FULLY HUMAN ANTI-VEGF ANTIBODIES AND METHODS OF USING

RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No. 60/981,808, filed October 22, 2007, and to U.S. Provisional Application No. 61/046,370, filed April 18, 2008. The disclosures of these applications are hereby incorporated by reference herein in their entirety, including drawings.

BACKGROUND

[0002] The vascular endothelial growth factors (VEGFs) are a major family of angiogenic proteins involved in endothelial cell activation, proliferation, and survival, particularly during retinal proliferative diseases and tumorigenesis. VEGFs belong to the VEGF-PDGF (platelet-derived growth factor) super-gene family, and are small glycoprotein dimers that bind receptors expressed on vascular and lymphatic endothelial cells. There are currently seven known ligands in the VEGF family: VEGF-A (VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E (viral-derived), and placental growth factor (PlGF)-1 and -2. These VEGF ligands mediate their effects by binding to one or more of the three known VEGF receptors (VEGF-Rs), each of which possess receptor tyrosine kinase activity. VEGF-R1 (Flt-1) is predominantly expressed on endothelial cells and monocytes, binds VEGF and VEGF-B, and appears to mediate endothelial and monocyte migration. VEGF-R2 (*i.e.*, human KDR or murine Flk-1) is mainly expressed on endothelial cells, is selective for VEGF (and particular fragments of VEGF-C and VEGF-D), and mediates VEGF-induced endothelial cell proliferation, survival, and migration, as well as vascular permeability. VEGF-R3 (Flt-4) is mainly expressed on lymphatic endothelial cells and binds VEGF-C and VEGF-D to promote lymphangiogenesis. VEGF-R1, -R2, and -R3 are each expressed on some tumor cells. Binding of VEGF to the VEGF receptors triggers receptor dimerization, leading to subsequent receptor activation and signal transduction. VEGF

binding to VEGF-R2 initiates a signal transduction pathway that is dominant in promoting angiogenesis. This pathway involves receptor activation with subsequent induction of intracellular signaling. Receptor activation in this case entails three basic events: (i) VEGF binding to VEGF-R2, (ii) receptor dimerization, and (iii) receptor autophosphorylation (and hence activation) of the receptor tyrosine kinase. Intracellular messengers such as phospholipase C and phosphatidylinositol-3-kinase bind directly to the autophosphorylated form of VEGF-R2 and become phosphorylated by the receptor tyrosine kinase, which subsequently triggers an intracellular cascade of signaling events leading to nuclear signals that ultimately promote cell proliferation, migration, and survival (anti-apoptosis), and increase vascular permeability.

[0003] Aberrant angiogenesis is associated with a variety of disease states, including cancer (Holash 2002). VEGF signaling has been verified to play a role in both normal vascular development and the pathologic angiogenesis associated with various diseases (Erikkson 1999; Ferrara 1999; Yancopoulos 2000). VEGF promotes vascular endothelial cell growth and increases vascular permeability (Ferrara 2004).

[0004] Previous studies have revealed elevated VEGF expression levels in a majority of tumor types (Berkman 1993; Brown 1993; Brown 1995; Dvorak 1995; Mattern 1996). Studies have also revealed increased VEGF levels in subjects with ocular angiogenic diseases such as wet AMD. Wet AMD accounts for only around 10% of total AMD cases, but causes approximately 90% of blindness arising from AMD. Wet AMD is characterized by choroidal neovascularization (CNV), the development of abnormal blood vessels beneath the retinal pigment epithelium layer of the retina. VEGF-A is believed to play a major role in the formation of these vessels, which leak beneath the macula and cause retinal distortion and vision deterioration.

[0005] Therapeutic anti-VEGF antibodies are currently available. For example, the humanized IgG1 monoclonal antibody Bevacizumab (a.k.a., Avastin®, sold by Genentech, San Francisco, CA; also referred to herein as BM-1) binds human VEGF with an affinity (K_D) of approximately 500 pM. While Bevacizumab has been used to treat a variety of cancers, there is a need in the art for antibodies with greater *in vivo* efficacy. Such antibodies present significant technical challenges and are highly elusive because merely increasing the binding affinity of a VEGF antibody does not necessarily increase its *in vivo* efficacy (Liang 2006).

SUMMARY

[0006] The present application addresses the need for VEGF antibodies with superior efficacy. Provided herein are therapeutic antibodies and antigen-binding fragments thereof, and compositions and methods of use for treating medical conditions such as cancer.

[0007] In certain embodiments, an antibody or antigen-binding fragment is provided that comprises an HCDR3 region selected from the group consisting of the HCDR3 of XPA.10.064, XPA.10.072, XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10. In certain embodiments, the antibody or antigen-binding fragment further comprises the HCDR1 and/or HCDR2 of XPA.10.064 or XPA.10.072.

[0008] In certain embodiments an antibody or antigen-binding fragment is provided that comprises LCDR1, LCDR2, and LCDR3 of XPA.10.072; LCDR1, LCDR2, and LCDR3 of XPA.10.064; HCDR1, HCDR2, and HCDR3 of XPA.10.072; HCDR1, HCDR2, and HCDR3 of XPA.10.064; HCDR1, HCDR2, and HCDR3 of XPA.10.064.03; HCDR1, HCDR2, and HCDR3 of XPA.10.064.04; HCDR1, HCDR2, and HCDR3 of XPA.10.064.06; HCDR1, HCDR2, and HCDR3 of XPA.10.064.07; and/or HCDR1, HCDR2, and HCDR3 of

XPA.10.064.10. In certain embodiments, the antibody or antigen-binding fragment comprises the heavy chain of XPA.10.072, XPA.10.064, XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, or XPA.10.064.10. In certain embodiments, the antibody or antigen-binding fragment comprises the light chain of XPA.10.064 or XPA.10.072.

[0009] In certain embodiments, an antibody or antigen-binding fragment is provided that comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19. In certain of these embodiments, the heavy chain variable region further comprises one or more of the amino acid sequences set forth in SEQ ID NO:6 and/or SEQ ID NO:7. In certain embodiments, the antibodies or antigen-binding fragments comprise a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24. In certain embodiments, the antibody or antigen-binding fragment further comprises a light chain variable region comprising one or more of the amino acid sequences set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11. In certain of these embodiments, the antibodies or antigen-binding fragments comprise a light chain comprising the amino acid sequence set forth in SEQ ID NO:5 or SEQ ID NO:3.

[0010] In certain embodiments, an antibody or antigen-binding fragment is provided that comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:17 and a light chain variable region comprising one or more of the amino acid sequences set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11. In certain embodiments, the heavy chain variable region of this antibody or antigen-binding fragment further comprises the amino acid sequences set forth in SEQ ID NO:6 and/or SEQ ID NO:7, and in certain of these

embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain comprising the amino acid sequence set forth in SEQ ID NO:5.

[0011] In certain embodiments, an antibody or antigen-binding fragment is provided that comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:18 and a light chain variable region comprising one or more of the amino acid sequences set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11. In certain embodiments, the heavy chain variable region of this antibody or antigen-binding fragment further comprises the amino acid sequences set forth in SEQ ID NO:6 and/or SEQ ID NO:7, and in certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:23 and a light chain comprising the amino acid sequence set forth in SEQ ID NO:5.

[0012] In certain embodiments, an antibody or antigen-binding fragment is provided that comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:15 and a light chain variable region comprising one or more of the amino acid sequences set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11. In certain embodiments, the heavy chain variable region of this antibody or antigen-binding fragment further comprises the amino acid sequences set forth in SEQ ID NO:6 and/or SEQ ID NO:7, and in certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:20 and a light chain comprising the amino acid sequence set forth in SEQ ID NO:5.

[0013] In certain embodiments, an antibody or antigen-binding fragment is provided that comprises a heavy chain variable region comprising the amino acid sequence set forth in

SEQ ID NO:16 and a light chain variable region comprising one or more of the amino acid sequences set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11. In certain embodiments, the heavy chain variable region of this antibody or antigen-binding fragment further comprises the amino acid sequences set forth in SEQ ID NO:6 and/or SEQ ID NO:7, and in certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:21 and a light chain comprising the amino acid sequence set forth in SEQ ID NO:5.

[0014] In certain embodiments, an antibody or antigen-binding fragment is provided that comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:19 and a light chain variable region comprising one or more of the amino acid sequences set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11. In certain embodiments, the heavy chain variable region of this antibody or antigen-binding fragment further comprises the amino acid sequences set forth in SEQ ID NO:6 and/or SEQ ID NO:7, and in certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:24 and a light chain comprising the amino acid sequence set forth in SEQ ID NO:5.

[0015] In certain embodiments, an antibody or antigen-binding fragment is provided that specifically binds VEGF and has one or more of the following properties: 1) K_D for binding to $hVEGF_{165}$ of about 10^{-10} M; 2) k_a for binding to $hVEGF_{165}$ of about 1.89 X 10^5; 3) k_d for binding to $hVEGF_{165}$ of about 1.73 X 10^{-5}; 4) binding affinity for $hVEGF_{165}$ that is about 4.25 times greater than the binding affinity of Bevacizumab for $hVEGF_{165}$; 5) inhibits HUVEC cell proliferation with an IC_{50} of about 154 pM; 6) inhibits HUVEC cell proliferation with an IC_{50} about 56% that of Bevacizumab; 7) K_D for binding to $hVEGF_{165}$ in the range of

about 1.97 X 10^{-10} M to about 3.49 X 10^{-11} M; 8) k_a for binding to $hVEGF_{165}$ in the range of about 1.5 X 10^5 to about 2.16 X 10^5 M; 9) k_d for binding to $hVEGF_{165}$ in the range of about 6.65 X 10^{-6} to about 2.94 X 10^{-5} M; 10) inhibits HUVEC cell proliferation with an IC_{50} in the range of about 129 pM to about 174 pM; 11) binding affinity for hVEGF that is at least about 10 times greater than the affinity of the antibody or antigen-binding fragment thereof for mVEGF; 12) competes with Bevacizumab for binding to $hVEGF_{165}$; 13) inhibits tumor growth as a function of percent tumor growth inhibition to the same or a greater degree than Bevacizumab when administered at the same or a lower dosage; and 14) delays tumor growth to a specified size for a greater duration than Bevacizumab when administered at the same or a lower dosage as Bevacizumab. In certain embodiments, an antibody or antigen-binding fragment is provided that binds to the same epitope on $hVEGF_{165}$ as an antibody comprising the amino acid sequence set forth in SEQ ID NO:8, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, or SEQ ID NO:19. In certain of these embodiments, the antibody or antigen-binding fragment binds the same epitope on $hVEGF_{165}$ as an antibody comprising the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24.

[0016] In certain embodiments, the antibodies or antigen-binding fragments bind $hVEGF_{165}$ with a K_D of ≤ 200 pM. In certain of these embodiments, the antibodies or antigen-binding fragments bind $hVEGF_{165}$ with a K_D of ≤ 150 pM, in other embodiments ≤ 100 pM, and in still other embodiments ≤ 50 pM. In certain embodiments, the antibodies or antigen-binding fragments provided herein that bind an epitope on $hVEGF_{165}$ that overlaps at least partially with the epitope bound by Bevacizumab.

[0017] In certain embodiments, the antibodies or antigen-binding fragments provided herein block binding of $hVEGF_{165}$ to a VEGF receptor, which in certain embodiments is

VEGF-R1 or VEGF-R2. In certain embodiments, the antibodies or antigen-binding fragments inhibit $hVEGF_{165}$-induced phosphorylation of a VEGF receptor. In certain embodiments, the antibodies or antigen-binding fragments binds $hVEGF_{165}$ with a K_D that is at least 10-fold greater (*i.e.*, at least ten-fold greater affinity, meaning at least 10-fold lower in number) than the K_D with which they bind $mVEGF_{165}$. In certain of these embodiments, the antibodies or antigen-binding fragments bind $hVEGF_{165}$ with a K_D at least 50-fold greater than the K_D with which they bind $mVEGF_{165}$, and in certain embodiments at least 100-fold greater.

[0018] In certain of the above embodiments, the antibodies or antigen-binding fragments disclosed herein comprise a κ light chain, a γ1 heavy chain, a γ2 heavy chain, a γ3 heavy chain, or a γ4 heavy chain constant region. In certain of these embodiments, the antibodies or antigen-binding fragments comprise an IgG2 constant region. In certain embodiments, the antibodies disclosed herein are full antibodies. In certain of these embodiments, the antibody may be a monoclonal antibody, polyclonal antibody, recombinant antibody, bispecific antibody, humanized antibody, chimeric antibody, labeled antibody, bivalent antibody, anti-idiotypic antibody, or fully human antibody. In certain embodiments, an antibody or antigen-binding fragment as provided herein may be a camelized single domain antibody, a diabody, a scFv, an scFv dimer, a dsFv, a $(dsFv)_2$, a dsFv-dsFv', an Fv fragment, a Fab, a Fab', a $F(ab')_2$, a ds diabody, a nanobody, a domain antibody, or a bivalent domain antibody.

[0019] In certain embodiments, methods are provided for inhibiting angiogenesis in a subject in need thereof by administering to said subject a therapeutically effective amount of one or more antibodies or antigen-binding fragments disclosed herein. In certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light

chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In other embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NOs:20, 21, 23, or 24 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In certain embodiments, the antibody or antigen-binding fragment is administered at a dosage of 5 mg/kg or less per administration. In certain of these embodiments, the antibody or antigen-binding fragment is administered at a dosage of 1 mg/kg or less per administration, in other embodiments 0.5 mg/kg or less, and in still other embodiments 0.1 mg/kg or less. In certain embodiments, the antibody or antigen-binding fragment is administered to the subject multiple times at an interval of once a day to once every two months. In certain of these embodiments, the antibody or antigen-binding fragment may be administered about once a week, about once every two weeks, about once a month, or about once every two months.

