a disorder or a predisposition toward the disorder, e.g., to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. Treating a cell refers to a reduction in an activity of a cell, e.g., ability of an endothelial cell to form tubes or vessels. A reduction does not necessarily require a total elimination of activity, but a reduction, e.g., a statistically significant reduction, in the activity or the number of the cell.

[0092] As used herein, the term "antibody" refers to a protein that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term "antibody" encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab fragments, $F(ab')_2$, a Fd fragment, a Fv fragments, and dAb fragments) as well as complete antibodies.

[0093] The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" (CDR), interspersed with regions that are more conserved, termed "framework regions" (FR). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E. A., et al. (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917). Kabat definitions are used herein. Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0094] An "immunoglobulin domain" refers to a domain from the variable or constant domain of immunoglobulin molecules. Immunoglobulin domains typically contain two β-sheets formed of about seven β-strands, and a conserved disulphide bond (see, e.g., A. F. Williams and A. N. Barclay 1988 *Ann. Rev Immunol.* 6:381-405). The canonical structures of hypervariable loops of an immunoglobulin variable can be inferred from its sequence, as described in Chothia et al. (1992) *J. Mol. Biol.* 227:799-817; Tomlinson et al. (1992) *J. Mol. Biol.* 227:776-798); and Tomlinson et al. (1995) EMBO J. 14(18):4628-38.

[0095] As used herein, an "immunoglobulin variable domain sequence" refers to an amino acid sequence which can form the structure of an immunoglobulin variable domain. For example, the sequence may include all or part of the amino acid sequence of a naturally-occurring variable domain. For example, the sequence may omit one, two or more N- or C-terminal amino acids, internal amino acids, may include one or more insertions or additional terminal amino acids, or may include other alterations. In one embodiment, a polypeptide that includes immunoglobulin variable domain sequence can associate with another immunoglobulin variable domain sequence to form a target binding structure (or "antigen binding site"), e.g., a structure that interacts with Tie1, e.g., binds to or inhibits Tie1.

[0096] The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. The heavy chain constant region includes three domains, CH1, CH2 and CH3. The light chain constant region includes a CL domain. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system. The term "antibody" includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). The light chains of the immunoglobulin may be of types kappa or lambda. In one embodiment, the antibody is glycosylated. An antibody can be functional for antibody-dependent cytotoxicity and/or complement-mediated cytotoxicity.

[0097] The term "monospecific antibody" refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope. This term includes a "monoclonal antibody" which refers to an antibody that is produced as a single molecular species, e.g., from a population of homogenous isolated cells. A "monoclonal antibody composition" refers to a preparation of antibodies or fragments thereof of in a composition that includes a single molecular species of antibody. In one embodiment, a monoclonal antibody is produced by a mammalian cell. One or more monoclonal antibody species may be combined.

[0098] One or more regions of an antibody can be human or effectively human. For example, one or more of the variable regions can be human or effectively human. For example, one or more of the CDRs can be human, e.g., HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3. Each of the light chain CDRs can be human. HC CDR3 can be human. One or more of the framework regions can be human, e.g., FR1, FR2, FR3, and FR4 of the HC or LC. In one embodiment, all the framework regions are human, e.g., derived from a human somatic cell, e.g., a hematopoietic cell that produces immunoglobulins or a non-hematopoietic cell. In one embodiment, the human sequences are germline sequences, e.g., encoded by a germline nucleic acid. One or more of the constant regions can be human or effectively human. In another embodiment, at least 70, 75, 80, 85, 90, 92, 95, or 98% of the framework regions (e.g., FR1, FR2, and FR3, collectively, or FR1, FR2, FR3, and FR4, collectively) or the entire antibody can be human or effectively human. For

example, FR1, FR2, and FR3 collectively can be at least 70, 75, 80, 85, 90, 92, 95, 98, or 99% identical to a human sequence encoded by a human germline V segment of a locus encoding a light or heavy chain sequence.

[0099] All or part of an antibody can be encoded by an immunoglobulin gene or a segment thereof. Exemplary human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin light chains (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH— terminus. Full-length immunoglobulin heavy chains (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). A light chain refers to any polypeptide that includes a light chain variable domain. A heavy chain refers to any polypeptide that a heavy chain variable domain.

[0100] The term "antigen-binding fragment" of a full-length antibody (or simply "antibody portion," or "fragment"), as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to a target of interest. Examples of binding fragments encompassed within the term "antigen-binding fragment" of a full length antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR) that retains functionality. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules known as single chain Fv (scFv). See e.g., Bird et al. (1988) *Science* 242:423-426; and Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883.

[0101] Antibody fragments can be obtained using any appropriate technique including conventional techniques known to those with skill in the art. The term "monospecific antibody" refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope. This term includes a "monoclonal antibody" or "monoclonal antibody composition," which as used herein refer to a preparation of antibodies or fragments thereof of single molecular composition. As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

[0102] In one embodiment, the HC or LC of an antibody includes sequences that correspond to an amino acid sequence encoded by a human germline sequence, e.g., the framework regions and/or in the CDRs. For example, the antibody can include sequences from the human DP47 antibody. In one embodiment, one or more codons for the antibody are altered relative to the germline nucleic acid sequence, but are chosen to encode the same amino acid sequence. Codons can be selected, e.g., to optimize expression in a particular system, create restriction enzyme sites, create a silent fingerprint, etc.

[0103] A "humanized" immunoglobulin variable region is an immunoglobulin variable region that includes sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human. Descriptions of "humanized" immunoglobulins include, for example, U.S. Pat. No. 6,407,213 and U.S. Pat. No. 5,693,762.

[0104] An "effectively human" immunoglobulin variable region is an immunoglobulin variable region that includes a sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human. An "effectively human" antibody is an antibody that includes a sufficient number of human amino acid positions such that the antibody does not elicit an immunogenic response in a normal human.

[0105] As used herein, "Tie complex" refers to either a heteromeric complex that includes Tie1 and Tie2 (and may include an angiopoietin (Ang)) or a homomeric complex of Tie1. The heteromeric Tie complex is formed in part by association of the extracellular domains of Tie1 and Tie2 and may also include Ang. As used herein, "complex members" refers to the proteins that are included in a heteromeric Tie complex. Accordingly, Tie1 and Tie2, and optionally Ang, are all complex members. The term "Ang" includes all angiopoietins, such as Ang1, Ang2, Ang3, and Ang4. The heteromeric Tie complex can include other proteins in addition to Tie1, Tie2, and Ang.

[0106] "Angiogenesis" includes all stages of vessel development (e.g., blood or lymphatic vessel development), including initial vessel formation and later vessel remodeling and morphological changes.

[0107] As used herein, the terms "agonist" and "antagonist" describe properties in context of a particular activity or effect. For example, the E3 or E3b antibody can be an agonist in the context of promoting Tie1 self-association (e.g., homodimerization), yet an antagonist in the context of decreasing or inhibiting Tie complex formation and tube formation by human umbilical vein endothelial cells (HUVECs). Likewise, an agent that is an agonist in the context of a Tie1 signaling pathway can be an antagonist in the context of endothelial cell sprouting, splitting, and tube formation.

[0108] The term "Tie1 ectodomain" refers to an extracellular region of a Tie1 protein, e.g., a region that includes about amino acids 25-759 of FIG. **1** (SEQ ID NO: 1). Other exemplary regions are regions that include one or more EGF-like domains (e.g., 214-256, 258-303, 303-345, 214-303, 258-345, or 214-345 of FIG. **1**); one or more Ig-Like C2-type domains (e.g., 43-105, 43-426, 372-426); one or more Fibronectin Type III repeats (e.g., 446-540, 543-639, 643-744, 446-639, 543-744, or 446-744 of FIG. **1**); and combinations thereof. The terms "first Ig-like C2-type domain" and "Ig 1" refer to the immunoglobulin-like domain in Tie1 or Tie2 that is located closest to the amino terminus of the protein relative to the other Ig-like C2-type domain (the second such domain). For example, for Tie1, the first Immunoglobulin-like C2-type domain is located at about residue 43 to about residue 105 and the second Ig-like C2-type domain is located at about residue 372 to about residue 426.

[0109] As used herein, "binding affinity" refers to the apparent association constant or K_a. The K_a is the reciprocal of the dissociation constant (K_d). A ligand may, for example,

have a binding affinity of at least 10^5, 10^6, 10^7 or 10^8 M^{-1} for a particular target molecule. Higher affinity binding of a ligand to a first target relative to a second target can be indicated by a higher K_a (or a smaller numerical value K_d) for binding the first target than the K_a (or numerical value K_d) for binding the second target. In such cases the ligand has specificity for the first target relative to the second target. Differences in binding affinity (e.g., for specificity or other comparisons) can be at least 1.5, 2, 5, 10, 50, 100, or 1000-fold. For example, a Tie1 ectodomain-binding agent may preferentially bind to Tie1 at least 1.5, 2, 5, 10, 50, 100, or 1000-fold better than to another antigen, e.g., Tie2, EGF, fibronectin, or human serum albumin.

[0110] Binding affinity can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (e.g., using a fluorescence assay). These techniques can be used to measure the concentration of bound and free ligand as a function of ligand (or target) concentration. The concentration of bound ligand ([Bound]) is related to the concentration of free ligand ([Free]) and the concentration of binding sites for the ligand on the target where (N) is the number of binding sites per target molecule by the following equation:

$$[\text{Bound}]=N\cdot[\text{Free}]/((1/Ka)+[\text{Free}])$$

[0111] Although quantitative measurements of Ka are routine, it is not always necessary to make an exact determination of K_a, though, since sometimes it is sufficient to obtain a qualitative measurement of affinity, e.g., determined using a method such as ELISA or FACS analysis, is proportional to K_a, and thus can be used for comparisons, such as determining whether a higher affinity is, e.g., 2, 5, 10, 20, or 50 fold higher than a reference. Binding affinity is typically evaluated in 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA and 0.005% (v/v) surfactant P20.

[0112] An "isolated composition" refers to a composition that is removed from at least 90% of at least one component of a natural sample from which the isolated composition can be obtained. Compositions produced artificially or naturally can be "compositions of at least" a certain degree of purity if the species or population of species of interests is at least 5, 10, 25, 50, 75, 80, 90, 95, 98, or 99% pure on a weight-weight basis.

[0113] An "epitope" refers to the site on a target compound that is bound by a ligand, e.g., an antigen-binding protein (e.g., a Fab or antibody). In the case where the target compound is a protein, for example, an epitope may refer to the amino acids that are bound by the ligand. Overlapping epitopes include at least one common amino acid residue.

[0114] As used herein, the term "substantially identical" (or "substantially homologous") is used herein to refer to a first amino acid or nucleotide sequence that contains a sufficient number of identical or equivalent (e.g., with a similar side chain, e.g., conserved amino acid substitutions) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have similar activities. In the case of antibodies, the second antibody has the same specificity and has at least 50% of the affinity of the same.

[0115] Sequences similar or homologous (e.g., at least about 85% sequence identity) to the sequences disclosed herein are also part of this application. In some embodiment, the sequence identity can be about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. Alternatively, substantial identity exists when the nucleic acid segments will hybridize under selective hybridization conditions (e.g., highly stringent hybridization conditions), to the complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

[0116] Calculations of "homology" or "sequence identity" between two sequences (the terms are used interchangeably herein) are performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0117] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch ((1970) *J. Mol. Biol.* 48:444-453) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation described herein) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0118] As used herein, the term "homologous" is synonymous with "similarity" and means that a sequence of interest differs from a reference sequence by the presence of one or more amino acid substitutions (although modest amino acid insertions or deletions) may also be present. Presently preferred means of calculating degrees of homology or similarity to a reference sequence are through the use of BLAST algorithms (available from the National Center of Biotechnology Information (NCBI), National Institutes of Health, Bethesda Md.), in each case, using the algorithm default or recommended parameters for determining significance of calculated sequence relatedness. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of E. Meyers and W. Miller ((1989)

CABIOS, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

[0119] The terms "polypeptide" or "peptide" (which may be used interchangeably) refer to a polymer of three or more amino acids linked by a peptide bond, e.g., between 3 and 30, 12 and 60, or 30 and 300, or over 300 amino acids in length. The polypeptide may include one or more unnatural amino acids. Typically, the polypeptide includes only natural amino acids. A "protein" can include one or more polypeptide chains. Accordingly, the term "protein" encompasses polypeptides. A protein or polypeptide can also include one or more modifications, e.g., a glycosylation, amidation, phosphorylation, and so forth. The term "small peptide" can be used to describe a polypeptide that is between 3 and 30 amino acids in length, e.g., between 8 and 24 amino acids in length.