[0020] In certain embodiments, methods are provided for treating a disease associated with aberrant angiogenesis in a subject (*e.g.*, a mammalian subject such as a human, primate, canine, rat, rabbit, or mouse) in need thereof comprising administering to said subject a therapeutically effective amount of one or more antibodies or antigen-binding fragments disclosed herein. In certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In other embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NOs:20, 21, 23, or 24 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In certain embodiments, the antibody or antigen-binding fragment is administered at a dosage of 5 mg/kg or less

per administration. In certain of these embodiments, the antibody or antigen-binding fragment is administered at a dosage of 1 mg/kg or less per administration, in other embodiments 0.5 mg/kg or less, and in still other embodiments 0.1 mg/kg or less. In certain embodiments, the antibody or antigen-binding fragment is administered to the subject multiple times at an interval of once a day to once every two months. In certain of these embodiments, the antibody or antigen-binding fragment may be administered about once a week, about once every two weeks, about once a month, or about once every two months.

[0021] In certain embodiments, methods are provided for treating an inflammatory disease associated with VEGF signaling in a subject (*e.g.*, a mammalian subject such as a human, primate, canine, rat, rabbit, or mouse) in need thereof comprising administering to said subject a therapeutically effective amount of one or more antibodies or antigen-binding fragments disclosed herein. In certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In other embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NOs:20, 21, 23, or 24 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In certain embodiments, the disease associated with VEGF signaling is rheumatoid arthritis, psoriasis, scleroderma, chronic obstructive pulmonary disease, or asthma. In certain embodiments, the antibody or antigen-binding fragment is administered at a dosage of 5 mg/kg or less per administration. In certain of these embodiments, the antibody or antigen-binding fragment is administered at a dosage of 1 mg/kg or less per administration, in other embodiments 0.5 mg/kg or less, and in still other embodiments 0.1 mg/kg or less. In

certain embodiments, the antibody or antigen-binding fragment is administered to the subject multiple times at an interval of once a day to once every two months. In certain of these embodiments, the antibody or antigen-binding fragment may be administered about once a week, about once every two weeks, about once a month, or about once every two months.

[0022] In certain embodiments, methods are provided for treating wet acute macular degeneration or diabetic retinopathy in a subject (*e.g.*, a mammalian subject such as a human, primate, canine, rat, rabbit, or mouse) in need thereof comprising administering to said subject a therapeutically effective amount of one or more antibodies or antigen-binding fragments disclosed herein. In certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In other embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NOs:20, 21, 23, or 24 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In certain embodiments, the antibody or antigen-binding fragment is administered at a dosage of 5 mg/kg or less per administration. In certain of these embodiments, the antibody or antigen-binding fragment is administered at a dosage of 1 mg/kg or less per administration, in other embodiments 0.5 mg/kg or less, and in still other embodiments 0.1 mg/kg or less. In certain embodiments, the antibody or antigen-binding fragment is administered to the subject multiple times at an interval of once a day to once every two months. In certain of these embodiments, the antibody or antigen-binding fragment may be administered about once a week, about once every two weeks, about once a month, or about once every two months.

[0023] In certain embodiments, methods are provided for treating a cancer associated with increased VEGF signaling in a subject (*e.g.*, a mammalian subject such as a human, primate, canine, rat, rabbit, or mouse) in need thereof comprising administering to said subject a therapeutically effective amount of one or more antibodies or antigen-binding fragments disclosed herein. In certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In other embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NOs:20, 21, 23, or 24 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In certain of these embodiments, the antibody or antigen-binding fragment further comprises a conjugate that is either a toxin, cytokine, or chemotherapeutic agent. In certain embodiments, the antibody or antigen-binding fragment is administered in combination with or linked to one or more chemotherapeutic agents. In certain embodiments, the subject is subjected to one or more additional therapeutic procedures such as surgical tumorectomy or anti-cancer radiation therapy. In certain embodiments, the antibody or antigen-binding fragment is administered at a dosage of 5 mg/kg or less per administration. In certain of these embodiments, the antibody or antigen-binding fragment is administered at a dosage of 1 mg/kg or less per administration, in other embodiments 0.5 mg/kg or less, and in still other embodiments 0.1 mg/kg or less. In certain embodiments, the antibody or antigen-binding fragment is administered to the subject multiple times at an interval of once a day to once every two months. In certain of these embodiments, the antibody or antigen-binding fragment may be administered about once a week, about once every two weeks, about once a month, or about once every two months.

[0024] In certain embodiments, a kit is provided comprising one or more antibodies or antigen-binding fragments as disclosed herein. In certain of these embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In other embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NOs:20, 21, 23, or 24 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In certain embodiments, the kit further comprises instructions for using the antibodies or antigen-binding fragments and/or for utilizing other components of the kit.

[0025] In certain embodiments, polynucleotides are provided that encode the amino acid sequences set forth in SEQ ID NOs:2-24. In certain other embodiments, vectors are provided that comprise these polynucleotides, and in certain other embodiments, host cells are provided that comprises these vectors. In certain embodiments, methods are provided for expressing one or more of the antibodies or antigen-binding fragments disclosed herein by culturing these host cells under conditions in which polynucleotides encoding the antibodies or antigen-binding fragments are expressed from a vector. In certain embodiments, the polynucleotides provided herein are operably associated with a promoter such as a CMV promoter in a vector. In certain embodiments, host cells comprising the vectors provided herein are Chinese hamster ovary cell.

[0026] In certain embodiments, pharmaceutical compositions are provided that comprise one or more antibodies or antigen-binding fragments as disclosed herein. In certain of these embodiments, the composition further comprises one or more physiologically tolerable components. In certain of these embodiments, the one or more physiologically tolerable components may be one or more pharmaceutically acceptable carriers, diluents,

adjuvants, excipients, or non-toxic auxiliary substances. In certain of these embodiments, the one or more physiologically tolerable components may comprise one or more antioxidants, which may be selected from methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxanisol, butylated hydroxytoluene, and/or propyl gallate. Likewise, in certain embodiments methods are provided for inhibiting oxidation and/or preventing degradation of VEGF binding affinity of the antibodies or antigen-binding fragments thereof provided herein using one or more antioxidants. In certain embodiments, the antibody or antigen-binding fragment for use in the composition comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5. In other embodiments, the antibody or antigen-binding fragment comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NOs:20, 21, 23, or 24 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5.

[0027] In certain embodiments, the use of one or more antibodies or antigen-binding fragments as provided herein in the manufacture of a medicament for treating a disease associated with aberrant angiogenesis, an inflammatory disease associated with aberrant angiogenesis, an inflammatory disease associated with VEGF signaling, wet acute macular degeneration, diabetic retinopathy, or cancer associated with increased VEGF signaling is provided.

[0028] In certain embodiments, the antibodies or antigen-binding fragments disclosed herein are provided for use in the treatment of a disease associated with aberrant angiogenesis, an inflammatory disease associated with aberrant angiogenesis, an inflammatory disease associated with VEGF signaling, wet acute macular degeneration,

diabetic retinopathy, or cancer associated with VEGF signaling (*e.g.*, a cancer exhibiting increased VEGF signaling relative to non-cancerous cells) is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] Figure 1: Heavy chain variable regions (including HCDRs) and light chain variable regions (including LCDRs) of XPA.10.064 and XPA.10.072

[0030] Figure 2: Biacore analysis of XPA.10.064 IgG2 binding to $hVEGF_{165}$.

[0031] Figure 3: Biacore analysis of XPA.10.072 IgG2 binding to $hVEGF_{165}$.

[0032] Figure 4: Biacore analysis of Bevacizumab binding to $hVEGF_{165}$.

[0033] Figure 5: Inhibition of $hVEGF_{165}$ binding to VEGF-R1 by Bevacizumab (BM1), XPA.10.064, and XPA.10.072.

[0034] Figure 6: Inhibition of $hVEGF_{165}$ binding to VEGF-R2 by Bevacizumab (BM1), XPA.10.064, and XPA.10.072.

[0035] Figure 7: Analysis of $hVEGF_{165}$ epitopes bound by Bevacizumab (BM1), XPA.10.064 (064), and XPA.10.072 (072).

[0036] Figure 8: Co-localization of XPA.10.064 and G153-694Panel A is shows the staining with XPA.10.064. Panel B shows staining with G153-694. Panel C shows staining with a nuclear dye. Panel D shows the merged image, where greater intensity (white) reflects colocalization.

[0037] Figure 9: Co-localization of XPA.10.072 and G153-694. Panel A is shows the staining with XPA.10.072. Panel B shows staining with G153-694. Panel C shows staining with a nuclear dye. Panel D shows the merged image, where greater intensity (white) reflects colocalization.

[0038] Figure 10: Inhibition of HUVEC proliferation by (A) XPA.10.064 IgG2 and (B) XPA.10.072 IgG2.

[0039] Figure 11: Treatment of HUVECs with a dose titration of $hVEGF_{165}$ leads to an increase in phosphorylation of VEGF-R2.

[0040] Figure 12: Treatment of HUVECs with a dose titration of $hVEGF_{165}$ plus Bevacizumab resulted in a decrease in VEGF-R2 phosphorylation.

[0041] Figure 13: XPA.10.064 (064) and XPA.10.072 (072) IgG2s inhibit $hVEGF_{165}$-induced phosphorylation of VEGF-R2.

[0042] Figure 14: Visual scoring system for Matrigel® plugs. Examples of each score 0 to 3 are shown.

[0043] Figure 15: Matrigel® plug assay showing the level of angiogenesis inhibition in the presence of Bevacizumab (BM-1), XPA.10.064 (064), and XPA.10.072 (072) at various dosages.

[0044] Figure 16: Inhibition of angiogenesis as determined by Matrigel® plug assay. Numbers are the mean of results from two blinded scorers. (A) no cells; (B) DU145 plus αKLH; (C) Bevacizumab (0.1 mg/kg); (D) Bevacizumab (1 mg/kg); (E) Bevacizumab (5 mg/kg); (F) XPA.10.064 (0.1 mg/kg); (G) XPA.10.064 (1 mg/kg); (H) XPA.10.064 (5 mg/kg); (I) XPA.10.072 (0.1 mg/kg); (J) XPA.10.072 (1 mg/kg); (K) XPA.10.072 (5 mg/kg).

[0045] Figure 17: Inhibition of A673 tumor growth *in vivo* by XPA.10.064 and XPA.072. (1) Vehicle only; (2) 0.5 mg/kg XPA.10.064 IgG2; (3) 5 mg/kg XPA.10.064 IgG2; (4) 0.5 mg/kg XPA.10.072 IgG2; (5) 5 mg/kg XPA.10.072 IgG2; (6) 5 mg/kg CHO.KLH IgG2; (7) 0.5 mg/kg Bevacizumab; (8) 5 mg/kg Bevacizumab.

[0046] Figure 18: Inhibition of HUVEC proliferation by XPA.10.064 IgG2 over four separate assays (#'s 1-4).

[0047] Figure 19: Inhibition of HUVEC proliferation by XPA.10.064.03 IgG2 over four separate assays (#'s 1-4).

[0048] Figure 20: Inhibition of HUVEC proliferation by XPA.10.064.06 IgG2 over four separate assays (#'s 1-4).

[0049] Figure 21: Inhibition of HUVEC proliferation by XPA.10.064.07 IgG2 over four separate assays (#'s 1-4).

[0050] Figure 22: Inhibition of HUVEC proliferation by XPA.10.064.10 IgG2 over four separate assays (#'s 1-4).

[0051] Figure 23: Inhibition of HUVEC proliferation by Bevacizumab IgG2 over four separate assays (#'s 1-4).

[0052] Figure 24: Inhibition of HUVEC proliferation by XPA.10.064.03 (■), XPA.10.064.04 (▲), XPA.10.064.06 (▼), XPA.10.064.07 (♦), XPA.10.064.10 (●), XPA.10.064 (□), and Bevacizumab (x, *). Results for each antibody are mean of four separate assays.

[0053] Figure 25: Inhibition of A673 tumor growth *in vivo* by XPA.10.064.06 and Bevacizumab through day 24. (●) 0.5 mg/kg isotype control antibody; (▼) 0.1 mg/kg Bevacizumab; (♦) 0.1 mg/kg XPA.10.064.06 IgG2; (■) 0.5 mg/kg Bevacizumab; (▲) 0.5 mg/kg XPA.10.064.06 IgG2; (□) 5 mg/kg Bevacizumab; and (Δ) 5 mg/kg XPA.10.064.06 IgG2. Arrows on X-axis indicate dosing days.

[0054] Figure 26: Inhibition of A673 tumor growth *in vivo* by XPA.10.064.06 and Bevacizumab through day 28. (●) 0.5 mg/kg isotype control antibody; (▼) 0.1 mg/kg Bevacizumab; (♦) 0.1 mg/kg XPA.10.064.06 IgG2; (■) 0.5 mg/kg Bevacizumab; (▲) 0.5 mg/kg XPA.10.064.06 IgG2; (□) 5 mg/kg Bevacizumab; and (Δ) 5 mg/kg XPA.10.064.06 IgG2. Arrows on X-axis indicate dosing days.

[0055] Figure 27: Inhibition of A673 tumor growth *in vivo* by XPA.10.064.06 and Bevacizumab through day 31. (●) 0.5 mg/kg isotype control antibody; (▼) 0.1 mg/kg Bevacizumab; (♦) 0.1 mg/kg XPA.10.064.06 IgG2; (■) 0.5 mg/kg Bevacizumab; (▲) 0.5

mg/kg XPA.10.064.06 IgG2; (□) 5 mg/kg Bevacizumab; and (Δ) 5 mg/kg XPA.10.064.06 IgG2. Arrows on X-axis indicate dosing days.

[0056] Figure 28: Effect of antibody methionine oxidation on $hVEGF_{165}$ binding. A. Biacore analysis of XPA.10.064 binding to $hVEGF_{165}$ in the presence or absence of methionine oxidation. B. Biacore analysis of XPA.10.064.06 binding to $hVEGF_{165}$ in the presence or absence of methionine oxidation.

[0057] Figure 29: Effect of methionine on XPA.10.064.06 binding to $hVEGF_{165}$ following thermal stress oxidizing conditions. A. Biacore analysis of XPA.10.064.06 binding to $hVEGF_{165}$ following thermal stress oxidation. B. Biacore analysis of XPA.10.064.06 binding to $hVEGF_{165}$ following thermal stress oxidation in the presence of methionine.