[0120] Statistical significance can be determined by any art known method. Exemplary statistical tests include: the Students T-test, Mann Whitney U non-parametric test, and Wilcoxon non-parametric statistical test. Some statistically significant relationships have a P value of less than 0.05, or 0.02. Particular ligands may show a difference, e.g., in specificity or binding that are statistically significant (e.g., P value <0.05 or 0.02).

[0121] "Angiogenesis-dependent cancers and tumors" are cancers and tumors that require, for their growth (expansion in volume and/or mass), an increase in the number and density of the blood vessels supplying them with blood.

[0122] "Regression" refers to the reduction of tumor mass and size, e.g., a reduction of at least 2, 5, 10, or 25%.

[0123] "Sensitizing tumor vasculature to a decrease in VEGF" refers to conditions in which the vascular demonstrates decreased growth and/or functionality when exposed to an agent that targets or negatively regulates a VEGF signaling pathway (e.g., a VEGF antagonist) then when the vasculature is exposed to the agents in the absence of the conditions.

[0124] As used herein, the term "a change" in tumor vasculature refers to a change in the physiology and/or function of a blood vessel within or surrounding a tumor. The change can be, but is not limited to, one or more of: a decrease or increase in tumor vascular growth, blood flow, blood volume, vessel permeability, tumor metabolism, microvessel density (MVD), pericyte coverage, or intratumoral pressure (i.e., interstitial fluid pressure or IFP). Decreases in tumor metabolism, pericyte coverage and blood flow indicate a decrease in vessel function. Increases in vessel permeability and IFP indicate a decrease in vessel function.

[0125] The followings are examples of known techniques that can be used to assess a change in tumor vasculature. Computed tomography (CT) performed with contrast agents can measure blood flow, blood volume and vessel permeability. Ultrasound such as Doppler ultrasound can also evaluate blood flow and blood volume. Magnetic resonance imaging (MRI) performed with contrast agents can measure blood volume and vessel permeability. In addition, positron emission tomography (PET), using a number of radiotracers such as H_2O^{15}, ^{11}CO and ^{18}FDG, can determine blood flow, blood volume and tumor metabolism. Endoscopy techniques such as flexible sigmoidoscopies can be used to measure IFP. Staining tumor biopsies with various antibodies can also evaluate a change in tumor vasculature. Using antibodies against, for example, PECAM, CD34 or von Willebrand Factor to visualize vessels, the number of vessels per micrometer square in areas of a tumor, i.e., MVD, can be determined. Double staining tumor biopsies with antibodies against PECAM and α-smooth muscle actin or NG2 can assess pericyte coverage of vessels.

[0126] The methods described herein can include monitoring tumor vasculature after the administration of a first agent and initiating administration of the second agent upon an indication that a change has occurred in the tumor vasculature. For example, in some aspects, the tumor vasculature in a subject can be monitored after the initiating of administration of a Tie1 ectodomain-binding agent (e.g., DX-2240 or DX-2220). Upon an indication of a change in tumor vasculature, administration of a VEGF antagonist (e.g., bevacizumab) can be initiated.

Tie1 Ectodomain-Binding Agents

[0127] Tie1 ectodomain-binding agents useful in accordance with the invention bind to an epitope of the Tie1 (e.g., human Tie1 ectodomain). In some embodiments, the Tie1 ectodomain-binding agent increases Tie complex formation. In some embodiments, the Tie1 ectodomain-binding agent increases tyrosine phosphorylation of Tie1. In some embodiments, the Tie1 ectodomain-binding agent induces down modulation of Tie1 from the surface of the cell.

[0128] Exemplary Tie1 ectodomain-binding proteins have been previously disclosed (see, e.g., U.S. Pat. No. 5,955,291 and U.S. Patent Publications Nos. 2005/0136053, 2006/0024297, and 2006/0057138, especially FIGS. 7-39 and Examples 28-30 of U.S. 2006/0057138), and include E3, E3b, M0044-A06; M0044-A11; M0044-B04; M0044-B05; M0044-B08; M0044-B09; M0044-B10; M0044-B12; M0044-C07; M0044-D01; M0044-E03; M0044-F03; M0044-F06; M0044-F09; M0044-G06; M0044-G07; M0044-G11; M0044-H03; M0044-H05; M0044-H07; M0044-H09; M0045-A02; M0045-A04; M0045-B01; M0045-B03; M0045-B11; M0045-C02; M0045-C11; M0045-C12; M0045-D01; M0045-D07; M0045-G01; M0045-G10; M0046-A11; M0046-B06; M0046-B10; M0046-G12; M0046-H03; M0046-H10; M0046-H11; M0047-B03; M0047-D01; M0047-D03; M0047-E10; M0047-G09; M0053-A02; M0053-A03; M0053-A05; M0053-A09; M0053-B09; M0053-B11; M0053-D03; M0053-D06; M0053-D12; M0053-E03; M0053-E04; M0053-E08; M0053-F04; M0053-F05; M0053-F06; M0053-F08; M0053-G04; M0053-G05; M0054-A08; M0054-B06; M0054-B08; M0054-C03; M0054-C07; M0054-E04; M0054-G01; M0054-G05; M0054-H10; M0055-A09; M0055-B11; M0055-B12; M0055-C05; M0055-C07; M0055-D03; M0055-D06; M0055-D12; M0055-E04; M0055-E06; M0055-E10; M0055-E12; M0055-F10; M0055-G02; M0055-G03; M0055-H04; M0056-A01; M0056-A06; M0056-B08; M0056-B09; M0056-C03; M0056-C04; M0056-E08; M0056-F01; M0056-F02; M0056-F10; M0056-F11; M0056-G03; M0056-G04; M0056-G08; M0056-G12; M0056-H04; M0056-H12; M0057-B05; M0057-H07; M0058-A09; M0058-D04; M0058-E09; M0058-F03; M0058-G03; M0058-H01; M0059-A02; M0059-A06; M0060-B02; M0060-H01; M0061-A03; M0061-C05; M0061-C06; M0061-F07; M0061-G12; M0061-H09; M0062-A 12; M0062-B05; M0062-B07; M0062-C08; M0062-D04; M0062-E02; M0062-E03; M0062-E11; M0062-F10; M0062-G06; and M0062-H01. Antibody E3 and variants thereof (e.g., DX-2220, DX-2240) and M0044-B08 induce

Tie complex formation, Tie1 tyrosine phosphorylation, and down modulation of Tie1 from the cell surface.

[0129] Additional or alternate Tie1 ectodomain-binding proteins may be isolated using techniques known in the art, including monoclonal antibody production from hybridomas made from B cells isolated from immunized animals (e.g., mice) or selection of display libraries. Display libraries useful for identifying Tie1 ectodomain-binding agents may display peptides (e.g., structured peptides, such as peptides constrained by a disulphide bond; see, e.g., U.S. Patent Publication No. 2006/0084113), or antibodies (e.g., Fabs; see, e.g., Hoet et al., 2005, *Nat. Biotech.* 23(3):344-48). Tie1 ectodomain (or a portion thereof, such as an EGF domain, a fibronectin repeat, or an Ig-superfamily domain (e.g., a Ig-like C2-type 2 domain)) may be used to identify display library members which bind to the Tie1 ectodomain. For example, Tie1 ectodomain may be recombinantly expressed, attached to a support, then mixed with the display library (e.g., a phage library displaying antibodies). Those members of the library which bind to the Tie1 ectodomain target are then isolated and further characterized. Such techniques are known in the art and are described in U.S. Patent Publication No. 2006/0057138.

[0130] Activity of additional/alternate Tie1 ectodomain-binding agents may be assayed using a variety of assays, including the Tie1/EpoR chimeric BaF3 cell assay described in Example 2 of U.S. 2006/0057138. Additional assays include tubulogenesis assays (e.g., Jones M K et al., 1999, Nature Medicine 5:1418-1423), measurements of Tie1 ectodomain-binding agent-induced tyrosine phosphorylation of Tie1 (e.g., phosphorylation of the tyrosine in the motif YVN at about amino acid 1117), as well as in vivo models (e.g., tumor xenograft or orthotopic tumor grafting).

[0131] Tie1 ectodomain-binding antibodies may be modified in order to make the variable regions of the antibody more similar to one or more germline sequences. For example, an antibody can include one, two, three or more amino acid substitutions, e.g., in a framework or CDR region, to make it more similar to a reference germline sequence. Exemplary germline reference sequences for Vkappa include: O12/O2, O18/O8, A20, A30, L14, L1, L15, L4/18a, L5/L19, L8, L23, L9, L24, L11, L12, O11/O1, A17, A1, A18, A2, A19/A3, A23, A27, A11, L2/L16, L6, L20, L25, B3, B2, A26/A10, and A14. See, e.g., Tomlinson et al. (1995) EMBO J. 14(18):4628-3. A germline reference sequence for the HC variable domain can be based on a sequence that has particular canonical structures, e.g., 1-3 structures in the H1 and H2 hypervariable loops. The canonical structures of hypervariable loops of an immunoglobulin variable domain can be inferred from its sequence, as described in Chothia et al. (1992) *J. Mol. Biol.* 227:799-817; Tomlinson et al. (1992) *J. Mol. Biol.* 227:776-798); and Tomlinson et al. (1995) EMBO J. 14(18):4628-38. Exemplary sequences with a 1-3 structure include: DP-1, DP-8, DP-12, DP-2, DP-25, DP-15, DP-7, DP-4, DP-31, DP-32, DP-33, DP-35, DP-40, 7-2, hv3005, hv3005f3, DP-46, DP-47, DP-58, DP-49, DP-50, DP-51, DP-53, and DP-54.

[0132] In some embodiments, the Tie1 ectodomain-binding agent is an aptamer. The term nucleic acid "aptamer," as used herein, refers to a nucleic acid molecule which has a conformation that includes an internal non-duplex nucleic acid structure of at least 5 nucleotides. An aptamer can be a single-stranded nucleic acid molecule which has regions of self-complementarity.

[0133] Aptamers can be screened in vitro since a selected aptamer can be recovered by standard nucleic acid amplification procedures. The method can be enhanced, e.g., in later rounds of selection, by splitting selected aptamers into pools and modifying each aptamer in the pool with a detectable label such as a fluorophore. Pools having aptamers that functionally alter the properties of the label can be identified. Such pools can be repeatedly split and reanalyzed to identify the individual aptamers with the desired properties (see, e.g., Jhaveri et al. *Nature Biotechnol.* 18:1293).

[0134] In addition, aptamers can be screened for activity in vivo. For example, shuffled nucleic acids can be cloned into an expression vector that is introduced into cells. RNA aptamers resulting from the expressed shuffled nucleic acids can be screened for a biological activity. Cells having the activity can be isolated and the expression vector for the selected RNA aptamer recovered.

[0135] An important feature of therapeutic oligomers (e.g., aptamers) is the design of the backbone of the administered oligomer. In some embodiments, the backbone contains internucleoside linkages that are stable in vivo and is structured such that the oligomer is resistant to endogenous nucleases, such as nucleases that attack the phosphodiester linkage. At the same time, the oligomer retains its ability to hybridize to the target DNA or RNA (Agarwal, K. L. et al. (1979) *Nucleic Acids Res.* 6:3009; Agarwal, S. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:7079). Modified oligonucleotides can be constructed using alternate internucleoside linkages. Several of these exemplary linkages are described in Uhlmann, E. and Peyman, A. (1990) *Chemical Reviews* 90:543-584. Among these are methylphosphonates (wherein one of the phosphorus-linked oxygens has been replaced by methyl); phosphorothioates (wherein sulphur replaces one of these oxygens) and various amidates (wherein NH_2 or an organic amine derivative, such as morpholidates or piperazidates, replace an oxygen). These substitutions confer enhanced stability. WO 91/15500 teaches various oligonucleotide analogs in which one or more of the internucleotide linkages are replaced by a sulfur based linkage, typically sulfamate diesters, which are isosteric and isoelectric with the phosphodiester. WO 89/12060 similarly discloses linkages containing sulfides, sulfoxides, and sulfones. WO 86/05518 suggests a variant of stereoregular polymeric 3',5'linkages. U.S. Pat. No. 5,079,151 discloses a msDNA molecule of branched RNA linked to a single strand DNA via a 2',5' phosphodiester linkage. U.S. Pat. No. 5,264,562 describes modified linkages of the formula —Y'CX'$_2$ Y'— wherein Y' is independently O or S and wherein each X' is a stabilizing substituent and independently chosen. Morpholino-type internucleotide linkages are described in U.S. Pat. No. 5,034,506 and in some cases give rise to an increased affinity of the oligomer for complementary target sequences. U.S. Pat. Nos. 5,264,562 5,596,086 disclose modified oligonucleotides having modified nucleoside linkages which are capable of strong hybridization to target RNA and DNA.