[0058] Figure 30: Effect of methionine on XPA.10.064.06 binding affinity for $hVEGF_{165}$ following oxidation. A. ELISA analysis of XPA.10.064.06 binding to $hVEGF_{165}$ following oxidation by thermal stress in the presence or absence of methionine. B. ELISA analysis of XPA.10.064.06 binding to $hVEGF_{165}$ following oxidation by chemical stress in the presence or absence of methionine.

[0059] Figure 31: Structure of the XPA.10.064 heavy chain plus light chain vector pMXSP117. The XPA.10.064 heavy chain plus light chain vector pMXSP119 has the same structure, but with the *his*D gene instead of the *neo* gene. A. Circular structure. B. Linearized structure following digestion with XbaI.

[0060] Figure 32: XPA.10.064.06 heavy and light chain variable region modular expression vectors. A. Structure of heavy chain vector. B. Structure of light chain vector.

DETAILED DESCRIPTION

[0061] The following description of the invention is merely intended to illustrate various embodiments of the invention. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in

the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein.

Abbreviations

[0062] The following abbreviations are used herein: ADCC, antibody-dependent cellular cytotoxicity; AMD, age-related macular degeneration; BDS, bulk drug substance; BM-1, Bevacizumab; CDC, complement-dependent cytotoxicity; CNV, choroidal neovascularization; COPD, chronic obstructive pulmonary disease; DF, diafiltration; EDTA, ethylenediaminetetraacetic acid; GMP, Good Manufacturing Practices; HAMA, human anti-mouse antibodies; HIC, hydrophobic interaction chromatography; HUVEC, human umbilical vein endothelial cell; hVEGF, human VEGF; MCB, Master Cell Bank; mpk, mg/kg; mVEGF, murine VEGF; PD, pharmacodynamics; PK, pharmacokinetics; RA, rheumatoid arthritis; RIA, radioimmunoprecipitation; UF, ultrafiltration; VEGF, vascular endothelial growth factor; VEGF-R, vascular endothelial growth factor receptor; VHL, von Hippel/Lindau; X-reactivity, cross-reactivity.

Definitions

[0063] The term "antibody" as used herein includes any monoclonal antibody, polyclonal antibody, multispecific antibody, or bispecific (bivalent) antibody that binds to a specific antigen. A complete antibody comprises two heavy chains and two light chains. Each heavy chain consists of a variable region and a first, second, and third constant region, while each light chain consists of a variable region and a constant region. Mammalian heavy chains are classified as α, δ, ε, γ, and μ, and mammalian light chains are classified as γ or κ. The antibody has a "Y" shape, with the stem of the Y consisting of the second and third constant regions of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable region and first constant region of a single heavy

chain bound to the variable and constant regions of a single light chain. The variable regions of the light and heavy chains are responsible for antigen binding. The variables region in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light (L) chain CDRs including LCDR1, LCDR2, and LCDR3, heavy (H) chain CDRs including HCDR1, HCDR2, HCDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, Chothia, or Al-Lazikani (Al-Lazikani 1997; Chothia 1985; Chothia 1987; Chothia 1989; Kabat 1987; Kabat 1991). The three CDRs are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of α, δ, ε, γ, and μ heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as IgG1 (γ1 heavy chain), IgG2 (γ2 heavy chain), IgG3 (γ3 heavy chain), IgG4 (γ4 heavy chain), IgA1 (α1 heavy chain), or IgA2 (α1 heavy chain).

[0064] An antibody or antigen-binding fragment thereof that is "bivalent" comprises two antigen-binding sites. The two antigen binding sites may bind to the same antigen, or they may each bind to a different antigen, in which case the antibody or antigen-binding fragment is characterized as "bispecific."

[0065] The term "antigen-binding fragment" as used herein refers to an antibody fragment such as for example a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide

stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody binds. In certain embodiments, an antigen-binding fragment may comprise one or more CDRs from a particular human antibody grafted to a framework region from one or more different human antibodies.

[0066] "Fab" with regard to an antibody refers to that portion of the antibody consisting of a single light chain (both variable and constant regions) bound to the variable region and first constant region of a single heavy chain by a disulfide bond.

[0067] "Fab'" refers to a Fab fragment that includes a portion of the hinge region.

[0068] "$F(ab')_2$ refers to a dimer of Fab.

[0069] "Fc" with regard to an antibody refers to that portion of the antibody consisting of the second and third constant regions of a first heavy chain bound to the second and third constant regions of a second heavy chain via disulfide bonding. The Fc portion of the antibody is responsible for various effector functions such as ADCC, and CDC, but does not function in antigen binding.

[0070] "Fv" with regard to an antibody refers to the smallest fragment of the antibody to bear the complete antigen binding site. An Fv fragment consists of the variable region of a single light chain bound to the variable region of a single heavy chain.

[0071] "Single-chain Fv antibody" or "scFv" refers to an engineered antibody consisting of a light chain variable region and a heavy chain variable region connected to one another directly or via a peptide linker sequence (Houston 1988).

[0072] "Single-chain Fv-Fc antibody" or "scFv-Fc" refers to an engineered antibody consisting of a scFv connected to the Fc region of an antibody.

[0073] "Camelized single domain antibody," "heavy chain antibody," or "HCAb" refers to an antibody that contains two V_H domains and no light chains (Riechmann 1999; Muyldermans 2001; WO94/04678; WO94/25591; U.S. Patent No. 6,005,079). Heavy chain antibodies were originally derived from *Camelidae* (camels, dromedaries, and llamas). Although devoid of light chains, camelized antibodies have an authentic antigen-binding repertoire (Hamers-Casterman 1993; Nguyen 2002; Nguyen 2003). The variable domain of a heavy chain antibody (VHH domain) represents the smallest known antigen-binding unit generated by adaptive immune responses (Koch-Nolte 2007).

[0074] A "nanobody" refers to an antibody fragment that consists of a VHH domain from a heavy chain antibody and two constant domains, CH2 and CH3.

[0075] "Diabodies" include small antibody fragments with two antigen-binding sites, wherein the fragments comprise a V_H domain connected to a V_L domain in the same polypeptide chain (V_H-V_L or V_H-V_L) (see, *e.g.*, Holliger 1993; EP404097; WO93/11161). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain, thereby creating two antigen-binding sites.

[0076] A "domain antibody" refers to an antibody fragment containing only the variable region of a heavy chain or the variable region of a light chain. In certain instances, two or more V_H domains are covalently joined with a peptide linker to create a bivalent domain antibody. The two V_H domains of a bivalent domain antibody may target the same or different antigens.

[0077] In certain embodiments, a "$(dsFv)_2$" comprises three peptide chains: two V_H moieties linked by a peptide linker and bound by disulfide bridges to two V_L moieties.

[0078] In certain embodiments, a "bispecific ds diabody" comprises V_{H1}-V_{L2} (linked by a peptide linker) bound to V_{L1}-V_{H2} (also linked by a peptide linker) via a disulfide bridge between V_{H1} and V_{L1}.

[0079] In certain embodiments, a "bispecific dsFv" or dsFv-dsFv'" comprises three peptide chains: a V_{H1}-V_{H2} moiety wherein the heavy chains are linked by a peptide linker (*e.g.*, a long flexible linker) and bound to V_{L1} and V_{L2} moieties, respectively, via disulfide bridges, wherein each disulfide paired heavy and light chain has a different antigen specificity.

[0080] In certain embodiments, an "scFv dimer" is a bivalent diabody comprising V_H-V_L (linked by a peptide linker) dimerized with another V_H-V_L moiety such that V_H's of one moiety coordinate with the V_L's of the other moiety and form two identical binding sites. In other embodiments, an "scFv dimer" is a bispecific diabody comprising V_{H1}-V_{L2} (linked by a peptide linker) associated with V_{L1}-V_{H2} (also linked by a peptide linker) such that V_{H1} and V_{L1} coordinate and V_{H2} and V_{L2} coordinate and each coordinated pair has a different antigen specificity.

[0081] The term "epitope" as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody binds. Two antibodies may bind the same epitope within an antigen if they exhibit competitive binding for the antigen. For example, if an antibody or antigen-binding fragment as disclosed herein competes with Bevacizumab for VEGF binding, the antibody may be, but is not necessarily, considered to bind the same epitope as Bevacizumab.

[0082] "VEGF" or "VEGF ligand" as used herein refers to one of the seven currently known VEGF ligands: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E (viral-derived), or placental growth factor (PlGF)-1 or -2. With regard to VEGF-A, there are currently four known splicing isoforms, with each demonstrating unique biological functions. The 165 amino acid isoform ($VEGF_{165}$, SEQ ID NO:1) exists in both heparin-bound and soluble forms.

The 121 amino acid isoform ($VEGF_{121}$), which is missing a fragment corresponding to the region between residues 115 and 159 of $VEGF_{165}$, exists in soluble form only. The longer 189 and 206 amino acid isoforms ($VEGF_{189}$ and $VEGF_{206}$, respectively) retain the ability to bind heparin. The antibodies and antigen-binding fragments provided herein exhibit high binding affinity for $hVEGF_{165}$, but in certain embodiments may cross-react or exhibit low-level binding affinity to a non-human VEGF protein or to other VEGF isoforms.

[0083] "VEGF signaling" as used herein includes intracellular events induced by VEGF binding to one or more VEGF receptors, such as receptor phosphorylation (*e.g.*, tyrosine phosphorylation), binding of intracellular signaling molecules (*e.g.*, PLCγ; phospholipase C-γ) to the receptor or to other intracellular signaling molecules, the initiation of a signaling cascade, and/or the initiation of a biological response (*e.g.*, induction of gene expression and changes in the physiology or development (*e.g.*, proliferation) of the cell).

[0084] "Cancer" or "cancerous condition" as used herein refers to any medical condition mediated by neoplastic or malignant cell growth, proliferation, or metastasis, and includes both solid cancers and non-solid cancers such as leukemia. "Tumor" as used herein refers to a solid mass of neoplastic and/or malignant cells.

[0085] "Treating" or "treatment" of a condition as used herein includes preventing or alleviating a condition, slowing the onset or rate of development of a condition, reducing the risk of developing a condition, preventing or delaying the development of symptoms associated with a condition, reducing or ending symptoms associated with a condition, generating a complete or partial regression of a condition, curing a condition, or some combination thereof. With regard to cancer, "treating" or "treatment" may refer to inhibiting or slowing neoplastic or malignant cell growth, proliferation, or metastasis, preventing or delaying the development of neoplastic or malignant cell growth, proliferation, or metastasis, or some combination thereof. With regard to a tumor, "treating" or "treatment"

includes eradicating all or part of a tumor, inhibiting or slowing tumor growth and metastasis, preventing or delaying the development of a tumor, or some combination thereof.

[0086] The term "specifically binds" as used herein refers to a non-random binding reaction between two molecules, such as for example between an antibody and a ligand. As used herein, an antibody or antigen-binding fragment that specifically binds a first ligand may exhibit cross-reactivity or low level binding affinity with a second ligand. In certain embodiments, an antibody or antigen-binding fragment that specifically binds a ligand binds the ligand with a binding affinity (K_D) of $\leq 10^{-7}$ M (*e.g.*, $5x10^{-8}$ M, 10^{-8} M, $5x10^{-9}$ M, 10^{-9} M, 10^{-10} M). K_D as used herein refers to the ratio of the dissociation rate to the association rate (k_{off}/k_{on}), may be determined using methods known in the art (*e.g.*, using Biacore or Kinexa techniques). In certain embodiments, an antibody or antigen-binding fragment that specifically binds to a ligand binds to that ligand with a binding affinity of no less than 10 fold higher (*e.g.*, ≥10 fold, ≥15 fold, ≥20 fold, ≥50 fold, $\geq 10^2$ fold, $\geq 10^3$ fold, or $\geq 10^4$ fold) than the binding affinity with which the antibody binds to a second ligand. In other embodiments, an antibody that specifically binds a ligand such as $hVEGF_{165}$ binds the ligand with a binding affinity (K_D) of $\leq 10^{-7}$ M, but exhibits no detectable binding affinity for a second ligand such as $mVEGF_{165}$.

[0087] An "isolated" substance has been altered by the hand of man from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide is "isolated" if it has been sufficiently separated from the coexisting materials of its natural state so as to exist in a substantially pure state.

[0088] The term "vector" as used herein refers to a vehicle into which a polynucleotide encoding a protein may be operably inserted so as to bring about the expression of that protein. A vector may be used to transform, transduce, or transfect a host cell so as to bring about expression of the genetic element it carries within the host cell. Examples of vectors include plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. Categories of animal viruses used as vectors include retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40). A vector may contain a variety of elements for controlling expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selectable elements, and reporter genes. In addition, the vector may contain an origin of replication. A vector may also include materials to aid in its entry into the cell, including but not limited to a viral particle, a liposome, or a protein coating.

[0089] The phrase "host cell" as used herein refers to a cell into which an exogenous polynucleotide and/or a vector has been introduced. A host cell may be selected from a variety of cell types, including for example bacterial cells such as *E. coli* or *B. subtilis* cells, fungal cells such as yeast cells or *Aspergillus* cells, insect cells such as *Drosophila* S2 or *Spodoptera* Sf9 cells, or animal cells such as fibroblasts, CHO cells, COS cells, NSO cells, HeLa cells, BHK cells, HEK 293 cells, or human cells.

[0090] A "disease associated with aberrant angiogenesis" as used herein refers to any condition that is caused by, exacerbated by, or otherwise linked to increased angiogenesis, specifically increased angiogenesis associated with or mediated by VEGF signaling. Such conditions include cancers mediated by cells that are dependent on neo-

angiogenesis for growth, proliferation, or metastasis, diseases of the eye such as for example wet AMD, and inflammatory conditions such as for example rheumatoid arthritis, psoriasis, scleroderma, chronic obstructive pulmonary disease (COPD), or asthma.

[0091] The ability to "block binding" or "compete for binding" as used herein refers to the ability of an antibody or antigen-binding fragment to inhibit the binding interaction between two molecules to any detectable agree. In certain embodiments, an antibody or antigen-binding fragment that blocks binding between two molecules inhibits the binding interaction between the two molecules by at least 50%. In certain embodiments, this inhibition may be greater than 60%, in certain embodiments greater than 70%, in certain embodiments greater than 80%, and in certain embodiments greater than 90%. In certain embodiments, the binding interaction being inhibited may be that of Bevacizumab to $hVEGF_{165}$. In certain other embodiments, the interaction being inhibited may be that of $hVEGF_{165}$ or any other VEGF ligand to VEGF-R1 and/or VEGF-R2.