VEGF Antagonist Agents

[0136] VEGF antagonist agents for use in the instant invention are VEGF pathway antagonists. VEGF pathway antagonists include inhibitors of a VEGF (e.g., VEGF-A, -B, or -C, for example bevacizumab), modulators of VEGF expression (e.g., INGN-241, oral tetrathiomolybdate, 2-methoxyestradiol, 2-methoxyestradiol nanocrystal dispersion, bevasiranib sodium, PTC-299, Veglin), inhibitors of a VEGF receptor

(e.g., KDR or VEGF receptor III (Flt4), for example anti-KDR antibodies, VEGFR2 antibodies such as CDP-791, IMC-1121B, VEGFR2 blockers such as CT-322), VEGFR3 antibodies such as hF4-3C5, modulators of VEGFR expression (e.g., VEGFR1 expression modulator Sirna-027) or inhibitors of VEGF receptor downstream signaling.

[0137] Exemplary inhibitors of VEGF include bevacizumab, pegaptanib, ranibizumab, NEOVASTAT®, AE-941, VEGF Trap, and PI-88.

[0138] Exemplary VEGF receptor antagonists include inhibitors of VEGF receptor tyrosine kinase activity. 4-[4-(1-Amino-1-methylethyl)phenyl]-2-[4-(2-morpholin-4-yl-ethyl)phenylamino]pyrimidine-5-carbonitrile (JNJ-17029259) is one of a structural class of 5-cyanopyrimidines that are orally available, selective, nanomolar inhibitors of the vascular endothelial growth factor receptor-2 (VEGF-R2). Additional examples include: PTK-787/ZK222584 (Astra-Zeneca), SU5416, SU11248 (Pfizer), and ZD6474 ([N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine]), vandetanib, cediranib, AG-013958, CP-547632, E-7080, XL-184, L-21649, and ZK-304709. Other VEGF antagonist agents are broad specificity tyrosine kinase inhibitors, e.g., SU6668 (see, e.g., Bergers, B. et al., 2003 J. Clin. Invest. 111:1287-95), sorafenib, sunitinib, pazopanib, vatalanib, AEE-788, AMG-706, axitinib, BIBF-1120, SU-14813, XL-647, XL-999, ABT-869, BAY-57-9352, BAY-73-4506, BMS-582664, CEP-7055, CHIR-265, OSI-930, and TKI-258. Also useful are agents that down regulate VEGF receptors on the cell surface, such as fenretinide, and agents which inhibit VEGF receptor downstream signaling, such as squalamine

[0139] Protein Production

[0140] Standard recombinant nucleic acid methods can be used to express Tie1 ectodomain-binding proteins and VEGF antagonist agents which are proteins. See, for example, the techniques described in Sambrook & Russell, *Molecular Cloning: A Laboratory Manual,* 3rd Edition, Cold Spring Harbor Laboratory, N.Y. (2001) and Ausubel et al., Current Protocols in Molecular Biology (Greene Publishing Associates and Wiley Interscience, N.Y. (1989). Generally, a nucleic acid sequence encoding the binding protein is cloned into a nucleic acid expression vector. If the protein includes multiple polypeptide chains, each chain can be cloned into an expression vector, e.g., the same or different vectors, that are expressed in the same or different cells. Methods for producing antibodies are also provided below.

[0141] Some antibodies, e.g., Fabs, can be produced in bacterial cells, e.g., *E. coli* cells. For example, if the Fab is encoded by sequences in a phage display vector that includes a suppressible stop codon between the display entity and a bacteriophage protein (or fragment thereof), the vector nucleic acid can be shuffled into a bacterial cell that cannot suppress a stop codon. In this case, the Fab is not fused to the gene III protein and is secreted into the media.

[0142] Antibodies can also be produced in eukaryotic cells. In one embodiment, the antibodies (e.g., scFv's) are expressed in a yeast cell such as *Pichia* (see, e.g., Powers et al. (2001) *J Immunol Methods.* 251:123-35), *Hanseula*, or *Saccharomyces.*

[0143] In one embodiment, antibodies are produced in mammalian cells. Preferred mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO cells) (including dhfr− CHO cells, described in Urlaub and Chasin (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp (1982) *Mol. Biol.* 159:601-621), lymphocytic cell lines, e.g., NS0 myeloma cells, SP2 cells, COS cells, HEK 293T cells, and a cell from a transgenic animal, e.g., a transgenic mammal. For example, the cell is a mammary epithelial cell.

[0144] In addition to the nucleic acid sequence encoding the immunoglobulin domain, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically, the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr$^-$ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection). Another exemplary expression system is the glutamine synthase (GS) vector system available from Lonza Group Ltd. CH (see, e.g., Clark et al. (2004) BioProcess International 2(4):48-52; Barnes et al. (2002) Biotech Bioeng. 81(6):631-639).

[0145] In an exemplary system for recombinant expression of an antibody, or antigen-binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr− CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G.

[0146] The codon usage can adapted to the codon bias of the host cell, e.g., for CHO cells it can be adapted for the codon bias *Cricetulus griseus* genes. In addition, regions of very high (>80%) or very low (<30%) GC content can be avoided where possible. During the optimization process following cis-acting sequence motifs were avoided: internal TATA-boxes; chi-sites and ribosomal entry sites; AT-rich or GC-rich sequence stretches; ARE, INS, CRS sequence elements; repeat sequences and RNA secondary structures; and (cryptic) splice donor and acceptor sites, branch points. Two STOP codons can be used to ensure efficient termination. The codon optimization of the sequence can be evaluated according to Sharp, P. M., Li, W. H., Nucleic Acids Res. 15 (3), 1987). The standard codon adaptation index (CAI) can be used. Rare codons include those with a quality class between 0-40.

[0147] Codon-altered (e.g., codon-optimized) sequences can be used to produce an antibody. An exemplary method includes providing a mammalian cell that includes an antibody-coding nucleic acid and expressing the nucleic acid in the cell, e.g., maintaining the cell under conditions in which the protein is expressed. The antibody-coding nucleic acid can be providing in a mammalian expression vector, e.g., a vector that is introduced into the cell. The cell can be a non-human mammalian cell, e.g., a CHO cell.

[0148] For antibodies that include an Fc domain, the antibody production system preferably synthesizes antibodies in which the Fc region is glycosylated. For example, the Fc domain of IgG molecules is glycosylated at asparagine 297 in the CH2 domain.

[0149] This asparagine is the site for modification with biantennary-type oligosaccharides. It has been demonstrated that this glycosylation is required for effector functions mediated by Fcγ receptors and complement C1q (Burton and Woof (1992) *Adv. Immunol.* 51:1-84; Jefferis et al. (1998) *Immunol. Rev.* 163:59-76). In a preferred embodiment, the Fc domain is produced in a mammalian expression system that appropriately glycosylates the residue corresponding to asparagine 297. The Fc domain can also include other eukaryotic post-translational modifications.

[0150] Antibodies can also be produced by a transgenic animal. For example, U.S. Pat. No. 5,849,992 describes a method of expressing an antibody in the mammary gland of a transgenic mammal. A transgene is constructed that includes a milk-specific promoter and nucleic acids encoding the antibody of interest and a signal sequence for secretion. The milk produced by females of such transgenic mammals includes, secreted-therein, the antibody of interest. The antibody can be purified from the milk, or for some applications, used directly.

[0151] It is also possible to produce antibodies that bind to Tie1 ectodomain by immunization, e.g., using an animal, e.g., with natural, human, or partially human immunoglobulin loci. Such an antibody can be of any allotype, e.g., a,z allotype, f allotype, or non-A allotype. Non-human antibodies can also be modified to include substitutions for human immunoglobulin sequences, e.g., consensus human amino acid residues at particular positions, e.g., at one or more of the following positions (preferably at least five, ten, twelve, or all): (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, and/or (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 78H, 91H, 92H, 93H, and/or 103H (according to the Kabat numbering). See, e.g., U.S. Pat. No. 6,407,213.

Pharmaceutical Compositions

[0152] The Tie1 ectodomain-binding agent and VEGF antagonist agent are typically administered in the methods of the invention as pharmaceutical compositions. A "pharmaceutical composition" of an agent is the agent formulated with a pharmaceutically acceptable carrier. Pharmaceutical compositions encompass labeled binding proteins (e.g., for in vivo imaging) as well as therapeutic compositions.

[0153] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the binding protein may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

[0154] A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S. M., et al. (1977) *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[0155] Pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. For Tie1 ectodomain-binding agents and VEGF antagonist agents that are proteins, the typical preferred formulations are in the form of injectable or infusible solutions, such as compositions similar to those used for administration of humans with antibodies. For such proteinaceous agents, the preferred mode of administration is typically parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In some embodiments, the proteinaceous Tie1 ectodomain-binding agent and/or VEGF antagonist agent is administered by intravenous infusion, e.g., at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m^2 or 7 to 25 mg/m^2, or by injection. In other embodiments, the proteinaceous Tie1 ectodomain-binding agent and/or VEGF antagonist agent is administered by intramuscular or subcutaneous injection.

[0156] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[0157] The Tie1 ectodomain-binding agent and/or VEGF antagonist agent can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the agent in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients

from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0158] The route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known. See, e.g., *Sustained and Controlled Release Drug Delivery Systems*, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0159] In certain embodiments, the binding protein may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound described herein by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[0160] Pharmaceutical compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a pharmaceutical can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Pat. No. 5,399,163, 5,383,851, 5,312,335, 5,064,413, 4,941,880, 4,790,824, or 4,596,556. Examples of implants and modules include: U.S. Pat. No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Pat. No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Pat. No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Pat. No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Pat. No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Pat. No. 4,475,196, which discloses an osmotic drug delivery system. Of course, many other such implants, delivery systems, and modules are also known.

[0161] In certain embodiments, a binding protein described herein can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic protein crosses the BBB (if desired), it can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may include one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V. V. Ranade (1989) *J. Clin. Pharmacol.* 29:685).

[0162] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms can be dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0163] The pharmaceutical compositions may be prepared using a therapeutically effective amount or a prophylactically effective amount of an target-binding protein described herein. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the binding protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects. A therapeutically effective amount preferably inhibits a measurable parameter, e.g., inflammation or tumor growth rate by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit a measurable parameter, e.g., cancer, can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition in vitro by assays known to the skilled practitioner.

[0164] Also within the scope of the invention are kits including (a) a Tie1 ectodomain-binding agent, (b) a VEGF antagonist agent, and (c) instructions for use in accordance with the methods disclosed herein. The instructions for therapeutic applications include suggested dosages and/or modes of administration in a patient with (a) a cancer or neoplastic disorder, (b) an inflammatory disorder (e.g., rheumatoid arthritis), or an ocular disorder. The kit can further contain at least one additional reagent, such as an additional therapeutic agent, (e.g., a cytotoxic chemotherapy agent) formulated as appropriate, in one or more separate pharmaceutical preparations.

Stabilization and Retention

[0165] In some embodiments, the Tie1 ectodomain-binding agent or VEGF antagonist agent is physically associated with a moiety that improves its stabilization and/or retention in circulation, e.g., in blood, serum, lymph, or other tissues.

[0166] For example, the Tie1 ectodomain-binding agent or VEGF antagonist agent can be associated with a polymer,

e.g., a substantially non-antigenic polymers, such as polyalkylene oxides or polyethylene oxides. Suitable polymers will vary substantially by weight. Exemplary polymers include polymers having molecular number average weights ranging from about 200 to about 35,000, from about 1,000 to about 15,000, and 2,000 to about 12,500, but can rang higher, (e.g., up to about 500,000 D), and in some embodiments is at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. The molecular weight chosen can depend upon the effective size of the conjugate to be achieved, the nature (e.g. structure, such as linear or branched) of the polymer, and the degree of derivatization.