Fully human VEGF antibodies and antigen-binding fragments

[0092] Fully human antibodies have several potential advantages over murine, chimeric, or humanized antibodies in terms of both safety and efficacy. First, their lack of non-human residues makes fully human antibodies less likely to generate a host immune response following administration. Second, fully human antibodies generally exhibit lower clearance rates than other antibody types. This decreased clearance rate allows for the use of lower dosage amounts and frequencies.

[0093] Provided herein are anti-hVEGF antibodies and antigen-binding fragments thereof that have been characterized as possessing superior anti-tumor activity *in vivo*. This represents an unexpected and surprising discovery because of the uncertainty associated with developing antibodies with superior *in vitro* antigen binding characteristics as a function of K_D in combination with superior *in vivo* biological effects. Indeed, it is known

that antibodies with particularly high *in vitro* binding affinity for VEGF do not necessarily possess high *in vivo* efficacy (Liang 2006). Therefore, identification of antibodies with high *in vivo* efficacy such as those disclosed herein is highly unpredictable and requires extensive scientific experimentation.

[0094] Disclosed herein are the fully human parental antibodies XPA.10.064 and XPA.072, both of which specifically bind $hVEGF_{165}$. As discussed below, the parental XPA.10.064 antibody was affinity matured to generate antibodies with high *in vivo* efficacy. XPA.10.064 and XPA.10.072 were identified by panning a phage display scFv library with $hVEGF_{165}$. The heavy and light chain variable region sequences of XPA.10.072 are set forth below and in SEQ ID NOs:2 and 3, respectively, and the heavy and light chain variable region sequences of XPA.10.064 are set forth below and in SEQ ID NOs:4 and 5, respectively. The XPA.10.072 and XPA.10.064 heavy chain variable regions as set forth in SEQ ID NOs:2 and 4, respectively, contain CDRs at residues 31-35 (HCDR1, SEQ ID NO:6), 50-66 (HCDR2, SEQ ID NO:7), and 99-108 (HCDR3, SEQ ID NO:8). The XPA.10.072 light chain variable region as set forth in SEQ ID NO:3 contains CDRs at residues 26-35 (LCDR1, SEQ ID NO:9), 51-57 (LCDR2, SEQ ID NO:10), and 90-100 (LCDR3, SEQ ID NO:11). The XPA.10.064 light chain variable region as set forth in SEQ ID NO:5 contains CDRs at residues 23-35 (LCDR1, SEQ ID NO:12), 51-57 (LCDR2, SEQ ID NO:13), and 90-100 (LCDR3, SEQ ID NO:14).

[0095] Amino acid sequences of the mature XPA.10.072 and XPA.10.064 heavy and light chains (lacking signal sequences) (CDRs are underlined):

[0096] XPA.10.072 heavy chain:

RLQLVQSGAEVRKPGASVKVSCKASGYSFTGHYIHWARQAPGQGLEWMGWINPYSGG TNFPREFQGRVTMTRDTSVNTVYMELTRLTSDDTSVYYCARDHRIVGGLDYWGQGTLV TVSS (SEQ ID NO:2).

[0097] XPA.10.072 light chain:

QSVLTQPPSASGTPGQRVTISCSGSSSNLGSNFVYWYQQLPGTAPKLLIYRNHQRPSGV PDRFSGSKSGTSASLAISGLRSEDEADYYCASWDDSLRVVVFGGGTKLTVL (SEQ ID NO:3).

[0098] XPA.10.064 heavy chain:

EVQLVQSGAEVRKPGASVKVSCKASGYSFTGHYIHWVRQAPGQGLEWMGWINPYSGG TNFPREFQGRVTMTRDTSVNTVYMELTRLTSDDTSVYYCARDHRIVGGLDYWGQGTLV TVSS (SEQ ID NO:4).

[0099] XPA.10.064 light chain

SYVLTQPPSASGTPGQRVTISCSGSSSNIGINYVYWYQQLPGTAPKLLIYRNDQRPSGVP DRFSGSKSGTSASLAISGLRSEDEADYYCATWDDSLSGVVFGGGTKVTVL (SEQ ID NO:5).

[00100] Based on the ability of XPA.10.064 and XPA.10.072 scFvs to bind $hVEGF_{165}$ with high affinity in an ELISA and to inhibit binding of $hVEGF_{165}$ to VEGF-R1 and VEGF-R2, the antibodies were selected for conversion to scFv-Fc and IgG2 for additional functional studies. XPA.10.064 and XPA.10.072 scFv-Fcs and IgG2s all exhibited similar high binding affinity for $hVEGF_{165}$ as determined by Biacore analysis. Both antibodies also bound $hVEGF_{121}$. XPA.10.064 and XPA.10.072 IgG2s displayed only weak binding to $mVEGF_{165}$. The binding affinities of XPA.10.064 and XPA.10.072 IgG2s for $hVEGF_{165}$ were 1.24-1.71 nM and 1.66-1.7 nM, respectively. Biacore analysis also revealed that XPA.10.064 and XPA.10.072 IgG2s block binding of $hVEGF_{165}$ to VEGF-R1 and VEGF-R2 to a degree similar to that observed for Bevacizumab. The ability of XPA.10.064 and XPA.10.072 to inhibit VEGF signaling was confirmed by ELISA experiments showing that both antibodies inhibit $hVEGF_{165}$-induced VEGF-R2 phosphorylation.

[00101] Epitope analysis suggested that XPA.10.064 and XPA.10.072 bind linear epitopes on $hVEGF_{165}$, and that these epitopes may overlap to at least partially with the epitopes bound by Bevacizumab. Immunohistochemical analysis revealed that, unlike Bevacizumab, XPA.10.064 and XPA.10.072 both exhibit broad range tissue cross-reactivity. Both antibodies inhibited HUVEC proliferation, and both inhibited angiogenesis and tumor growth *in vivo*. A summary of the characteristics of XPA.10.064 and XPA.10.072 is set forth in Table 1.

Table 1: Summary of XPA.10.064 and XPA.10.072 characteristics

	XPA.10.064	**XPA.10.072**	**Bevacizumab**
HUVEC Proliferation Potency (HPP): HEK293-$hVEGF_{165}$, IgG2 (IC_{50})	0.07 µg/ml (0.66 in 2nd assay)	0.68 µg/ml	0.04 µg/ml
HPP: sf21-$hVEGF_{165}$, scFvFc (IC_{50})	0.41 µg/ml	>1 µg/ml	0.06 µg/ml
HPP: sf21-$hVEGF_{165}$, IgG2 (IC_{50})	0.15 µg/ml	0.24 µg/ml	0.05 µg/ml
Binding to $hVEGF_{165}$ (K_D)	1.24-1.71 nM (IgG2)	1.66-1.70 nM (IgG2)	0.425-0.605 nM (IgG2)
Binding to $mVEGF_{165}$ (K_D)	Weak binding (> 100 nM) by Biacore analysis	Weak binding (> 100 nM) by Biacore analysis	No detectable binding by Biacore analysis
Human/murine cross-reactivity	No	No	No
Blocks binding of $hVEGF_{165}$ to VEGF-R1	Yes (scFv-Fc)	Yes (scFv-Fc)	Yes
Blocks binding of $hVEGF_{165}$ to VEGF-R2	Yes (scFv-Fc)	Yes (scFv-Fc)	Yes
Binds different $hVEGF_{165}$ epitope than Bevacizumab	No	No	--
Conformational vs. linear epitope binding	Linear	Linear	Linear

MPA (% αKLH cntr; 2 x 5 mg/kg)	20	26	38

[00102] Affinity maturation was carried out on the heavy chain CDRs of XPA.10.064 in order to generate mutagenized versions of the fully human antibodies with improved binding and efficacy. An antibody library was generated by randomly mutagenizing XPA.10.064 HCDR3 in blocks of five amino acids, and the library was screened for binding to $hVEGF_{165}$ using phage display techniques. The HCDR3 sequences of five affinity matured IgGs (XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10) are set forth below and in SEQ ID NOs:15-19, respectively.

[00103] XPA.10.064.03: DQMVHGGLDY (SEQ ID NO:15).

[00104] XPA.10.064.04: DEMQNGGLDY (SEQ ID NO:16).

[00105] XPA.10.064.06: DEMTRGGLDY (SEQ ID NO:17).

[00106] XPA.10.064.07: DEMHVGGLDY (SEQ ID NO:18).

[00107] XPA.10.064.10: DEMVWGGLDY (SEQ ID NO:19).

[00108] Each of the XPA.10.064 affinity matured clones bound $hVEGF_{165}$ with a higher affinity than the parental XPA.10.064 antibody or Bevacizumab as determined by Biacore analysis, and exhibited only weak binding to $mVEGF_{165}$. The affinity matured clones also exhibited the ability to inhibit HUVEC proliferation to a greater extent than parental XPA.10.064. In addition, XPA.10.064.06 exhibited the ability to inhibit tumor growth *in vivo* in a Rhabdomyosarcoma tumor growth model to a greater extent than Bevacizumab at dosages as low as 0.1 mg/kg. The percent tumor growth inhibition following administration of XPA.10.064.06 at 0.1 mg/kg and 0.5 mg/kg was approximately the same as that obtained by administering Bevacizumab at dosages at least five times greater. When administered at the same dosage, XPA.10.064.06 inhibited tumor growth to a specified size for a duration at least two to three times longer than Bevacizumab. As discussed above, this increase in *in vivo* efficacy was unexpected because increasing the

affinity of a VEGF antibody does not necessarily correlate with an increase in *in vivo* tumor growth reduction efficacy (Liang 2006).

[00109] Provided herein in certain embodiments are antibodies and antigen-binding fragments that comprise the HCDR3 sequence of XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, or XPA.10.064.10 as set forth in SEQ ID NOs:15-19, respectively. In certain of these embodiments, the antibodies or antigen-binding fragments may further comprise the HCDR1 (GHYIH) and/or HCDR2 (WINPYSGGTNFPREFQG) sequence of XPA.10.064 as set forth in SEQ ID NOs:6 and 7, respectively. In certain embodiments, the antibodies or antigen-binding fragments provided herein comprise the heavy chain variable sequence of XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, or XPA.10.064.10 as set forth below and in SEQ ID NOs:20-24, respectively. In certain embodiments, the antibodies or antigen-binding fragments further comprise one or more of the LCDR sequences of XPA.10.064 as set forth in SEQ ID NOs:12-14. In certain of these embodiments, the antibodies or antigen-binding fragments comprise the light chain variable sequence of XPA.10.064 as set forth in SEQ ID NO:5. Also disclosed herein in certain embodiments are antibodies and antigen-binding fragments thereof that bind to the same epitope bound by the affinity-matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, or XPA.10.064.10. The amino acid sequences of the mature affinity matured XPA.10.064 heavy chains (lacking signal sequences) are set forth below and in SEQ ID NOs:20-24 (CDRs underlined):

[00110] XPA.10.064.03:

EVQLVQSGAEVRKPGASVKVSCKASGYSFTGHYIHWVRQAPGQGLEWMGWINPYSGG TNFPREFQGRVTMTRDTSVNTVYMELTRLTSDDTSVYYCARDQMVHGGLDYWGQGTL VTVSS (SEQ ID NO:20).

[00111] XPA.10.064.04:

EVQLVQSGAEVRKPGASVKVSCKASGYSFTGHYIHWVRQAPGQGLEWMGWINPYSGG TNFPREFQGRVTMTRDTSVNTVYMELTRLTSDDTSVYYCARDEMQNGGLDYWGQGTL VTVSS (SEQ ID NO:21).

[00112] XPA.10.064.06:

EVQLVQSGAEVRKPGASVKVSCKASGYSFTGHYIHWVRQAPGQGLEWMGWINPYSGG TNFPREFQGRVTMTRDTSVNTVYMELTRLTSDDTSVYYCARDEMTRGGLDYWGQGTLV TVSS (SEQ ID NO:22).

[00113] XPA.10.064.07:

EVQLVQSGAEVRKPGASVKVSCKASGYSFTGHYIHWVRQAPGQGLEWMGWINPYSGG TNFPREFQGRVTMTRDTSVNTVYMELTRLTSDDTSVYYCARDEMHVGGLDYWGQGTLV TVSS (SEQ ID NO:23).

[00114] XPA.10.064.10:

EVQLVQSGAEVRKPGASVKVSCKASGYSFTGHYIHWVRQAPGQGLEWMGWINPYSGG TNFPREFQGRVTMTRDTSVNTVYMELTRLTSDDTSVYYCARDEMVWGGLDYWGQGTL VTVSS (SEQ ID NO:24). CDRs are underlined.

[00115] In certain embodiments, the antibodies and antigen-binding fragments provided herein comprise the HCDR3 sequence of XPA.10.064.06 or XPA.10.064.07 as set forth in SEQ ID NOs:17 and 18, respectively. In certain of these embodiments, the antibodies or antigen-binding fragments may further comprise the HCDR1 and/or HCDR2 sequence of XPA.10.064 as set forth in SEQ ID NOs:6 and 7, respectively. In certain embodiments, the antibodies or antigen-binding fragments provided herein comprise the heavy chain variable sequence of XPA.10.064.06 or XPA.10.064.07 as set forth in SEQ ID NOs:22 and 23, respectively. In certain embodiments, the antibodies or antigen-binding fragments further comprise one or more of the LCDR sequences of XPA.10.064 as set

forth in SEQ ID NOs:12-14. In certain of these embodiments, the antibodies or antigen-binding fragments comprise the light chain variable sequence of XPA.10.064 as set forth in SEQ ID NO:5.

[00116] Provided herein in certain embodiments are antibodies or antigen-binding fragments thereof that specifically bind VEGF and have one or more of the following properties: (1) a K_D for $hVEGF_{165}$ of about 10^{-10} M (*i.e.*, similar to the average K_D exhibited by the affinity matured XPA.10.064 antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10); (2) a k_a for $hVEGF_{165}$ of about 1.89 X 10^5 (*i.e.*, similar to the average K_a exhibited by the affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10); (3) a k_d for $hVEGF_{165}$ of about 1.73 X 10^{-5} (*i.e.,* similar to the average k_d exhibited by the affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10); (4) a binding affinity (as a function of K_D) for $hVEGF_{165}$ that is greater than that of Bevacizumab (*i.e.*, about 4.25 X 10^{-10}) to about the same degree that the binding affinities of the affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 for $hVEGF_{165}$ are greater than that of Bevacizumab; (5) the ability to inhibit HUVEC cell proliferation with an IC_{50} of about 154 pM (*i.e.*, similar to the average IC_{50} exhibited by the affinity matured antibodies XPA.10.064.03, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10); (6) the ability to inhibit HUVEC cell proliferation with an IC_{50} about 56% that of Bevacizumab; (7) a K_D for $hVEGF_{165}$ from about 1.97 X 10^{-10} M to about 3.49 X 10^{-11} M (*i.e.*, within the range exhibited by the affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10); (8) a k_a for $hVEGF_{165}$ from about 1.5 X 10^5 to about 2.16 X 10^5 (*i.e.*, within the range exhibited by the affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and

XPA.10.064.10); (9) a k_d for $hVEGF_{165}$ from about 6.65 X 10^{-6} to about 2.94 X 10^{-5} (*i.e.*, within the range exhibited by the affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10); (10) the ability to inhibit HUVEC cell proliferation with an IC_{50} of about 129 pM to about 174 pM (*i.e.*, within the range exhibited by the affinity matured antibodies XPA.10.064.03, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10); (11) a binding affinity for $hVEGF_{165}$ that is at least about 10 times greater than the binding affinity of the antibody or antigen-binding fragment for $mVEGF_{165}$; and (12) the ability to compete with Bevacizumab for $hVEGF_{165}$ binding.

[00117] Further provided herein are complexes comprising one or more of the antibodies or antigen-binding fragments disclosed herein and one or more VEGF ligands or antigenic fragments thereof. These complexes may be formed *in vitro* or *in vivo*. For example, in certain embodiments, such complexes may be formed when an antibody or antigen-binding fragment as disclosed herein is administered to a subject and binds to VEGF in the body of the subject.

[00118] The affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 as provided herein were generated by random mutagenesis of XPA.10.064 HCDR3 and subsequent binding and functional assays. In certain embodiments, the methionine residue in XPA.10.064.06 HCDR3 may be substituted with another amino acid residue, such as for example an alanine, lysine, proline, threonine, or leucine residue. Examples of such mutated HCDR3 sequences are set forth in SEQ ID NOs:25-29. Therefore, in certain embodiments, antibodies and antigen-binding fragments are provided that comprise the HCDR3 sequence set forth in any of SEQ ID NOs:25-29. In certain of these embodiments, the antibodies or antigen-binding fragments may further comprise the HCDR1 and/or HCDR2 sequence of XPA.10.064 as set forth in SEQ ID NOs:6 and 7, respectively. In certain embodiments,

the antibodies or antigen-binding fragments may further comprise one or more of the LCDR sequences of XPA.10.064 as set forth in SEQ ID NOs:12-14. In certain of these embodiments, the antibodies or antigen-binding fragments may comprise the light chain variable sequence of XPA.10.064 as set forth in SEQ ID NO:5.

[00119] In certain embodiments, additional affinity matured versions of the parental antibody XPA.10.064 are generated by mutagenizing one or more residues of XPA.10.064 HCDR1, HCDR2, LCDR1, LCDR2, and/or LCDR3. Therefore, in certain embodiments, antibodies and antigen-binding fragments are provided that comprise one or more CDR sequences of XPA.10.064, wherein the one or more CDR sequences contain one or more amino acid substitutions. Antibodies and antigen-binding fragments generated in this manner may be screened for binding to $hVEGF_{165}$ in order to identify affinity matured antibodies with improved binding characteristics. Antibodies with favorable binding characteristics may be subjected to one or more functional assays to determine their ability to, for example, inhibit HUVEC proliferation, angiogenesis, tumor growth, and/or $hVEGF_{165}$-induced phosphorylation of VEGF-R2.

[00120] In certain embodiments, the antibodies or antigen-binding fragments provided herein bind $hVEGF_{165}$ with a greater affinity than that of Bevacizumab for $hVEGF_{165}$. For example, in certain embodiments, the antibodies or antigen-binding fragments provided herein bind $hVEGF_{165}$ with a $K_D \leq 500$ pM. In certain of these embodiments, the antibodies or antigen-binding fragments bind $hVEGF_{165}$ with a $K_D \leq 200$ pM, in other embodiments ≤ 150 pM, in other embodiments ≤ 100 pM, and in still other embodiments ≤ 50 pM.

[00121] In certain embodiments, the antibodies and antigen-binding fragments provided herein exhibit no detectable binding affinity or weak binding affinity for $mVEGF_{165}$. In certain of these embodiments, the antibodies or antigen-binding fragments

exhibit a K_D for $mVEGF_{165}$ ≥ about 100 nM. In certain embodiments, the antibodies and antigen-binding fragments exhibit a binding affinity for $hVEGF_{165}$ that is at least 10-fold greater (*e.g.*, 20-fold, 30-fold, or 40-fold greater) than the binding affinity of the antibody or antigen-binding fragment for $mVEGF_{165}$. In certain of these embodiments, the binding affinity of the antibody or antigen-binding fragment for $hVEGF_{165}$ is at least 50-fold greater (*e.g.*, 60-fold, 70-fold, 80-fold, or 90-fold greater) than the binding affinity of the antibody or antigen-binding fragment for $mVEGF_{165}$, and in certain embodiments at least 100-fold greater (*e.g.*, 110-fold, 120-fold, 150-fold, 175-fold, 200-fold, 250-fold, 300-fold, 400-fold, or 500-fold greater).

[00122] Based on their high binding affinity for $hVEGF_{165}$ and $hVEGF_{121}$ and their ability to block VEGF binding to VEGF-R1 and VEGF-R2 and VEGF-induced receptor phosphorylation, the antibodies and antigen-binding fragments provided herein may be used to inhibit VEGF signaling. On this basis, the antibodies and antigen-binding fragments may be used to treat various conditions associated with VEGF expression and/or signaling.

[00123] The antibodies and antigen-binding fragments provided herein have been found to inhibit HUVEC proliferation and to inhibit angiogenesis. Therefore, the antibodies and antigen-binding fragments may be used to treat various conditions associated with increased angiogenesis. For example, the antibodies and antigen-binding fragments may be used to treat cancer by inhibiting the proliferation of blood vessels from a tumor site and thus inhibiting tumor growth. Likewise, the antibodies and antigen-binding fragments may used to treat cancer by destroying blood vessels at a tumor site, resulting in tumor necrosis. The efficacy of the antibodies and antigen-binding fragments disclosed herein for the treatment of cancer has been confirmed *in vivo*.

[00124] In certain embodiments, the antibodies and antigen-binding fragments provided herein inhibit angiogenesis and/or tumor growth at a level similar to or greater than Bevacizumab. In *in vivo* A673 Rhabdomyosarcoma tumor growth inhibition experiments, XPA.10.064 and XPA.10.072 inhibited tumor growth to a degree that was similar to or greater than Bevacizumab at all dosages tested. The affinity matured antibody XPA.10.064.06 inhibited tumor growth more effectively than Bevacizumab. As a function of percent tumor growth inhibition, XPA.10.064.06 was approximately five-fold more effective than Bevacizumab at reducing tumor growth. Likewise, XPA.10.064.06 delayed tumor growth to a specified size for a significantly longer duration than Bevacizumab when the antibodies were administered at the same dosage. Therefore, in certain embodiments, the antibodies and antigen-binding fragments disclosed herein inhibit tumor growth as measured by percent tumor growth inhibition or duration of tumor growth delay at least twice as effectively as Bevacizumab when administered at the same or similar dosages. In certain of these embodiments, the antibodies and antigen-binding fragments disclosed herein inhibit tumor growth at least three times as effectively as Bevacizumab, in other embodiments at least four times as effectively as Bevacizumab, in other embodiments at least five times as effectively as Bevacizumab, and in other embodiments more than five times as effectively as Bevacizumab. Likewise, in certain embodiments the antibodies and antigen-binding fragments disclosed herein inhibit tumor growth to a degree approximately equal to or greater than Bevacizumab when administered at a lower dosage than Bevacizumab. In certain of these embodiments, the antibodies and antigen-binding fragments disclosed herein inhibit tumor growth to a degree approximately equal to or greater than Bevacizumab when administered at one-half the dosage of Bevacizumab, in other embodiments at one-third the dosage of Bevacizumab, in other embodiments at one-fourth the dosage of Bevacizumab, and in

other embodiments at one-fifth the dosage of Bevacizumab. The antibodies and antigen-binding fragments provided herein may exhibit similar or improved pharmacokinetic (PK) properties as compared to Bevacizumab. For example, the antibodies or antigen-binding fragments may exhibit increased circulating half-life or decreased immunogenicity as compared to Bevacizumab. In certain embodiments wherein the antibodies or antigen-binding fragments exhibit similar or improved pharmacokinetic properties versus Bevacizumab, the antibodies or antigen-binding fragments may be administered over a longer interval than Bevacizumab without exhibiting negative effects associated with increased intervals of Bevacizumab administration.

[00125] The antibodies and antigen-binding fragments disclosed herein may be used in the treatment of any condition associated with aberrant angiogenesis controlled at least in part by VEGF signaling. These conditions, which are generally associated with increased VEGF expression levels, include ocular diseases associated with increased angiogenesis, such as wet AMD or proliferative retinopathies such as diabetic retinopathy, diabetic kidney disease and other diabetic vascular proliferative diseases, cystic fibrosis, and various tumor types (Amoroso 1997; McColley 2000; Khamaisi 2003).

[00126] Cancerous conditions and tumor types that may be treated using the antibodies or antigen-binding fragments disclosed herein include but are not limited to carcinoma, blastoma, sarcoma, germ cell tumor, or hematological or lymphoid malignancy such as leukemia, lymphoma, or multiple myeloma. More specifically, cancerous conditions and tumor types that may be treated using the antibodies disclosed herein include but are not limited to squamous cell cancer, lung cancer (*e.g.*, small cell lung cancer, non-small cell lung cancer (NSCLC), adenocarcinoma of the lung, or squamous cell carcinoma of the lung), cancer of the peritoneum, liver cancer (*e.g.*, hepatocellular carcinoma/hepatoma), gastric or stomach cancer (*e.g.*, gastrointestinal cancer), pancreatic

cancer, brain tumor (*e.g.*, glioblastoma/glioblastoma multiforme (GBM), non-glioblastoma brain tumor, or meningioma), glioma (*e.g.*, ependymoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, or mixed glioma such as oligoastrocytoma), cervical cancer, ovarian cancer, liver cancer (*e.g.*, hepatoblastoma, hepatocellular carcinoma/hepatoma, or hepatic carcinoma), bladder cancer (*e.g.*, urothelial cancer), breast cancer, colon cancer, colorectal cancer, rectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer (*e.g.*, rhabdoid tumor of the kidney), prostate cancer, vulval cancer, penile cancer, anal cancer (*e.g.*, anal squamous cell carcinoma), thyroid cancer, head and neck cancer (*e.g.*, nasopharyngeal cancer), skin cancer (*e.g.*, melanoma or squamous cell carcinoma), osteosarcoma, Ewing's sarcoma, chondrosarcoma, soft tissue sarcoma (*e.g.*, rhabdomyosarcoma, fibrosarcoma, Kaposi's sarcoma), carcinoid cancer, eye cancer (*e.g.*, retinoblastoma), mesothelioma, lymphocytic/lymphoblastic leukemia (*e.g.*, acute lymphocytic/lymphoblastic leukemia (ALL) of both T-cell lineage and B-cell precursor lineage, chronic lymphoblastic/lymphocytic leukemia (CLL), acute myelogenous/myeloblastic leukemia (AML), including mast cell leukemia, chronic myelogenous/myelocytic/myeloblastic leukemia (CML), hairy cell leukemia (HCL), Hodgkin's disease, non-Hodgkin's lymphoma, chronic myelomonocytic leukemia (CMML), follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Burkitt's lymphoma (BL), mycosis fungoides, Sezary syndrome, cutaneous T-cell lymphoma, mast cell neoplasm, medulloblastoma, nephroblastoma, solitary plasmacytoma, myelodysplastic syndrome, chronic and non-chronic myeloproliferative disorder, central nervous system tumor, pituitary adenoma, vestibular schwannoma, primitive neuroectodermal tumor, ependymoma, choroid plexus papilloma, polycythemia vera, thrombocythemia, idiopathic myelofibrosis, and pediatric cancers such as pediatric sarcomas (*e.g.*, neuroblastoma, rhabdomyosarcoma, and

osteosarcoma). In addition, tumors can be malignant (*e.g.*, cancers) or benign (*e.g.*, hyperplasia, cyst, pseudocyst, hamartoma, and benign neoplasm).

[00127] Tumor types that may be treated using the antibodies or antigen-binding fragments disclosed herein also include cancers associated with a particular biomarker. For example, a biomarker includes, but is not limited to, mutations in the von Hippel-Lindau (VHL) tumor suppressor gene and/or overexpression of Hypoxia-inducible factor-1α (HIF-1α). In certain embodiments, the antibodies can be used to treat cancers displaying mutations in the VHL tumor suppressor gene. Mutations in the VHL gene result in the constitutive stabilization of hypoxia-inducible transcription factors 1α and 2α, which bind to enhancer elements in the VEGF gene and stimulate angiogenesis (Harris 2000). VHL mutant tumor types that may be treated using the antibodies disclosed herein include, for example, central nervous system hemangioblastomas, retinal hemangioblastomas, endolymphatic sac tumors, clear cell renal cell cancers and/or renal cysts, pheochromocytomas, pancreatic cysts, neuroendocrine tumors, and epididymal and broad ligament cystadenomas. Subjects are first selected or screened for the presence of VHL gene mutations through known methods such as the molecular detection using a mutation specific nested reverse transcription polymerase chain reaction or a nested single strand conformational polymorphism analysis (Ashida 2003). The identified subjects are then subject to the treatment with the antibodies or antigen-binding fragments as disclosed herein. In other embodiments, the antibodies can be used to treat cancers displaying overexpression of HIF-1α in a subject. The HIF-1α overexpression can be examined through biopsies of a tissue (*e.g.*, brain, breast, cervical, esophageal, oropharyngeal, ovarian, and prostate tissues). The identified subjects are selected and subject to the treatment with the antibodies or antigen-binding fragments, or with the antibodies or antigen-binding fragments in combination with HIF-1α inhibitors such as 2-

methoxyestradiol, 4-O-methylsarcerneol, manassantin A, manassantin B1, NSC-134754, NSC-643735, topotecan, SCH66336, PX-478, R115777, Cetuximab, 103D5R, and NSAID (Kimbro 2006). Provided herein are methods for selecting a subject for therapy with the antibodies or antigen-binding fragments disclosed herein if the subject is observed to possess one or more of the biomarkers discussed above (*e.g.*, VHL gene mutation) and, optionally, treating said subject with the antibody or antibody-binding fragment.