[0167] Polymers useful for modification of the Tie1 ectodomain-binding agent or VEGF antagonist agent include water soluble polymers for, e.g., hydrophilic polyvinyl polymers, e.g. polyvinylalcohol and polyvinylpyrrolidone. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained. Additional useful polymers include polyoxyalkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene (Pluronics); polymethacrylates; carbomers; branched or unbranched polysaccharides which comprise the saccharide monomers D-mannose, D- and L-galactose, fucose, fructose, D-xylose, L-arabinose, D-glucuronic acid, sialic acid, D-galacturonic acid, D-mannuronic acid (e.g. polymannuronic acid, or alginic acid), D-glucosamine, D-galactosamine, D-glucose and neuraminic acid including homopolysaccharides and heteropolysaccharides such as lactose, amylopectin, starch, hydroxyethyl starch, amylose, dextrane sulfate, dextran, dextrins, glycogen, or the polysaccharide subunit of acid mucopolysaccharides, e.g. hyaluronic acid; polymers of sugar alcohols such as polysorbitol and polymannitol; heparin or heparan.

[0168] Other compounds can also be attached to the same polymer, e.g., a cytotoxin, a label, or another targeting agent, e.g., another target-binding agent or an unrelated agent. Mono-activated, alkoxy-terminated polyalkylene oxides (PAO's), e.g., monomethoxy-terminated polyethylene glycols (mPEG's); C_{1-4} alkyl-terminated polymers; and bis-activated polyethylene oxides (glycols) can be used for crosslinking. See, e.g., U.S. Pat. No. 5,951,974.

[0169] In its most common form poly(ethylene glycol), PEG, is a linear or branched polyether terminated with hydroxyl groups and having the general structure:

$$HO—(CH_2CH_2O)_n—CH_2CH_2—OH$$

PEG can be synthesized by anionic ring opening polymerization of ethylene oxide initiated by nucleophilic attack of a hydroxide ion on the epoxide ring. Particularly useful for polypeptide modification is monomethoxy PEG, mPEG, having the general structure:

$$CH_3O—(CH_2CH_2O)_n—CH_2CH_2—OH$$

For further description, see, e.g., Roberts et al. (2002) *Advanced Drug Delivery Reviews* 54:459-476.

[0170] The covalent crosslink can be used to attach a target-binding agent (e.g., a protein) to a polymer, for example, crosslinking to the N-terminal amino group and epsilon amino groups found on lysine residues, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. The polymer may be covalently bonded directly to the target-binding protein without the use of a multifunctional (ordinarily bifunctional) crosslinking agent. Covalent binding to amino groups is accomplished by known chemistries based upon cyanuric chloride, carbonyl diimidazole, aldehyde reactive groups (PEG alkoxide plus diethyl acetal of bromoacetaldehyde; PEG plus DMSO and acetic anhydride, or PEG chloride plus the phenoxide of 4-hydroxybenzaldehyde, activated succinimidyl esters, activated dithiocarbonate PEG, 2,4,5-trichlorophenylcloroformate or P-nitrophenylcloroformate activated PEG). Carboxyl groups can be derivatized by coupling PEG-amine using carbodiimide. Sulfhydryl groups can be derivatized by coupling to maleimido-substituted PEG (e.g. alkoxy-PEG amine plus sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) (WO 97/10847) or PEG-maleimide commercially available from Shearwater Polymers, Inc., Huntsville, Ala.). Alternatively, free amino groups on the binding protein (e.g. epsilon amino groups on lysine residues) can be thiolated with 2-imino-thiolane (Traut's reagent) and then coupled to maleimide-containing derivatives of PEG, e.g., as described in Pedley et al., Br. J. Cancer, 70: 1126-1130 (1994).

[0171] Functionalized PEG polymers that can be attached to a Tie1 ectodomain-binding agent or a VEGF antagonist agent are available, e.g., from Shearwater Polymers, Inc. (Huntsville, Ala.). Such commercially available PEG derivatives include, e.g., amino-PEG, PEG amino acid esters, PEG-hydrazide, PEG-thiol, PEG-succinate, carboxymethylated PEG, PEG-propionic acid, PEG amino acids, PEG succinimidyl succinate, PEG succinimidyl propionate, succinimidyl ester of carboxymethylated PEG, succinimidyl carbonate of PEG, succinimidyl esters of amino acid PEGs, PEG-oxycarbonylimidazole, PEG-nitrophenyl carbonate, PEG tresylate, PEG-glycidyl ether, PEG-aldehyde, PEG vinylsulfone, PEG-maleimide, PEG-orthopyridyl-disulfide, heterofunctional PEGs, PEG vinyl derivatives, PEG silanes, and PEG phospholides. The reaction conditions for coupling these PEG derivatives may vary depending on the Tie1 ectodomain-binding agent or a VEGF antagonist agent involved, the desired degree of PEGylation, and the PEG derivative utilized. Some factors involved in the choice of PEG derivatives include: the desired point of attachment (such as lysine or cysteine R-groups), hydrolytic stability and reactivity of the derivatives, stability, toxicity and antigenicity of the linkage, suitability for analysis, etc. Specific instructions for the use of any particular derivative are available from the manufacturer.

[0172] The conjugates of a Tie1 ectodomain-binding agent or a VEGF antagonist agent and a polymer can be separated from the unreacted starting materials, e.g., by gel filtration or ion exchange chromatography, e.g., HPLC. Heterologous species of the conjugates are purified from one another in the same fashion. Resolution of different species (e.g., containing one or two PEG residues) is also possible, e.g., due to the difference in the ionic properties of unreacted amino acids. See, e.g., WO 96/34015.

[0173] A target binding protein can also be physically associated with a protein that provides a stabilizing or retention function, e.g., an albumin, e.g., human serum albumin. US 2004/0171794 describes exemplary methods for physically associating a protein with serum albumin. For exemplary, human albumin sequences or fragments thereof, see EP 201 239, EP 322 094 WO 97/24445, WO95/23857 especially the mature form of human albumin as shown in SEQ ID NO:18 of US 2004/0171794 and WO 01/79480 or albumin from other vertebrates or fragments thereof, or analogs or variants of

these molecules or fragments thereof. Other exemplary human serum albumin proteins can include one or both of the following sets of point mutations Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to Ala, Lys-413 to Gln, and Lys-414 to Gln (see, e.g., International Publication No. WO95/23857, with reference to SEQ ID NO: 18 of US 2004/0171794).

Methods of Use

[0174] The invention provides methods for ameliorating at least one symptom of an angiogenesis-related disorder by initiating the administration of a Tie1 ectodomain-binding agent and a VEGF antagonist agent at different times. In some aspects, the Tie1 ectodomain-binding agent is administered and then the VEGF antagonist is administered. The Tie1 ectodomain-binding agent is administered for a period prior to the first administration of the VEGF antagonist agent, which period may range from 1 day up to 35 days (e.g., 5, 7, 10, 14, 20, 21, 28, or 30 days) prior to the first administration of the VEGF antagonist agent, or may be upon indication of a change in the tumor vasculature or of tumor growth. The period can be calculated based, e.g., upon the first administration of the Tie1 ectodomain-binding agent for a given treatment cycle.

[0175] In some embodiments, the subject is in need of reduced angiogenesis, or identified as such. For example, the subject has a neoplastic disorder, e.g., a metastatic cancer. For example, the subject has an angiogenesis-dependent cancer or tumor. The tumor can be a solid tumor, e.g., a tumor at least 1, 2, 3, 5, 8 or 10 mm in diameter. In one embodiment, the solid tumor has a hypoxic core. The method can include, prior to administering the antagonist, evaluating the subject and detecting a solid tumor in the subject.

[0176] In some embodiments, administration of the Tie1 ectodomain-binding agent is continued following the first administration of the VEGF antagonist agent, while in other embodiments, the administration of the Tie1 ectodomain-binding agent is discontinued upon initiation of VEGF antagonist agent administration.

[0177] In some embodiments, the Tie1 ectodomain-binding agent or the VEGF antagonist agent is administered in an amount effective to individually reduce angiogenesis in the subject or otherwise treat a disorder in a subject (e.g., ameliorate a symptom of the disorder). In other embodiments the Tie1 ectodomain-binding agent or the VEGF antagonist agent is administered in an amount that is less than an amount effective to individually reduce angiogenesis in the subject or otherwise treat or prevent a disorder in a subject. In other embodiments the VEGF antagonist agent is administered in an amount that is less than an amount effective to individually reduce angiogenesis in the subject or otherwise treat or prevent a disorder in a subject (e.g., when the VEGF antagonist agent is bevacizumab and the disorder is non-squamous, non-small cell lung cancer, dose of AVASTIN is less than 15 mg/kg). In some embodiments the Tie1 ectodomain-binding agent and the VEGF antagonist agent are administered in synergistically effective amounts (e.g., amounts which, when compared to either compound administered alone, result in a synergistic effect).

[0178] Tie1 ectodomain-binding agents (e.g., the Tie1 ectodomain-binding proteins disclosed herein) can potentiate the activity of an agent that targets the VEGF pathway (e.g., a VEGF-A-binding antibody such as bevacizumab). Accordingly, in one combination therapy for the treatment of cancer, the second therapy is an agent that inhibits VEGF pathway signaling, such as a VEGF-A-binding antibody (e.g., bevacizumab) which is administered at a dose that is less than the dose of the VEGF-pathway inhibiting agent when administered in the absence of a Tie1 ectodomain-binding protein. For example, when a Tie1 ectodomain-binding protein is used in a combination therapy with a VEGF antagonist (e.g., an anti-VEGF-A antibody), the dose of the VEGF antagonist may be reduced from the dose of the VEGF antagonist when administered not in combination with Tie1 ectodomain-binding protein (e.g., is at least 10%, 25%, 40%, or 50% less than the dose of the VEGF antagonist agent when administered not in combination with a Tie1 ectodomain-binding protein). For example, the dose of bevacizumab (AVASTIN®), when administered in a combination therapy with Tie1 ectodomain-binding protein (but without other chemotherapeutic agents) is less than about 15, 13.5, 11.25, 9, or 7.5 mg/kg. Bevacizumab dosage is reduced when administered in combination with chemotherapeutic agents (e.g., bolus-IFL (irinotecan 125 mg/m2 IV, 5-fluorouracil 500 mg/m2 IV, and leucovorin 20 mg/m2 IV given once weekly for 4 weeks every 6 weeks), FOLFOX4 (Day 1: oxaliplatin 85 mg/m2 and leucovorin 200 mg/m2 concurrently IV, then 5-FU 400 mg/m2 IV bolus followed by 600 mg/m2 continuously IV; Day 2: leucovorin 200 mg/m2 IV, then 5-FU 400 mg/m2 IV bolus followed by 600 mg/m2 continuously IV; repeated every 2 weeks), or 5-FU), to either 5 or 10 mg/kg. Accordingly, in combination, the dose of bevacizumab, when given in combination with a Tie1 ectodomain-binding protein as disclosed herein, may be reduced to less than about 10, 9, 7.5, 6, or 5 mg/kg (when the dose of bevacizumab in the absence of a Tie1 ectodomain-binding protein is 10 mg/kg) or less than about 5, 4.5, 3.75, 3, or 2.5 mg/kg (when the dose of bevacizumab in the absence of a Tie1 ectodomain-binding protein is 5 mg/kg).

[0179] Angiogenesis-related disorders include, but are not limited to, neoplastic disease (e.g., solid tumors, tumor metastases, and benign tumors, particularly neoplastic disease requiring a blood supply or angiogenesis); inflammatory disorders (e.g., rheumatoid arthritis, lupus, restenosis, psoriasis, graft v. host response, or multiple sclerosis); ocular angiogenic diseases, for example, retinal disorders (e.g., a proliferative retinopathy, such as diabetic retinopathy, ischemic retinopathy, or retinopathy of prematurity); choroidal neovascularization; lens neovascularization; corneal neovascularization; iridial neovascularization; or conjunctival neovascularization, macular degeneration (e.g., wet and/or dry forms of age-related macular degeneration), corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis; Osler-Webber Syndrome; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; and wound granulation.