[00128] Other conditions that may be treated by the antibodies and antigen-binding fragments described herein include inflammatory conditions such as rheumatoid arthritis, psoriasis, scleroderma, chronic obstructive pulmonary disease, and asthma. In certain embodiments, the antibodies or antigen-binding fragments provided herein may be used to treat a condition that has become resistant to treatment with Bevacizumab.

[00129] The antibodies and antigen-binding fragments provided herein may be utilized in various non-therapeutic uses. In certain embodiments, the antibodies or antigen-binding fragments may be used as affinity purification agents to purify $hVEGF_{165}$, other VEGF isoforms, or fragments thereof. In these embodiments, the antibodies or antigen-binding fragments may be immobilized on a solid phase such as a resin or filter paper using methods known in the art. The antibodies or antigen-binding fragments may also be used to precipitate $hVEGF_{165}$, other VEGF isoforms, or fragments thereof from solution. In other non-therapeutic embodiments, the antibodies or antigen-binding fragments may be used in various *in vitro* or *in vivo* diagnostic or detection applications. In certain of these embodiments, the antibodies or antigen-binding fragments may be conjugated to a detectable label. In other embodiments, the antibodies or antigen-binding fragments may not be conjugated to a detectable label, but may be detected using a labeled secondary antibody that binds to the antibody. In certain embodiments, the antibodies or antigen-binding fragments disclosed herein may be used to detect $hVEGF_{165}$

expression. In certain of these embodiments, the antibodies or antigen-binding fragments may be used to diagnose a condition associated with increased $hVEGF_{165}$ expression. For example, the antibody or antigen-binding fragment may be contacted with a biological sample from a subject in order to diagnose a condition associated with increased $hVEGF_{165}$ expression in the subject. Likewise, the antibody or antigen-binding fragment may be administered to the subject directly, with binding to $hVEGF_{165}$ detected using methods known in the art.

[00130] Mutant epitope binding studies show that the antibodies and antigen-binding fragments disclosed herein may bind linear epitopes on VEGF that may overlap at least partially with the epitope recognized by Bevacizumab. Therefore, in certain embodiments, the antibodies or antigen-binding fragments disclosed herein may bind to an epitope consisting of or comprising residues 79-94 of $hVEGF_{165}$ (SEQ ID NO:1). Likewise, the antibodies or antigen-binding fragments may bind an epitope that completely or partially overlaps with the sequence corresponding to residues 79-94 of SEQ ID NO:1. In certain embodiments the antibodies or antigen-binding fragments disclosed herein competitively inhibit Bevacizumab binding to $hVEGF_{165}$.

[00131] The antibodies or antigen-binding fragments provided herein may have a terminal half-life ($t_{1/2}$) in humans that is similar to or greater than that of Bevacizumab, which has a half-life of 17-21 days (Ferrara 2004). For example, in certain embodiments, the antibodies or antigen-binding fragments provided herein may have a terminal half-life of about 21 days, 28 days, 35 days, or 60 days. Terminal half-life, which refers to the time that it takes the plasma concentration of an administered antibody to decrease by one half, may be calculated using methods known in the art. In certain embodiments, the terminal half-life of the antibodies or antigen-binding fragments disclosed herein may be at

least 17 days. In certain other embodiments, it may be 17-21 days, and in certain of these embodiments it may be greater than 21 days.

[00132] The antigen-binding fragments disclosed herein may comprise a fragment or fragments of an antibody, such as for example a Fab, Fab', $F(ab')_2$, Fv, or scFv fragment. These fragments can be produced from antibodies using methods well known in the art, such as for example proteolytic cleavage with enzymes such as papain to produce Fab fragments or pepsin to produce $F(ab')_2$ fragments. In certain embodiments, the antibodies or antigen-binding fragments disclosed herein may comprise one or more CDRs from SEQ ID NOs:2-5 or 20-24 grafted to one or more human framework regions.

[00133] The antibodies or antigen-binding fragments disclosed herein may be administered alone or in combination with one or more additional therapeutic agents. For example, the antibodies or antigen-binding fragments disclosed herein may be administered in combination with chemotherapy, radiation therapy, surgery for the treatment of cancer (*e.g.*, tumorectomy), one or more anti-emetics or other treatments for complications arising from chemotherapy, or any other therapeutic agent for use in the treatment of cancer or any medical disorder mediated by elevated VEGF expression and/or signaling. In certain of these embodiments, an antibody or antigen-binding fragment as disclosed herein that is administered in combination with one or more additional therapeutic agents may be administered simultaneously with the one or more additional therapeutic agents, and in certain of these embodiments the antibody or antigen-binding fragment and the additional therapeutic agent(s) may administered as part of the same pharmaceutical composition. However, an antibody or antigen-binding fragment administered "in combination" with another therapeutic agent does not have to be administered simultaneously with or in the same composition as the agent. An antibody or antigen-binding fragment administered prior to or after another agent is

considered to be administered "in combination" with that agent as the phrase is used herein, even if the antibody or antigen-binding fragment and second agent are administered via different routes. Where possible, additional therapeutic agents administered in combination with the antibodies or antigen-binding fragments disclosed herein are administered according to the schedule listed in the product information sheet of the additional therapeutic agent, or according to the Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed; Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002)) or protocols well known in the art.

[00134] Those of skill in the art will recognize that a variety of conjugates may be linked to the antibodies or antigen-binding fragments provided herein (see, for example, "Conjugate Vaccines", Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr. (eds.), Carger Press, New York, (1989)). These conjugates may be linked to the antibodies or antigen-binding fragments by covalent binding, affinity binding, intercalation, coordinate binding, complexation, association, blending, or addition, among other methods. In certain embodiments, the antibodies and antigen-binding fragments disclosed herein may be engineered to contain specific sites outside the epitope binding portion that may be utilized for binding to one or more conjugates. For example, such a site may include one or more reactive amino acid residues, such as for example cysteine or histidine residues, to facilitate covalent linkage to a conjugate. In certain embodiments, the antibodies may be linked to a conjugate indirectly, or through another conjugate. For example, the antibody or antigen-binding fragments may be conjugated to biotin, then indirectly conjugated to a second conjugate that is conjugated to avidin.

[00135] In certain embodiments, conjugates linked to the antibodies or antigen-binding fragments disclosed herein may comprise one or more agents meant to alter one or more pharmacokinetic (PK) properties of the antibody or antigen-binding fragment,

such as for example polyethylene glycol (PEG) to increase the half-life or decrease the immunogenicity of the antibody or antigen-binding fragment (see, *e.g.*, Katre 1990).

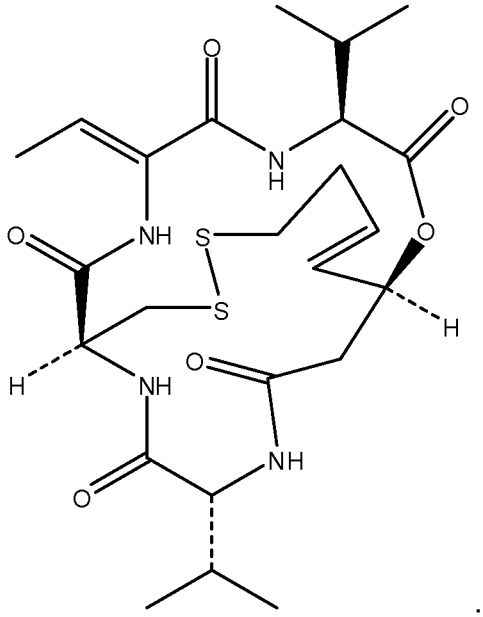
[00136] In certain embodiments, conjugates linked to the antibodies or antigen-binding fragments disclosed herein may comprise one or more detectable labels. Such labels include, but are not limited to, radioactive isotopes such as ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{35}S, ^{3}H, ^{111}In, ^{112}In, ^{14}C, ^{64}Cu, ^{67}Cu, ^{86}Y, ^{88}Y, ^{90}Y, ^{177}Lu, ^{211}At, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, and ^{32}P, other lanthanides, luminescent labels, fluorescent labels such as for example fluorescein, rhodamine, dansyl, phycoerythrin, or Texas Red, and enzyme-substrate labels such as for example horseradish peroxidase, alkaline phosphatase, or β-D-galactosidase.

[00137] In certain embodiments, compositions are provided comprising antibodies or antigen-binding fragments disclosed herein linked to or in combination with one or more cytokines, which include proteins that act on a cell as an intercellular mediator. Example of cytokines include but are not limited to lymphokines, monokines, human growth hormone, N-methionyl human growth hormone, bovine growth hormone, parathyroid hormone, thyroxine, insulin, proinsulin, relaxin, prorelaxin, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), hepatic growth factor, fibroblast growth factor, prolactin, placental lactogen, tumor necrosis factor α and β, mullerian-inhibiting substance, mouse gonadotropin-associated peptide, inhibin, activin, integrin, thrombopoietin (TPO), nerve growth factors such as NGF-β, platelet growth factor, transforming growth factors such as TGF-α and TGF-β, insulin-like growth factor I and II, erythropoietin (EPO), osteoinductive factors, interferons such as interferon-α, -β, and –γ, colony stimulating factors such as macrophage-CSF, granulocyte macrophage CSF, and granulocyte-CSF, interleukins such IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, and IL-12, tumor necrosis factors such as TNF-α and TNF-β, and other polypeptide factors. The antibodies or antigen-binding fragments disclosed herein

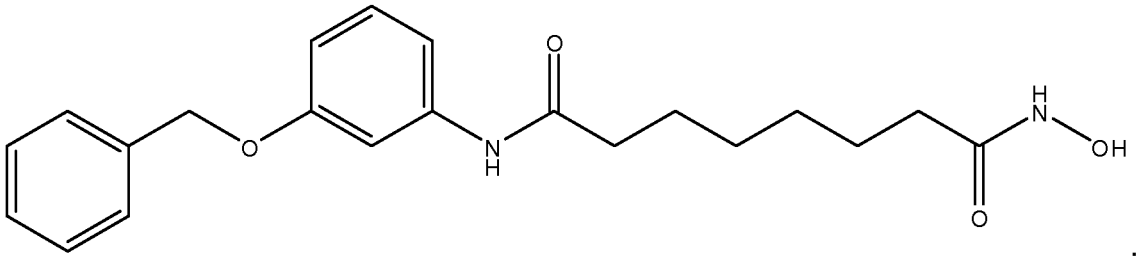
may be provided and/or administered in combination with any cytokine, including any of those listed above.

[00138] In certain embodiments, compositions are provided comprising antibodies or antigen-binding fragments disclosed herein linked to or in combination with one or more chemotherapeutic agents. Examples of chemotherapeutic agents include, but are not limited to, ALT-110, AMN-107 (Nilotinib), amrubicin, ARQ-197, atrasentan (Xinlay®), AV-299, AZD 1152, AZD 2171, batabulin, BIO-111, BIO-140, calcitriol, CC 8490, cilengitide, dasatinib, decatanib, DN-101, edotecarin, enzastaurin, erlotinib, everolimus, gimatecan, gossypol (*e.g.*, gossypol acetate), GSK461364, GSK690693, IL13-PE38QQR, INO 1001, IPdR, ipilimumab, KRX-0402, Lep-etu, lonafarnib, lucanthone, LY 317615, MK-0457, MLN8054, neuradiab, nolatrexed, oblimersen, ofatumumab, ON 0910.Na, oregovomab, panitumumab, pazopanib, PHA-739358, R-763, RTA 744, rubitecan, Sdx 102, talampanel, temsirolimus, tesmilifene, tetrandrine, ticilimumab, TKI-258, TLK 286, trabectedin, vandetanib, vitespan, Xr 311, zanolimumab, 131-I-TM-601, and zolendronate, histrelin, azacitidine, dexrazoxane, alemtuzumab, lenalidomide, gemtuzumab, ketoconazole, nitrogen mustard, ibritumomab tiuxetan, decitabine, hexamethylmelamine, bexarotene, tositumomab, arsenic trioxide, editronate, cyclosporine, Edwina-asparaginase, and strontium 89.