[0180] Benign tumors include, but are not limited to hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas. Solid tumors include, but are not limited to malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, gastrointestinal (e.g., colon), and genitourinary tract (e.g., renal, urothelial cells), pharynx, as well as adenocarcinomas which include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, and pancreatic cancer. Still further examples of solid tumors that can be treated include: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastrointestinal system carcinomas, colon carcinoma, pancreatic cancer, breast cancer, genitourinary system carcinomas, ovarian cancer,

prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, endocrine system carcinomas, testicular tumor, lung carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

[0181] When the angiogenesis-related disorder is a neoplastic disorder, amelioration of a symptom of the disorder is elimination, reduction, stabilization, as determined by clinical measurements (e.g., MRI, CT, diagnostic radiometry (e.g., bone scan), and the like) in the amount of the neoplastic disorder (e.g., tumor size), or reduction in the growth rate or number of tumors (e.g., metastases). Other parameters that can be affected include activities of daily living, such as pain (e.g., patient reported pain using a visual or numerical scale).

[0182] In some embodiments, the angiogenesis-related disorder is an inflammatory disorder, e.g., rheumatoid arthritis, psoriasis, rheumatoid or rheumatic inflammatory disease, or other chronic inflammatory disorders, such as chronic asthma, arterial or post-transplantational atherosclerosis, and endometriosis. Other angiogenesis-related disorders that can be treated include those that have deregulated or undesired angiogenesis, such as ocular neovascularization, e.g., retinopathies (including diabetic retinopathy and age-related macular degeneration) hemangioblastoma, hemangioma, and arteriosclerosis.

[0183] Psoriasis is a chronic skin disease, characterized by scaling and inflammation. When psoriasis develops, typically patches of skin thicken, redden, and become covered with silvery scales, referred to as plaques. Psoriasis most often occurs on the elbows, knees, scalp, lower back, face, palms, and soles of the feet. The disease also may affect the fingernails, toenails, and the soft tissues inside the mouth and genitalia. About 10 percent of people with psoriasis have joint inflammation that produces symptoms of arthritis. Patients can be evaluated using a static Physician Global Assessment (sPGA), and receive a category score ranging from six categories between clear and very severe. The score is based on plaque, scaling, and erythema. The therapeutic methods herein can be used to achieve an improvement for at least one of these indicia.

[0184] Rheumatoid arthritis ("RA") is a chronic inflammatory disease that causes pain, swelling, stiffness, and loss of function, primarily the joints. RA frequently begins in the synovium, the membrane that surrounds a joint creating a protective sac. In many individuals suffering from RA, leukocytes infiltrate from the circulation into the synovium causing continuous abnormal inflammation (e.g., synovitis). Consequently, the synovium becomes inflamed, causing warmth, redness, swelling, and pain. The collagen in the cartilage is gradually destroyed, narrowing the joint space and eventually damaging bone. The inflammation causes erosive bone damage in the affected area. During this process, the cells of the synovium grow and divide abnormally, making the normally thin synovium thick and resulting in a joint that is swollen and puffy to the touch. RA can be assessed by a variety of clinical measures. Some exemplary indicia include the total Sharp score (TSS), Sharp erosion score, and the HAQ disability index. The therapeutic methods herein can be used to achieve an improvement for at least one of these indicia.

[0185] As used herein, an amount of a Tie1 ectodomain-binding agent or VEGF antagonist agent effective to treat (e.g., ameliorate at least one symptom of) a disorder, or a "therapeutically effective amount" refers to an amount of the Tie1 ectodomain-binding agent or VEGF antagonist agent which is effective, upon single or multiple-dose administration to a subject, in prolonging curing, alleviating, relieving or improving a subject with a disorder as described herein beyond that expected in the absence of such treatment. In some cases, a therapeutically effective amount can be ascertained by evaluating the ability of the binding agent to reduce tumor size of a xenograft in a nude mouse model relative to an untreated control mouse. As used herein, "inhibiting the growth" of a tumor or other neoplasm refers to slowing, interrupting, arresting or stopping its growth and metastases and does not necessarily indicate a total elimination of the neoplastic growth.

[0186] An exemplary, non-limiting range for a therapeutically effective amount of an antibody described herein is 0.1-20 mg/kg, more preferably 1-10 mg/kg. The target-binding antibody can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m^2 or about 5 to 30 mg/m^2. For Tie1 ectodomain-binding agents and VEGF antagonist agents smaller in molecular weight than an antibody, appropriate amounts can be proportionally less. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0187] Subjects that can be treated include human and non-human animals. For example, the human can be a human patient having a disorder characterized by abnormal cell proliferation or cell differentiation. The term "non-human animals" includes all vertebrates, e.g., non-mammals (such as chickens, amphibians, reptiles) and mammals, such as non-human primates, sheep, dog, cow, pig, etc.

[0188] Methods of administering Tie1 ectodomain-binding agents, VEGF antagonist agents and other agents (e.g., cytotoxic chemotherapy agents) are also described in "Pharmaceutical Compositions". Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used.

Combination Therapies

[0189] The treatment methods disclosed herein can be used in combination with one or more additional treatment modalities, including, but not limited to: surgery; radiation therapy, and chemotherapy.

[0190] With reference to the methods disclosed herein, the term "combination" refers to the use of one or more additional agents or therapies to treat the same patient, wherein the use or action of the agents or therapies overlap in time. The additional agents or therapies can be administered at the same time as the Tie1 ectodomain-binding protein and/or VEGF antagonist agent are administered, or sequentially in any order. Sequential administrations are administrations that are given at different times. The time between administration of the one agent and another agent can be minutes, hours, days, or weeks.

[0191] The additional agent or therapy can also be another anti-cancer agent or therapy. Nonlimiting examples of anti-cancer agents include, e.g., anti-microtubule agents, topoisomerase inhibitors, antimetabolites, mitotic inhibitors, alkylating agents, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis, radiation, and antibodies against other tumor-associated antigens (including naked antibodies, immunotoxins and radioconjugates). Examples of the particular classes of anti-cancer agents are provided in detail as follows: antitubulin/antimicrotubule, e.g., paclitaxel, vincristine, vinblastine, vindesine, vinorelbin, taxotere; topoisomerase I inhibitors, e.g., irinotecan, topotecan, camptothecin, doxorubicin, etoposide, mitoxantrone, daunorubicin, idarubicin, teniposide, amsacrine, epirubicin, merbarone, piroxantrone hydrochloride; antimetabolites, e.g., 5-fluorouracil (5-FU), methotrexate, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, cytarabine/Ara-C, trimetrexate, gemcitabine, acivicin, alanosine, pyrazofurin, N-Phosphoracetyl-L-Asparate=PALA, pentostatin, 5-azacitidine, 5-Aza 2'-deoxycytidine, ara-A, cladribine, 5-fluorouridine, FUDR, tiazofurin, N-[5-[N-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-N-methylamino]-2-thenoyl]-L-glutamic acid; alkylating agents, e.g., cisplatin, carboplatin, mitomycin C, BCNU=Carmustine, melphalan, thiotepa, busulfan, chlorambucil, plicamycin, dacarbazine, ifosfamide phosphate, cyclophosphamide, nitrogen mustard, uracil mustard, pipobroman, 4-ipomeanol; agents acting via other mechanisms of action, e.g., dihydrolenperone, spiromustine, and desipeptide; biological response modifiers, e.g., to enhance anti-tumor responses, such as interferon; apoptotic agents, such as actinomycin D; and anti-hormones, for example anti-estrogens such as tamoxifen or, for example antiandrogens such as 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl)propionanilide.

[0192] A combination therapy can include administering an agent that reduces the side effects of other therapies. The agent can be an agent that reduces the side effects of anti-cancer treatments. For example, the agent can be leucovorin (e.g., in combination with 5-fluorouracil). A combinational therapy can also include administering an agent that reduces the frequency of administration of other therapies. The agent can be an agent that decreases growth of tumor after the anti-cancer effects of other therapies have decreased.

[0193] The following examples are not to be construed as limiting.

EXAMPLES

Example 1

Exemplary Tie1 Ectodomain-Binding Antibody Sequences

[0194] The following are exemplary sequences of immunoglobulin light chain and heavy chain variable domains:

[0195] 806C-M0044-B08

806C-M0044-B08
L-Variable AA:
(SEQ ID NO:2)
QDIQMTQSPSFLSASVGDRVTISCRASQYISIYLNWYQQRPGEAPKLLIN

AASSLQSGDPSRFSGSGSGTDFTLTINSLQPDDFATYYCQQYKSYPLTFG

EGTKVEIK

-continued
L-Variable (DNA):
(SEQ ID NO:3)
CAAGACATCCAGATGACCCAGTCTCCATCCTTCCTGTCCGCATCTGTAGG

AGACAGAGTCACCATCTCTTGCCGGGCAAGTCAGTACATCAGCATATATT

TGAATTGGTATCAGCAGAGACCAGGGGAAGCCCCTAAACTCCTGATCAAT

GCTGCATCCAGTTTGCAAAGTGGGGACCCATCAAGGTTCAGTGGCAGTGG

ATCTGGGACAGATTTCACTCTCACCATCAACAGCCTGCAGCCTGATGATT

TTGCAACTTATTACTGCCAACAGTATAAGAGTTACCCCCTCACTTTCGGC

GAGGGGACCAAGGTGGAGATCAAA

H-Variable AA:
(SEQ ID NO:4)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSAYGMGWVRQAPGKGLEWVSV

ISPSGGQTSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTALYYCAGGD

RYGPLHYWGQGTLVTVSS

H-Variable (DNA):
(SEQ ID NO:5)
GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTC

TTTACGTCTTTCTTGCGCTGCTTCCGGATTCACTTTCTCTGCTTACGGTA

TGGGTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGGTTTCTGTT

ATCTCTCCTTCTGGTGGCCAGACTTCTTATGCTGACTCCGTTAAAGGTCG

CTTCACTATCTCTAGAGACAACTCTAAGAATACTCTCTACTTGCAGATGA

ACAGCTTAAGGGCTGAGGACACCGCCTTGTATTACTGTGCGGGAGGGGAC

AGGTATGGACCCTTGCACTACTGGGGCCAGGGAACCCTGGTCACCGTCTC

AAGC

[0196] DX-2220 is a full length, IgG1, germlined human anti-Tie1 antibody E3b. The sequence of DX-2220 is as follows:

DX2220 Light Chain Amino Acid Sequence:
(SEQ ID NO:6)
DIQMTQSPSSLSASVGDRVTITCRASQGIGHYLAWYQQKPGKVPKLLIYT

ASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQFNSYPHTFGQ

GTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV

DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG

LSSPVTKSFNRGEC

DX-2220 Heavy Chain Amino Acid Sequence:
(SEQ ID NO:7)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSMYGMVWVRQAPGKGLEWVSV

ISPSGGNTGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARAP

RGYSYGYYYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK

DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT

YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP

KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN

STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ

VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV

-continued

LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

An exemplary DX-2220 Light Chain Nucleotide Sequence:

(SEQ ID NO:8)

GGCGTGCACTCTGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGC

ATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCGAGTCAGGGCATTG

GCCATTATTTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCTC

CTGATCTATACTGCATCCACTTTGCAATCAGGGGTCCCATCTCGGTTCAG

TGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGC

CTGAAGATGTTGCAACTTATTACTGTCAACAGTTTAATAGTTACCCTCAC

ACCTTCGGCCAAGGGACACGACTGGAGATTAAACGAACTGTGGCTGCACC

ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG

CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA

CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGT

CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA

CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC

ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGA

GTGTTAATAA

An exemplary DX-2220 Heavy Chain Nucleotide Sequence:

(SEQ ID NO:9)

GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTC

TTTACGTCTTTCTTGCGCTGCTTCCGGATTCACTTTCTCTATGTACGGTA

TGGTTTGGGTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGGTTTCTGTT

ATCTCTCCTTCTGGTGGCAATACTGGTTATGCTGACTCCGTTAAAGGTCG

CTTCACTATCTCTAGAGACAACTCTAAGAATACTCTCTACTTGCAGATGA

ACAGCTTAAGGGCTGAGGACACTGCAGTCTACTATTGTGCGAGAGCCCCA

CGTGGATACAGCTATGGTTACTACTACTGGGGCCAGGGAACCCTGGTCAC

CGTCTCAAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCCT

CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG

GACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGAC

CAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCCGGACTCTACT

CCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC

TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA

AGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAG

CACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCC

AAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT

GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG

GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC

AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCT

GAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC

CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG

-continued

GTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAG

CCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGT

GGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG

CTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAA

GAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG

CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

TGA

Example 2

Sequence of DX-2240: Germlined F Allotyped E3 Antibody

[0197] DX-2240 (Light, Heavy—Variable, Constant).

Variable region:

(SEQ ID NO:10)

DIQMTQSPSSLSASVGDRVTITCRASQGIGHYLAWYQQKPGKVPKLLIYT

ASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQFNSYPHTFGQ

GTRLEIK

Light constant:

(SEQ ID NO:11)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG

NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK

SFNRGEC

DX-2240 Heavy variable:

(SEQ ID NO:12)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSMYGMVWVRQAPGKGLEWVSV

ISPSGGNTGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARAP

RGYSYGYYYWGQGTLVTVSS

Heavy constant (CH1, Hinge, CH2, CH3):

(SEQ ID NO:13)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV

HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP

KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS

HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK

EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC

LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW

QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0198] The light chain can optionally further include the following signal sequence: MGWSCIILFLVATATGVHS (SEQ ID NO:14). The heavy chain can optionally further include the following signal sequence: MGWSCIIL-FLVATATGAHS (SEQ ID NO:15).

Example 3

Evaluation of Different Dosing Schedules of a Tie1 Ectodomain-Binding Protein and VEGF Antagonist Agents in a Pancreatic Cancer Xenograft Model

[0199] The antitumor activity of combinations of DX-2240 and bevacizumab (anti-VEGF antibody) or sorafenib (multi-

kinase inhibitor) were tested in a human tumor xenograft model. BxPC3 (human pancreatic cancer cell line) tumor fragments harvested from host nude mice (nu/nu) bearing subcutaneous BxPC-3 tumors were implanted subcutaneously into female nude mice (study animals) and allowed to form tumors. Mice were pair matched by tumor size and randomized into the various treatment groups when the tumors reached approximately 100 mm^3 (about 18 days following tumor implantation). The animals were assorted into the treatment groups shown in Table 1 ("Start" refers to the day on which treatment was begun). DX-2240 was administered by intraperitoneal (IP) injection at 20 mg/kg; bevacizumab was administered by IP injection at 10 mg/kg, sorafenib was administered orally at 60 mg/kg (for a total of 9 doses), and the control IgG (palivizumab, an antibody of the same isotype as DX-2240) was administered by IP injection at 20 mg/kg at the indicated dosing start dates and dosing regimens. An additional positive control group (group 13) was included: these animals were dosed with gemcitabine at 40 mg/kg Q3d for a total of four doses.

TABLE 1

	DX-2240		Bevacizumab		Sorafenib		Control	
Group	Start	Dosing	Start	Dosing	Start	Dosing	Start	Dosing
1							1	Q2d
2	1	Q2d						
3	1	Q2d	10	Q3d				
4	1	Q2d	20	Q3d				
6			10	Q3d			1	Q2d
7			20	Q3d			1	Q2d
9	1	Q8d	4	Q8d				
10			4	Q8d			1	Q8d
11	1	Q2d			15	Q1d		
12					15	Q1d	1	Q2d

[0200] Tumor size was evaluated every three to four days using calipers. Results are summarized graphically in FIGS. **2-4**. Tumor volume data (mm^3) is shown in Table 2.

TABLE 2

Day	Group 1	Group 2	Group 3	Group 4	Group 6	Group 7	Group 11	Group 12	Group 13
1	106.9	105.6	106.5	106.7	105.4	106.5	105.3	105.3	106.5
3	164.7	139.9	150.0	159.2	168.2	156.2	172.6	146.5	144.1
7	293.2	223.1	223.1	229.8	326.2	247.5	294.4	239.5	214.9
10	366.4	331.6	345.7	363.3	516.6	379.5	403.8	333.6	280.8
14	521.8	460.5	354.1	460.3	616.8	464.3	508.6	434.5	347.7
21	907.5	724.4	361.0	591.7	773.2	700.1	596.1	424.0	686.1
24	1062.0	954.1	386.0	570.6	927.7	777.4	522.7	348.2	848.1
28	1354.0	1171.1	437.5	626.3	1058.5	799.5	815.1	532.9	1084.8
31	1669.1	1351.4	535.7	777.8	1216.5	938.0	1081.3	830.0	1325.2

[0201] As single agents, DX-2240, bevacizumab and sorafenib all reduced tumor size as compared to control. Administration of DX-2240, followed by bevacizumab starting at day 10 resulted in a synergistic improvement in tumor size reduction, and the same combination of drugs with bevacizumab starting at day 20 showed a trend to synergistic improvement as well. In particular, it was noted that the sequential dosing of DX-2240 and bevacizumab led to a decreased rate of tumor growth leading to an increase in the number of stable tumors and regressions in the groups with ten or twenty day DX-2240 pretreatment (i.e., groups 3 and 4) relative to the control IgG and bevacizumab groups (groups 6 and 7). Table 3 shows calculated tumor growth rates.

TABLE 3

	Before Avastin (Day 10)	After Avastin	# of stable tumor growths or tumor regressions
DX-2240	23.9 ± 4.9	12.2 ± 3.3*	1/6
Control IgG	41.1 ± 9.0	33.3 ± 6.6	0/8
	Before Avastin (Day 21)#		
DX-2240	23.1 ± 6.6	18.6 ± 8.0	2/8
Control IgG	28.3 ± 5.2	23.8 ± 8.6	1/8

[0202] Combination of DX-2240 with sorafenib resulted in a small reduction in tumor size, although this effect was no greater than the control IgG/sorafenib combination. This may be due to hypersensitivity of this tumor type to sorafenib. It is anticipated that there is an optimal dose of sorafenib that would give a synergistic effect in some tumor types when administered with DX-2240.

Example 4

Evaluation of Different Dosing Schedules of a Tie1 Ectodomain-Binding Protein and VEGF Antagonist Agents in a Prostate Cancer Xenograft Model

[0203] DU145 prostate cancer cells were injected subcutaneously into athymic nu/nu mice and tumors were allowed to form. Animals were assorted into groups of 9-10 mice, then treated with DX-2240 (20 mg/kg by IP injection, Q2d), an isotype-matched antibody control (A2, 20 mg/kg by IP injection, Q2d), bevacizumab (12 mg/kg by IP injection, Q5d), DX-2240 20 mg/kg Q2d+bevacizumab (DX-2240 20 mg/kg Q2d+bevacizumab at 12 mg/kg, Q5d, starting at day 25), DX-2240 20 mg/kg Q4d+bevacizumab (DX-2240 20 mg/kg Q4d+bevacizumab at 12 mg/kg, Q5d, starting at day 25), DX-2240 10 mg/kg Q4d+bevacizumab (DX-2240 10 mg/kg Q4d+bevacizumab at 12 mg/kg, Q5d, starting at day 25), DX-2240 5 mg/kg Q4d+bevacizumab (DX-2240 5 mg/kg Q4d+bevacizumab at 12 mg/kg, Q5d, starting at day 25), DX-2240 1 mg/kg Q4d+bevacizumab (DX-2240 1 mg/kg Q4d+bevacizumab at 12 mg/kg, Q5d, starting at day 25),

DX-2240+sorafenib (DX-2240 at 20 mg/kg by IP injection, Q2d, sorafenib at 100 mg/kg orally (PO) daily for nine days (QDx9) starting at day 25 and 56), or A2+sorafenib (A2 at 20 mg/kg by IP injection, Q2d, sorafenib 100 mg/kg PO QDx9 starting at day 25 and 56). After day 34, mice in the DX-2240 only and A2 only groups were given an additional agent, and were no longer available as controls for this experiment. Tumor volume was measured twice weekly.

[0204] Tumor volume data is summarized in FIGS. **5** and **6**. Sequential dosing with DX-2240 followed by sorafenib in this model substantially decreased tumor growth (resulting in tumor shrinkage during the periods of co-administration of DX-2240 and sorafenib), and the decrease appeared to be synergistic at later time points (e.g., larger tumor sizes), as shown in FIG. **5**. This model appears to be highly sensitive to bevacizumab (dosing with bevacizumab alone at 12 mg/kg, Q5d, resulted in tumor shrinkage), so synergistic effects of sequential dosing with a Tie1 ectodomain-binding agent (DX-2240) could not be observed.

Example 5

Evaluation of Different Dosing Schedules of DX-2240 and VEGF Antagonist Agents in a Pancreatic Cancer Model

[0205] Schedule-dependent anti-tumor activity of DX-2240 in the BxPC-3 human pancreas cancer model were evaluated. DX-2240 and bevacizumab were co-administered on various schedules and activity compared to groups co-treated with bevacizumab and the control IgG (palivizumab). In addition, we examined changes in tumor vasculature and any potential effects of murine VEGF using an α-mouse VEGF binding antibody.

[0206] Female nude mice (nu/nu) were divided into 14 groups. Animals in groups 1-12 were implanted subcutaneously by trocar with BxPC3 tumor fragments harvested from growing tumors in nude mice hosts. Mice in groups 13-14 were injected subcutaneously with approximately 1×10^7 BxPC-3 cells from tissue culture. When injected tumors grew to approximately 104 mm^3 in size (about 19 days following implantation) and implanted tumors grew to approximately 110 mm^3 in size (about 22 days following implantation) animals were pair-matched by tumor size into treatment and control groups. Animals from groups 1 and 2 were used to evaluate tumor vasculature. Animals from groups 13 and 14 were used to compare effects of DX-2240 on tumors formed from cell injection.

[0207] Treatment was initiated after the animals were pair-matched (day 1). Animals in all groups were dosed by weight (10 ml/kg). The control IgG and DX-2240 were administered by IP injection at 20 mg/kg, and bevacizumab and the rat α-mouse VEGF IgG antibody were administered by IP injection at 10 mg/kg once every three days. Dosing was initiated on day −3, 1, 5, 6, 10 or 20. The various dosing schedules are shown in Table 4.

TABLE 4

Group	# Animals	Compound	Dose (mg/kg)	Schedule
1*	12	Control IgG	20	Q2D × 10 (Day 1)
2*	12	DX-2240	20	Q2D × 10 (Day 1)
3	6	Control IgG	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 13 (Day 1)
4	6	DX-2240	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 13 (Day 1)
5	8	Control IgG	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 12 (Day 5)
6	8	DX-2240	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 12 (Day 5)
7	8	Control IgG	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 10 (Day 10)
8	8	DX-2240	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 10 (Day 10)
9	8	Control IgG	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 7 (Day 20)
10	8	DX-2240	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 7 (Day 20)
11	8	Control IgG	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 7 (Day 20)
		Rat α-mouse VEGF	10	Q3D × 7 (Day 20)
12	8	DX-2240	20	Q2D × 19 (Day 1)
		Bevacizumab	10	Q3D × 7 (Day 20)
		Rat α-mouse VEGF	10	Q3D × 7 (Day 20)
13	8	Control IgG	20	Q2D × 21 (Day −3)
		Bevacizumab	10	Q3D × 11 (Day 6)
14	8	DX-2240	20	Q2D × 21 (Day −3)
		Bevacizumab	10	Q3D × 11 (Day 6)

*Animals used to evaluate tumor vasculature.

[0208] Individual and group mean tumor volumes were recorded twice weekly from day 1 until day 39. The mean tumor volumes for each group are shown in Table 5 and summarized in FIG. **7**.

TABLE 5

	G 1	G 2	G 3	G 4	G 5	G 6	G 7	G 8	G 9	G 10	G 11	G 12	G 13	G 14
−3													103.6	103.6
1	107.6	107.7	109.6	107.9	108.7	110.6	109.1	110.6	109.6	109.6	109.6	108.9	140.1	139.6
4	180.1	157.7	161.4	145.4	163.7	166.5	178.3	177.5	184.1	162.4	181.8	163.8	179.6	163.2
8	237.7	194.2	243.2	231.7	203.0	232.9	322.1	264.3	290.3	241.6	255.2	237.1	158.9	158.9
11	249.9	199.0	282.4	308.1	263.6	286.9	403.2	283.7	408.0	326.6	410.6	330.7	155.8	167.4
15	355.9	308.7	394.2	433.2	319.7	358.9	470.3	383.4	576.2	394.7	506.4	418.8	166.3	164.3
18	300.0	289.1	472.0	561.6	408.1	431.6	514.5	406.2	800.3	549.5	777.1	541.6	235.7	216.8
22			560.0	605.3	449.1	563.1	581.0	508.6	839.5	562.4	752.7	580.8	241.9	223.7
25			634.1	767.8	545.9	573.6	708.9	484.6	986.4	553.7	847.4	678.9	282.5	202.9
29			767.9	958.6	618.2	771.2	883.4	568.4	1092.3	624.7	1120.3	792.6	327.1	268.5
32			871.0	1069.1	581.8	783.6	915.5	645.6	1189.3	650.6	1242.5	820.2	309.7	273.3
37			1046.4	1169.6	709.0	859.4	1141.5	712.1	1602.5	708.6	1294.4	968.4	359.8	305.0
39			1032.2	1252.4	669.3	903.1	1255.3	755.3	1662.7	703.0	1363	996.3	391.7	323.5

[0209] Consistent with the results from the study described in Example 3 above, this study showed that administration of DX-2240, followed by bevacizumab starting at day 10 or day 20 resulted in a synergistic improvement in tumor size reduction as compared to the control groups, as shown in FIG. **8**. Groups co-administered the control IgG or DX-2240 on day 1 and bevacizumab initiated on day 1 or day 5 did not show significant differences in tumor growth inhibition, as shown in FIG. **9**.

[0210] Addition of the α-mouse VEGF antibody resulted in decreased tumor growth inhibition in DX-2240/bevacizumab treated animals (group 12), but the DX-2240/bevacizumab combination still decreased tumor growth as compared to the control IgG/bevacizumab combination (group 11). Tumors formed from cell injection (groups 13 and 14) were less sensitive to the DX-2240/bevacizumab combination as compared to implanted tumors evaluated at similar measured tumor volumes (~400 mm^3). However, it is unclear whether this decreased sensitivity would have remained once injected tumors reached their volume endpoints.

[0211] To determine changes in tumor vasculature, three animals from each of groups 1 and 2 were randomly selected for destructive sample collection, including tumor and serum on days 5, 10, 15, and 20. Animals were injected IV with 150 μL of a biotinylated-lectin solution (no injections on day 5 and 15). Five minutes later, animals were perfused by intracardiac injection with a 1% paraformaldehyde solution in PBS for 3 minutes. Tumors were then collected, placed in the perfusion solution, and sent immediately for analysis. Blood was collected by cardiac puncture and serum extracted, flash frozen in liquid nitrogen, and stored at −80° C. The data from the lectin staining study is summarized in Table 6 and FIG. **10**.

TABLE 6

	Control IgG Day 10	DX-2240 Day 10	Control IgG Day 20	DX-2240 Day 20
High intensity (3+)	19242	92134	7824	5922
Medium intensity (2+)	3148400	3468512	272402	205406
Low intensity (1+)	1214685	909706	222879	122022

[0212] The tumors from animals treated with DX-2240 exhibited less lectin staining as compared to tumors from the control animals. The results suggest that there was less functional tumor vasculature in DX-2240-treated animals.

Example 6

Evaluation of Different Dosing Schedules of DX-2240 and Sorafenib in a Prostate Tumor Model

[0213] Tumor growth inhibition was evaluated for treatment with DX-2240 and sorafenib on various schedules as compared to treatment with DX-2240 and the control IgG (palivizumab) in the DU 145 human prostate tumor model.

[0214] Male nude mice (nu/nu) were injected subcutaneously with DU 145 cells harvested from tissue culture (~1× 10^7 cells/mouse). When tumors grew to approximately 95 mm^3 in size (about 7 days following injection), animals were pair-matched by tumor size into treatment and control groups.

[0215] Treatment was initiated after the animals were paired-matched (day 1). Animals in control and treatment groups were dosed by weight (10 ml/kg). DX-2240 and the control IgG (palivizumab) were administered by IP injection either once every other day for seventeen treatments beginning day 1 or once every four days for nine treatments beginning day 1. Sorafenib was dosed per os via gavage once daily for nine treatments beginning day 15. To serve as a control, the control IgG was administered every other day for seventeen treatments beginning day 1. Dosing concentrations and schedules for the study are shown in Table 7. Individual and group mean tumor volumes were recorded twice weekly from day 1 until day 57. The results are shown in Table 8 and summarized in FIG. **11**.

[0216] Animals treated with DX-2240 and sorafenib showed reduction in tumor growth as compared to animals treated with the control IgG alone or DX-2240 alone. In particular, as shown in FIG. **12**, animals treated with DX-2240 (20 mg/kg; Q2Dx17; day 1) in combination with sorafenib (100 mg/kg; QDx9; day 15) continued to exhibit significantly decreased tumor growth after administration of the compounds has stopped, as compared to animals co-administered with the control IgG and sorafenib.

TABLE 7

Group	# Animals	Compound	Dose (mg/kg)	Route/Schedule
1	10	Palivizumab	20	IP/Q2D × 17 (Day 1)
2	10	DX-2240	20	IP/Q2D × 17 (Day 1)
3	10	Palivizumab	20	IP/Q2D × 17 (Day 1)
		Sorafenib	100	PO/QD × 9 (Day 15)
4	10	DX-2240	20	IP/Q2D × 17 (Day 1)
		Sorafenib	100	PO/QD × 9 (Day 15)
5	10	Palivizumab	30	IP/Q4D × 9 (Day 1)
		Sorafenib	100	PO/QD × 9 (Day 15)
6	10	DX-2240	1	IP/Q4D × 9 (Day 1)
		Sorafenib	100	PO/QD × 9 (Day 15)
7	10	DX-2240	3	IP/Q4D × 9 (Day 1)
		Sorafenib	100	PO/QD × 9 (Day 15)
8	10	DX-2240	10	IP/Q4D × 9 (Day 1)
		Sorafenib	100	PO/QD × 9 (Day 15)
9	10	DX-2240	30	IP/Q4D × 9 (Day 1)
		Sorafenib	100	PO/QD × 9 (Day 15)
10	10	Palivizumab	20	IP/Q2D × 17 (Day 1)
		Sorafenib	60	PO/QD × 9 (Day 15)
11	10	DX-2240	20	IP/Q2D × 17 (Day 1)
		Sorafenib	1	PO/QD × 9 (Day 15)
12	10	DX-2240	20	IP/Q2D × 17 (Day 1)
		Sorafenib	10	PO/QD × 9 (Day 15)
13	10	DX-2240	20	IP/Q2D × 17 (Day 1)
		Sorafenib	30	PO/QD × 9 (Day 15)
14	10	DX-2240	20	IP/Q2D × 17 (Day 1)
		Sorafenib	60	PO/QD × 9 (Day 15)

TABLE 8

	Group													
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	94.9	94.6	94.6	94.2	95.5	95.5	94.2	94.6	94.6	94.6	94.6	94.6	95.5	95.5
5	125.7	162.6	118.1	99.5	137.9	123.5	130.0	118.9	103.5	139.2	127.2	108.3	99.8	129.2

TABLE 8-continued

	Group													
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
9	195.1	269.3	199.8	186.3	239.8	205.2	196.6	182.9	174.4	224.7	232.2	180.8	173.4	243.5
13	293.5	417.3	309.0	229.3	368.1	326.1	318.6	312.5	261.4	334.9	303.6	264.1	266.7	317.1
15	320.9	460.0	383.4	259.4	389.8	316.2	329.4	348.2	308.3	367.8	390.5	406.0	273.9	370.6
19	400.7	553.9	263.6	164.6	273.5	224.5	261.0	225.2	190.9	249.0	447.6	426.7	271.1	294.1
23	417.8	644.4	157.0	126.8	187.3	151.3	174.4	167.9	156.9	186.1	506.1	430.5	185.1	208.7
26	487.6	707.1	191.6	150.2	201.1	164.2	204.3	204.4	188.1	183.1	585.6	452.5	208.9	239.2
29	450.5	737.8	193.4	134.9	192.6	144.7	204.5	172.4	175.6	198.5	507.8	424.6	215.6	180.9
33	501.1	866.7	233.1	143.7	202.1	169.6	245.8	225.3	226.8	225.2	673.7	512.8	260.9	259.8
36	529.3	1008.3	319.4	184.8	239.6	226.8	307.1	251.4	259.6	279.5	730.3	617.7	328.9	290.3
40			379.8	221.2	323.2	318.9	422.8	331.0	351.5	413.5	811.9	740.9	411.6	423.8
43			468.5	244.3	346.6	363.9	526.2	408.8	452.2	464.4	890.0	823.2	461.7	490.1
48			503.4	276.0	396.1	413.1	549.4	557.4	532.6	509.6	866.2	898.5	504.3	552.2
51			686.0	322.9	488.9	551.3	927.9	616.8	708.1	706.5	1152.5	1053.1	749.6	865.5
54			891.7	424.9	580.2	627.1	1086.3	801.9	937.4	869.3	1323.8	1266.9	977.3	1077.7
57			1045.8	499.0	669.2	861.7	1287.5	974.2	1055.2	907.9	1539.9	1398.7	1161.0	1116.6

[0217] Other embodiments are within the following claims:

SEQUENCE LISTING

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Leu Thr Asp Pro Gln Arg Phe Phe Leu Thr Cys Val Ser Gly Glu Ala
        35                  40                  45

Gly Ala Gly Arg Gly Ser Asp Ala Trp Gly Pro Pro Leu Leu Leu Glu
    50                  55                  60

Lys Asp Asp Arg Ile Val Arg Thr Pro Pro Gly Pro Pro Leu Arg Leu
65                  70                  75                  80

Ala Arg Asn Gly Ser His Gln Val Thr Leu Arg Gly Phe Ser Lys Pro
                85                  90                  95

Ser Asp Leu Val Gly Val Phe Ser Cys Val Gly Gly Ala Gly Ala Arg
            100                 105                 110

Arg Thr Arg Val Ile Tyr Val His Asn Ser Pro Gly Ala His Leu Leu
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Pro Asp Lys Val Thr His Thr Val Asn Lys Gly Asp Thr Ala Val Leu
    130                 135                 140

Ser Ala Arg Val His Lys Glu Lys Gln Thr Asp Val Ile Trp Lys Ser
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Asn Gly Ser Tyr Phe Tyr Thr Leu Asp Trp His Glu Ala Gln Asp Gly
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Thr Lys Glu Cys Pro Gly Cys Leu His Gly Gly Val Cys His Asp His
225                 230                 235                 240

Asp Gly Glu Cys Val Cys Pro Pro Gly Phe Thr Gly Thr Arg Cys Glu
              245                 250                 255

Gln Ala Cys Arg Glu Gly Arg Phe Gly Gln Ser Cys Gln Glu Gln Cys
          260                 265                 270

Pro Gly Ile Ser Gly Cys Arg Gly Leu Thr Phe Cys Leu Pro Asp Pro
      275                 280                 285

Tyr Gly Cys Ser Cys Gly Ser Gly Trp Arg Gly Ser Gln Cys Gln Glu
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              325                 330                 335

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Arg Ile Asn Cys Ala Ala Ala Gly Asn Pro Phe Pro Val Arg Gly Ser
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Ile Glu Leu Arg Lys Pro Asp Gly Thr Val Leu Leu Ser Thr Lys Ala
385                 390                 395                 400

Ile Val Glu Pro Glu Lys Thr Thr Ala Glu Phe Glu Val Pro Arg Leu
              405                 410                 415

Val Leu Ala Asp Ser Gly Phe Trp Glu Cys Arg Val Ser Thr Ser Gly
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Pro Leu Ala Ala Pro Arg Leu Leu Thr Lys Gln Ser Arg Gln Leu Val
  450                 455                 460

Val Ser Pro Leu Val Ser Phe Ser Gly Asp Gly Pro Ile Ser Thr Val
465                 470                 475                 480

Arg Leu His Tyr Arg Pro Gln Asp Ser Thr Met Asp Trp Ser Thr Ile
              485                 490                 495

Val Val Asp Pro Ser Glu Asn Val Thr Leu Met Asn Leu Arg Pro Lys
          500                 505                 510

Thr Gly Tyr Ser Val Arg Val Gln Leu Ser Arg Pro Gly Glu Gly Gly
      515                 520                 525

Glu Gly Ala Trp Gly Pro Pro Thr Leu Met Thr Thr Asp Cys Pro Glu
  530                 535                 540

Pro Leu Leu Gln Pro Trp Leu Glu Gly Trp His Val Glu Gly Thr Asp
545                 550                 555                 560

Arg Leu Arg Val Ser Trp Ser Leu Pro Leu Val Pro Gly Pro Leu Val
              565                 570                 575

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              645                 650                 655

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          660                 665                 670

Pro Ile Ser Lys Tyr Val Val Glu Val Gln Val Ala Gly Gly Ala Gly
      675                 680                 685

Asp Pro Leu Trp Ile Asp Val Asp Arg Pro Glu Glu Thr Ser Thr Ile
  690                 695                 700

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Ser Ile Gln Gly Leu Gly Asp Trp Ser Asn Thr Val Glu Glu Ser Thr
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          740                 745                 750

Ala Glu Glu Gly Leu Asp Gln Gln Leu Ile Leu Ala Val Val Gly Ser
      755                 760                 765

Val Ser Ala Thr Cys Leu Thr Ile Leu Ala Ala Leu Leu Thr Leu Val
  770                 775                 780

Cys Ile Arg Arg Ser Cys Leu His Arg Arg Arg Thr Phe Thr Tyr Gln
785                 790                 795                 800

Ser Gly Ser Gly Glu Glu Thr Ile Leu Gln Phe Ser Ser Gly Thr Leu
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Thr Leu Thr Arg Arg Pro Lys Leu Gln Pro Glu Pro Leu Ser Tyr Pro
          820                 825                 830

Val Leu Glu Trp Glu Asp Ile Thr Phe Glu Asp Leu Ile Gly Glu Gly
      835                 840                 845

Asn Phe Gly Gln Val Ile Arg Ala Met Ile Lys Lys Asp Gly Leu Lys
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His Pro Asn Ile Ile Asn Leu Leu Gly Ala Cys Lys Asn Arg Gly Tyr
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945                 950                 955                 960

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Cys Trp Arg Asp Arg Pro Tyr Glu Arg Pro Pro Phe Ala Gln Ile Ala
 1090                1095                1100

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tcaaggttca gtggcagtgg atctgggaca gatttcactc tcaccatcaa cagcctgcag    240

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50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95

Ala Gly Gly Asp Arg Tyr Gly Pro Leu His Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> SEQ ID NO 5
<211> LENGTH: 354
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

gaagttcaat tgttagagtc tggtggcggt cttgttcagc ctggtggttc tttacgtctt 60

tcttgcgctg cttccggatt cactttctct gcttacggta tgggttgggt tcgccaagct 120

cctggtaaag gtttggagtg ggtttctgtt atctctcctt ctggtggcca gacttcttat 180

gctgactccg ttaaaggtcg cttcactatc tctagagaca actctaagaa tactctctac 240

ttgcagatga acagcttaag ggctgaggac accgccttgt attactgtgc gggaggggac 300

aggtatggac ccttgcacta ctggggccag ggaaccctgg tcaccgtctc aagc 354

<210> SEQ ID NO 6
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly His Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Tyr Thr Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro His

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             85                  90                  95

Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala
          100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
      115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
  130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
             165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
          180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
      195                 200                 205

Phe Asn Arg Gly Glu Cys
  210


<210> SEQ ID NO 7
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Met Tyr
           20                  25                  30

Gly Met Val Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
       35                  40                  45

Ser Val Ile Ser Pro Ser Gly Gly Asn Thr Gly Tyr Ala Asp Ser Val
   50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
              85                  90                  95

Ala Arg Ala Pro Arg Gly Tyr Ser Tyr Gly Tyr Tyr Tyr Trp Gly Gln
          100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
      115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
  130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
             165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
          180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
      195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
  210                 215                 220
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Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
              245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
          260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
      275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
  290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
              325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
          340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
      355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
  370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
              405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
          420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
      435                 440                 445

Gly Lys
  450
```

<210> SEQ ID NO 8
<211> LENGTH: 660
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

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gacagagtca ccatcacttg ccgggcgagt cagggcattg gccattattt agcctggtat    120

cagcagaaac cagggaaagt tcctaagctc ctgatctata ctgcatccac tttgcaatca    180

ggggtcccat ctcggttcag tggcagtgga tctgggacag atttcactct caccatcagc    240

agcctgcagc ctgaagatgt tgcaacttat tactgtcaac agtttaatag ttaccctcac    300

accttcggcc aagggacacg actggagatt aaacgaactg tggctgcacc atctgtcttc    360

atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg    420

aataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc cctccaatcg    480

ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc    540

agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc    600

acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga gtgttaataa    660
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<210> SEQ ID NO 9
<211> LENGTH: 1353
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

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cctggtaaag gtttggagtg ggtttctgtt atctctcctt ctggtggcaa tactggttat 180

gctgactccg ttaaaggtcg cttcactatc tctagagaca actctaagaa tactctctac 240

ttgcagatga acagcttaag ggctgaggac actgcagtct actattgtgc gagagcccca 300

cgtggataca gctatggtta ctactactgg ggccagggaa ccctggtcac cgtctcaagc 360

gcctccacca agggcccatc ggtcttcccg ctagcaccct cctccaagag cacctctggg 420

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 480

tggaactcag gcgccctgac cagcggcgtc cacaccttcc cggctgtcct acagtcctcc 540

ggactctact ccctcagcag cgtagtgacc gtgccctcca gcagcttggg cacccagacc 600

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc 660

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 720

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 780

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 840

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 900

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 960

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 1020

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggatgag 1080

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1140

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1200

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1260

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1320

cagaagagcc tctccctgtc tccgggtaaa tga 1353

<210> SEQ ID NO 10
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly His Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Tyr Thr Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

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Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro His
               85                  90                  95

Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
          100                 105


<210> SEQ ID NO 11
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1               5                  10                  15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
           20                  25                  30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
       35                  40                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
   50                  55                  60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
               85                  90                  95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
          100                 105


<210> SEQ ID NO 12
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Met Tyr
           20                  25                  30

Gly Met Val Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
       35                  40                  45

Ser Val Ile Ser Pro Ser Gly Gly Asn Thr Gly Tyr Ala Asp Ser Val
   50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
               85                  90                  95

Ala Arg Ala Pro Arg Gly Tyr Ser Tyr Gly Tyr Tyr Tyr Trp Gly Gln
          100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
      115                 120


<210> SEQ ID NO 13
<211> LENGTH: 330
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
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1               5                  10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330


<210> SEQ ID NO 14
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5                  10                  15

Val His Ser
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<210> SEQ ID NO 15
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Ala His Ser

What is claimed:

1. A method of treating an angiogenesis-related disorder, the method comprising administering to a subject a first agent comprising a Tie1 ectodomain-binding agent before administering to the subject a second agent comprising a VEGF antagonist.

2. The method of claim **1**, wherein the first agent is administered about 1 day to 35 days before administration of the second agent.

3. The method of claim **1**, wherein the first agent is administered about 1, 4, 6, 8 or 10 days before administration of the second agent.

4. The method of claim **1**, wherein the first agent is administered about 2 weeks before administration of the second agent.

5. The method of claim **1**, wherein the first agent is administered about 10 days before administration of the second agent.

6. The method of claim **1**, wherein the first agent is administered about 20 days before administration of the second agent.

7. The method of claim **1**, wherein administration of the first agent is continued after the administration of the second agent.

8. The method of claim **1**, wherein administration of the first agent is discontinued after the administration of the second agent.

9. The method of claim **1**, wherein the Tie1 ectodomain-binding agent is DX-2240 or DX-2220.

10. The method of claim **1**, wherein the VEGF antagonist is bevacizumab.

11. The method of claim **1**, wherein the VEGF antagonist is sorafenib.

12. The method of claim **1**, wherein the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount effective to individually treat the angiogenesis-related disorder.

13. The method of claim **1**, wherein the Tie1 ectodomain-binding agent or the VEGF antagonist is administered in an amount less than an amount effective to individually treat the angiogenesis-related disorder.

14. The method of claim **1**, wherein the Tie1 ectodomain-binding agent and the VEGF antagonist are each administered in a synergistically effective amount to treat the angiogenesis-related disorder.

15. The method of claim **1**, wherein the angiogenesis-related disorder is cancer or tumor.

16. The method of claim **1**, wherein the angiogenesis-related disorder is prostate cancer or pancreatic cancer.

17. The method of claim **15**, further comprising a step of monitoring the subject for changes in tumor vasculature, and the second agent is administered when tumor vasculature exhibits any changes as compared to prior to the administration of the first agent.

18. The method of claim **15**, further comprising a step of monitoring the subject for change in tumor size.

19. The method of claim **15**, further comprising radiation therapy or chemotherapy.

20. A method of providing a post-operative adjuvant therapy, the method comprising administering a first agent comprising a Tie1 ectodomain-binding agent before administering a second agent comprising a VEGF antagonist, to a subject who has had surgery to remove a tumor.

21. The method of **20**, wherein the Tie1 ectodomain-binding agent is DX-2240.

22. The method of **20**, wherein the first agent is administered within 48 hours of surgery.

23. A method of treating an angiogenesis-related disorder, the method comprising administering to a subject a first agent comprising a VEGF antagonist before administering to the subject a second agent comprising a Tie1 ectodomain-binding agent.

24. The method of claim **23**, wherein the first agent is administered about 1 day to 35 days before administration of the second agent.

25. The method of claim **23**, wherein the Tie1 ectodomain-binding agent is DX-2240.

26. The method of claim **23**, wherein the VEGF antagonist is bevacizumab.

27. The method of claim **23**, wherein the VEGF antagonist is sorafenib.

28. The method of claim **23**, further comprising a step of monitoring the subject for changes in tumor vasculature, and the second agent is administered when tumor vasculature exhibits any changes as compared to prior to the administration of the first agent.

29. A method of sensitizing tumor vasculature to a decrease in VEGF, comprising administering to a subject a first agent comprising a Tie1 ectodomain-binding agent before administering to the subject a second agent comprising a VEGF antagonist.

30. The method of claim **29**, wherein the Tie1 ectodomain-binding agent is DX-2240.

31. The method of claim **29**, wherein the VEGF antagonist is bevacizumab.

32. The method of claim **29**, further comprising a step of monitoring the subject for changes in tumor vasculature, and

the second agent is administered when tumor vasculature exhibits any changes as compared to prior to the administration of the first agent.

33. A method of inhibiting tumor regrowth in a subject being administered a VEGF antagonist, the method comprising administering to the subject a first agent comprising a Tie1 ectodomain-binding agent before administering to the subject a second agent comprising the VEGF antagonist.

34. The method of claim **33**, wherein the Tie1 ectodomain-binding agent is DX-2240.

35. The method of claim **33**, wherein the VEGF antagonist is bevacizumab or sorafenib.

36. The method of claim **33**, further comprising a step of monitoring the subject for changes in tumor vasculature, and the second agent is administered when tumor vasculature exhibits any changes as compared to prior to the administration of the first agent.

37. The method of claim **33**, further comprising a step of monitoring the subject for tumor growth.

38. A method of decreasing frequency of administration of chemotherapy agent or radiation to a subject, the method comprising administering to the subject a first agent comprising a Tie1 ectodomain-binding agent and a second agent comprising a VEGF antagonist.

39. The method of claim **38**, wherein the Tie1 ectodomain-binding agent is DX-2240.

40. The method of claim **38**, further comprising a step of monitoring the subject for changes in tumor vasculature, and the second agent is administered when tumor vasculature exhibits any changes as compared to prior to the administration of the first agent.

41. The method of claim **39**, further comprising a step of monitoring the subject for tumor growth.

42. A method of treating an angiogenesis-related disorder, the method comprising administering to a subject of a first agent comprising a Tie2 ectodomain-binding agent and a second agent comprising a VEGF antagonist.

43. The method of claim **42**, wherein the second agent is administered after the administration of the first agent.

44. The method of claim **42**, wherein the VEGF antagonist is bevacizumab or sorafenib.

45. A kit comprising a first agent comprising a Tie1 ectodomain-binding agent, a second agent comprising a VEGF antagonist, and instructions for use in accordance with the method of claim **1**.

* * * * *