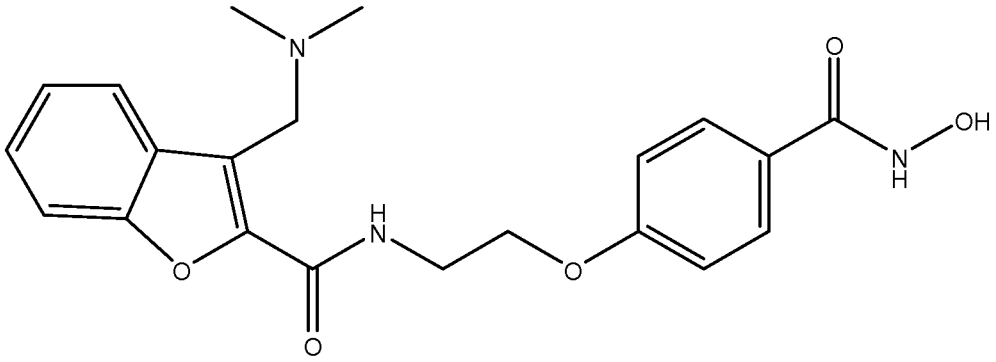
[00139] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with romidepsin (FK-228), which has the structure:



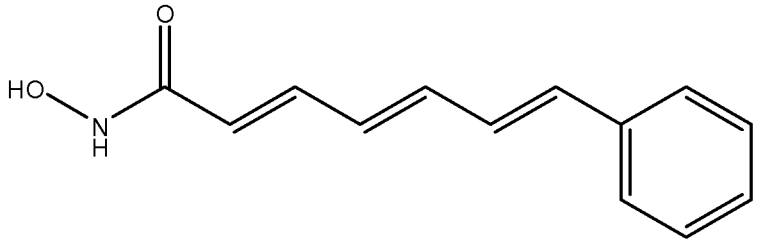
[00140] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with ADS-100380, which has the structure:



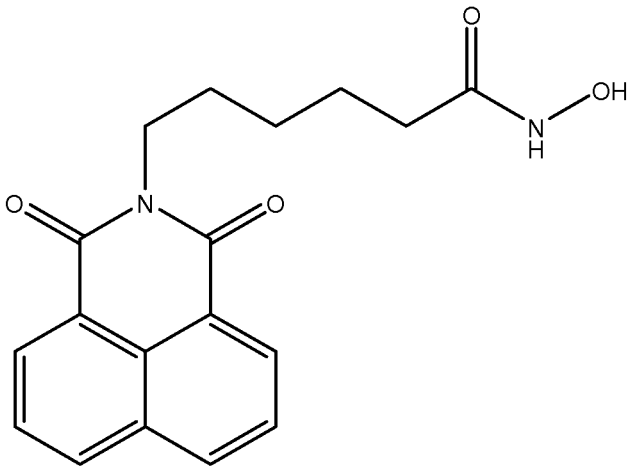
[00141] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with CG-781, which has the structure:



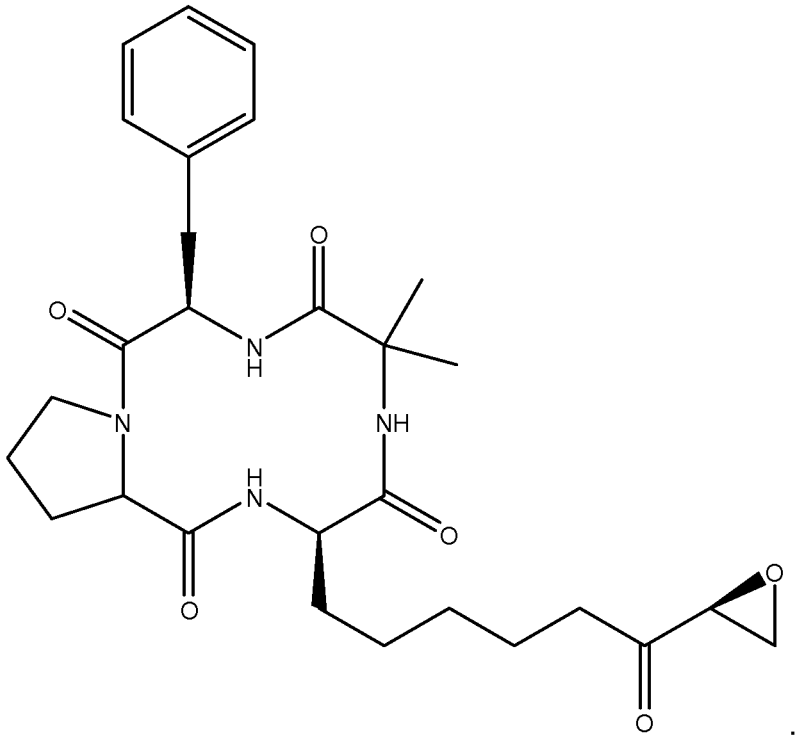
[00142] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with CG-1521, which has the structure:



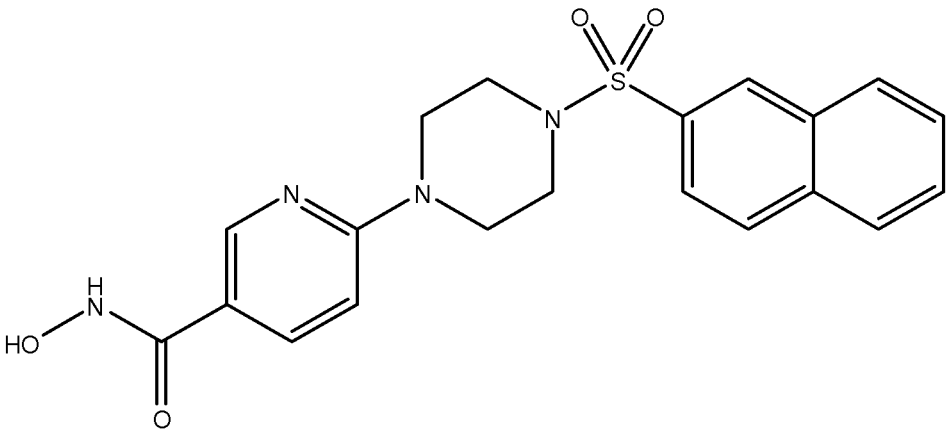
[00143] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with SB-556629, which has the structure:



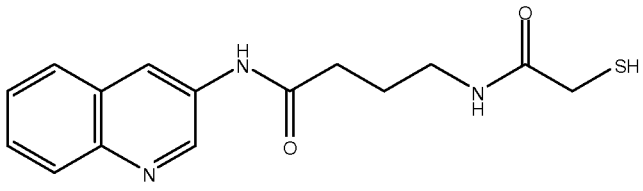
[00144] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with chlamydocin, which has the structure:

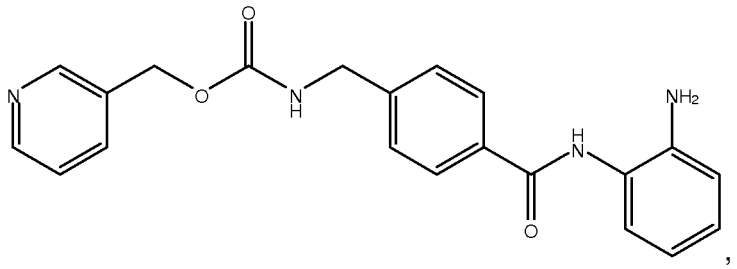


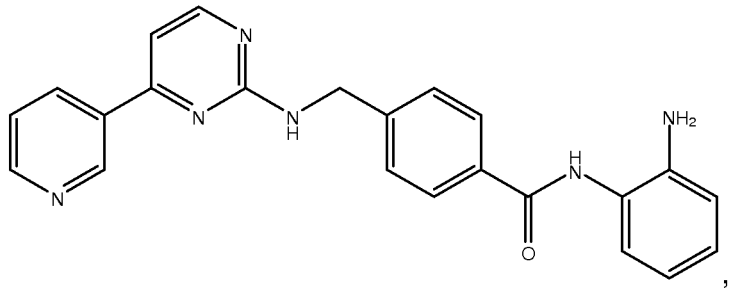
[00145] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with JNJ-16241199, which has the structure:

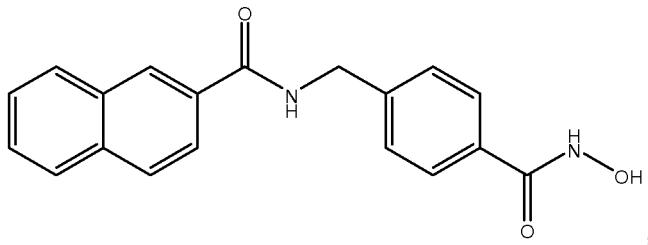


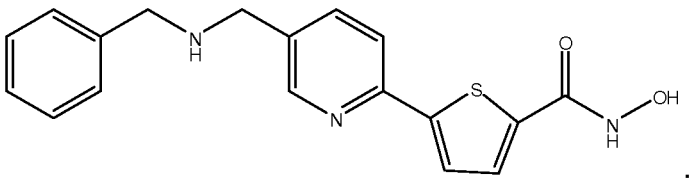
[00146] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with:



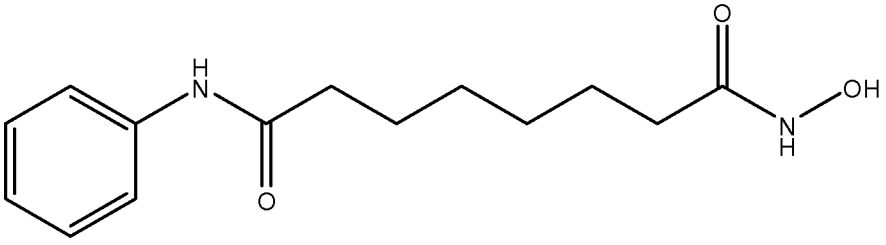

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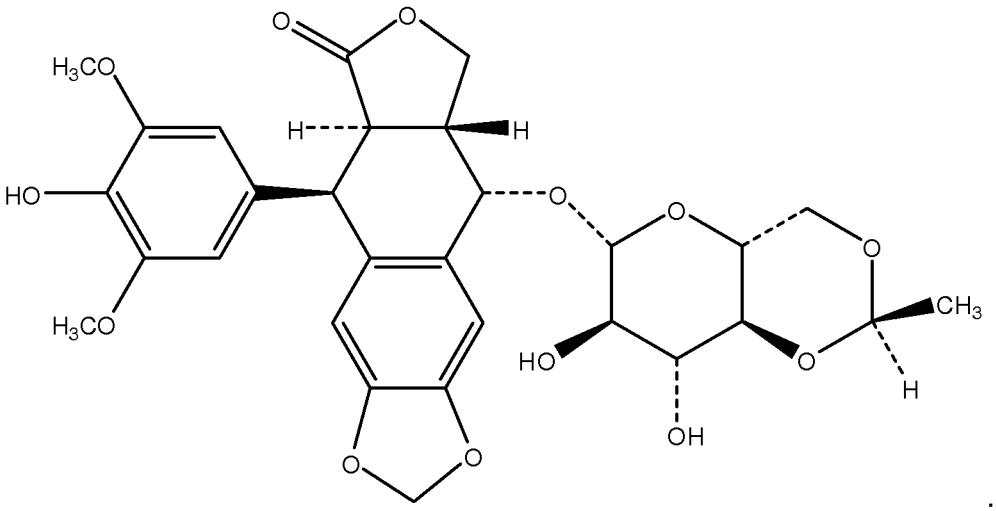

, or


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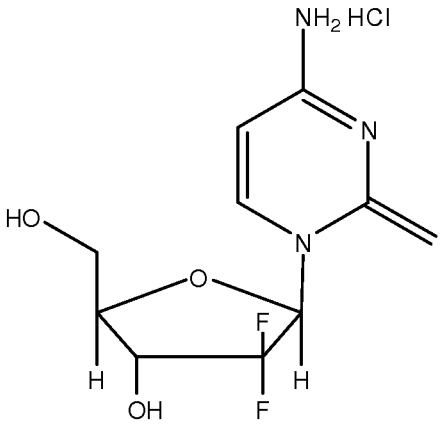
[00147] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with vorinostat (SAHA), which has the structure:


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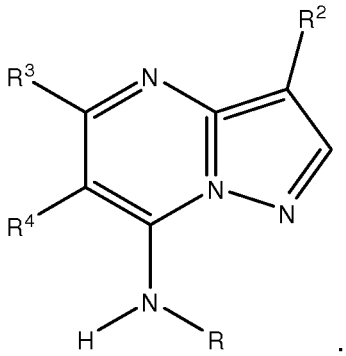
[00148] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with etoposide (VP-16), which has the structure:



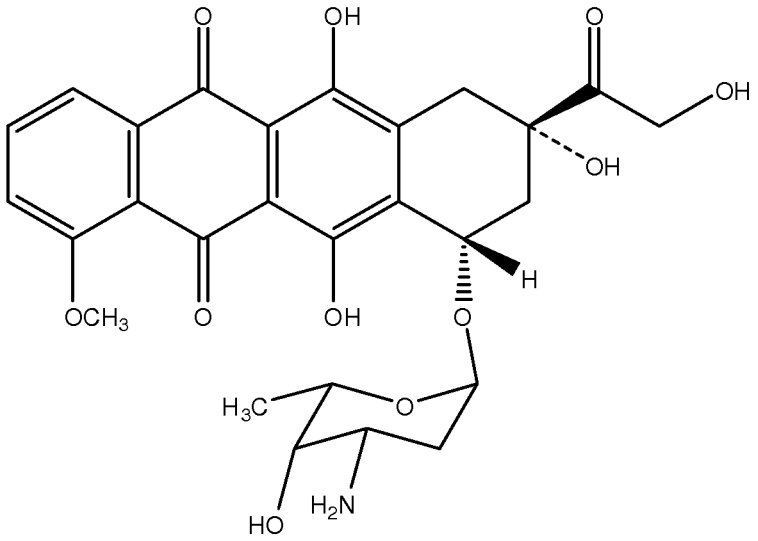
[00149] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with gemcitabine, which has the structure:



[00150] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with a compound disclosed in U.S. Patent Publication No. 2004/0209878A1, wherein the compound has the following core structure:

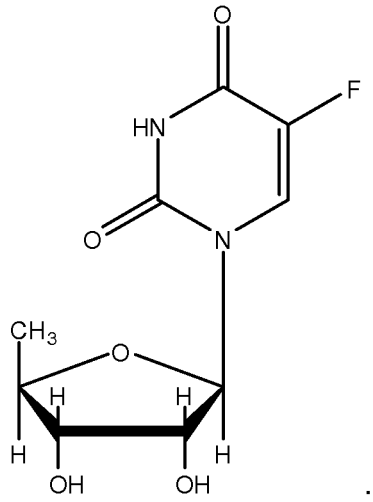


[00151] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or used in combination with doxorubicin (Adriamycin®), including Caelyx or Doxil® (doxorubicin HCl liposome injection; Ortho Biotech Products L.P., Raritan, NJ). Doxil® comprises doxorubicin in STEALTH® liposome carriers, which are composed of N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), fully hydrogenated soy phosphatidylcholine (HSPC), and cholesterol. Doxorubicin has the structure:



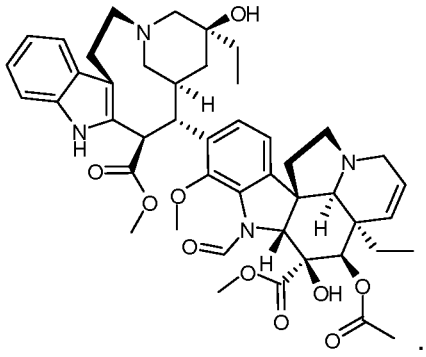
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[00152] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with 5'-deoxy-5-fluorouridine, which has the structure:

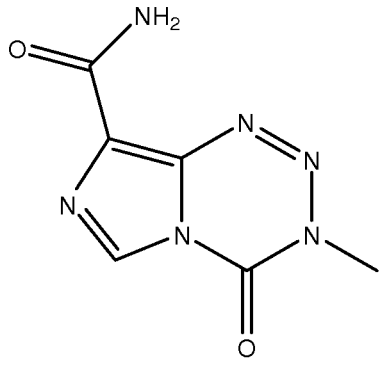


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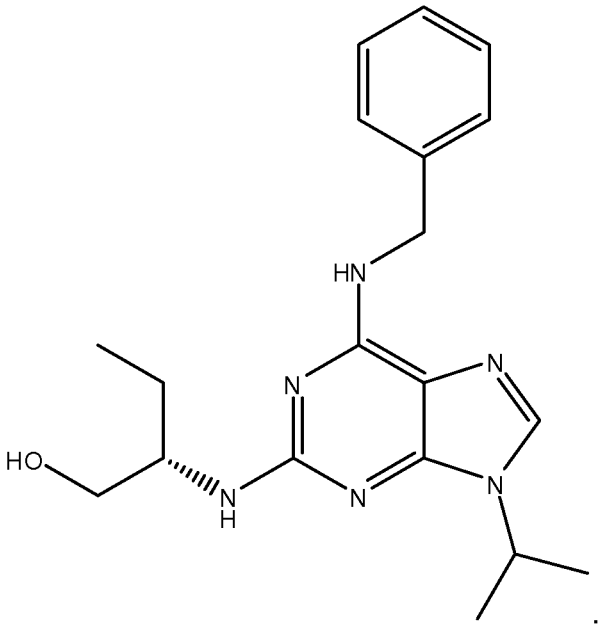
[00153] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with vincristine, which has the structure:



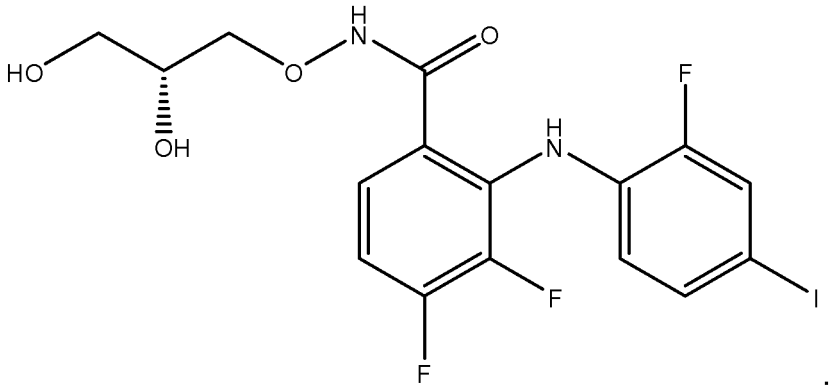
[00154] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with temozolomide (methazolastone), which has the structure:



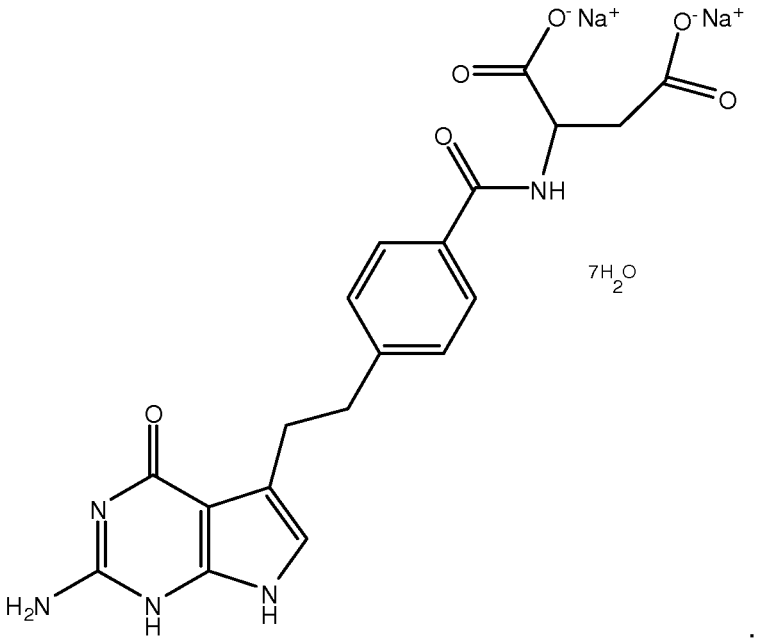
[00155] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with a CDK inhibitor, such as ZK-304709 or Seliciclib (R-roscovitine, CYC-202). Seliciclib has the structure:



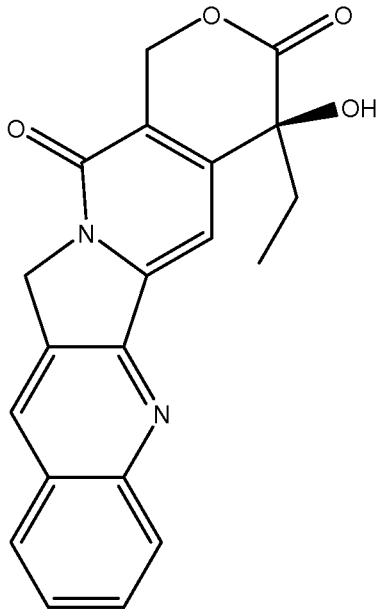
[00156] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with a MEK inhibitor, such as PD0325901 or AZD-6244 (ARRY-142886). PD0325901 has the structure:



[00157] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with capecitabine (5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl]-cytidine) or Pemetrexed (L-Glutamic acid, N -[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate), which has the structure:

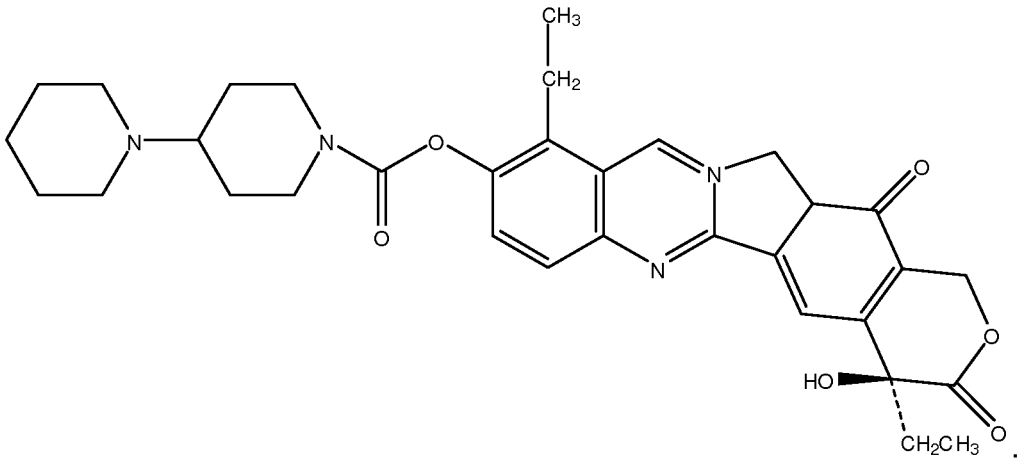


[00158] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with camptothecin (Beisler 1971; Stork 1971). Camptothecin has the structure:

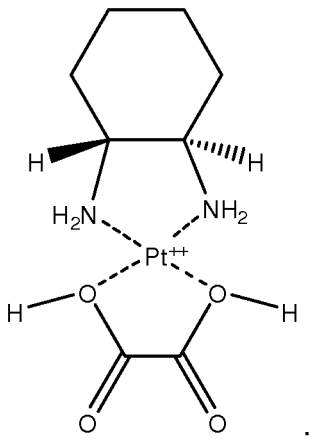


[00159] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with irinotecan (sold as Camptosar®; Pharmacia &

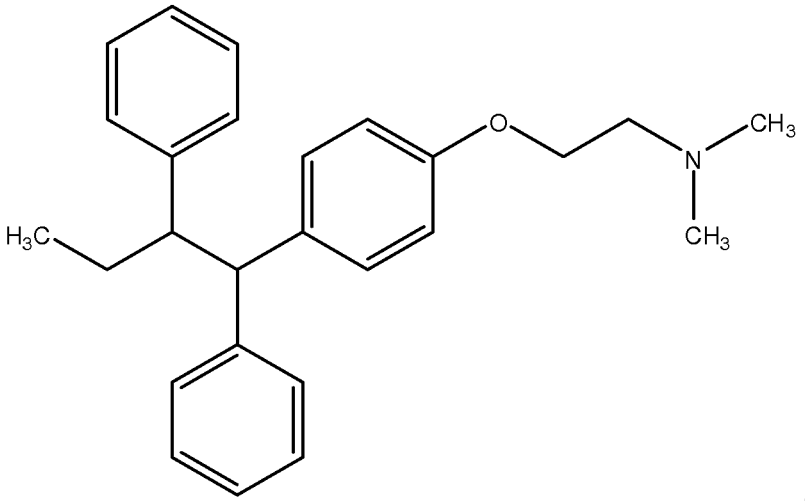
Upjohn Co.; Kalamazoo, MI); a combination of irinotecan, 5-fluorouracil, and leucovorin; or PEG-labeled irinotecan. Irinotecan has the structure:



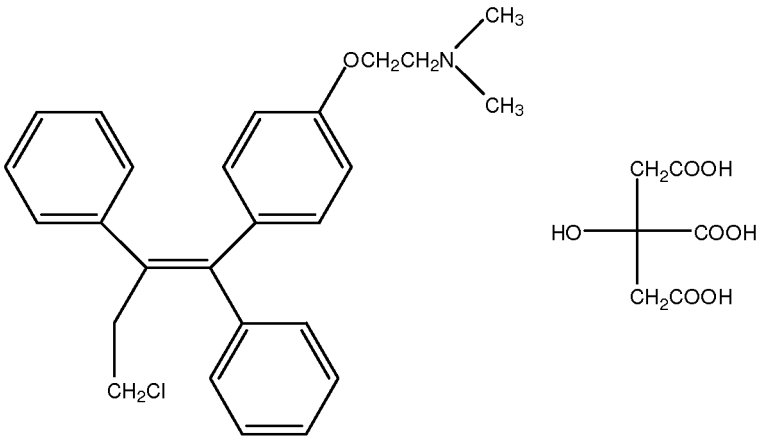
[00160] In certain embodiments, an antibody or antigen-binding fragment provided herein is associated with the FOLFOX regimen, which consists of oxaliplatin together with infusional fluorouracil and folinic acid (Chaouche 2000; de Gramont 2000). Oxaliplatin has the structure:



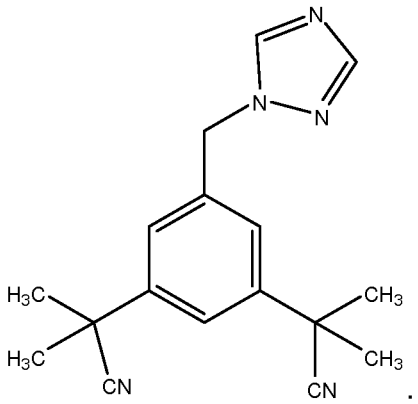
[00161] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with an antiestrogen such as tamoxifen sold as Nolvadex® by AstraZeneca Pharmaceuticals LP; Wilmington , DE) or toremifene citrate (sold as Fareston® by Shire US, Inc.; Florence, KY). Tamoxifen has the structure:



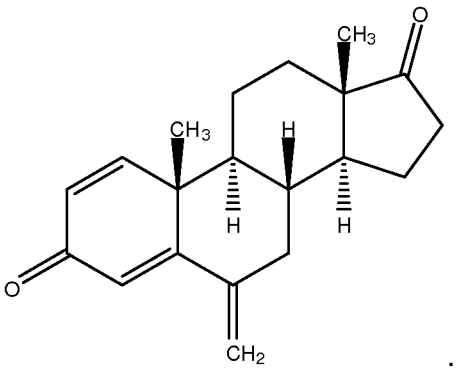
[00162] Toremifene citrate has the structure:



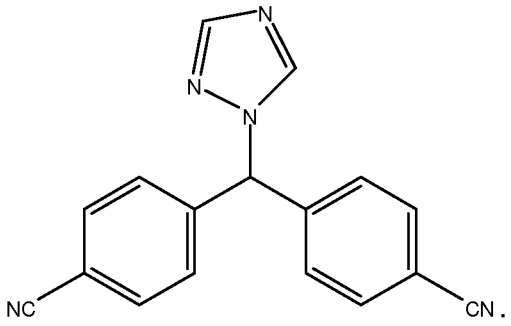
[00163] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with an aromatase inhibitor such as anastrazole (sold as Arimidex® by AstraZeneca Pharmaceuticals LP; Wilmington, DE), exemestane (sold as Aromasin® by Pharmacia Corporation; Kalamazoo, MI), or letrozole (sold as Femara® by Novartis Pharmaceuticals Corporation; East Hanover, NJ). Anastrazole has the structure:



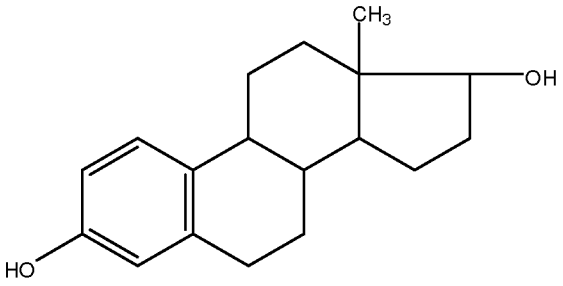
[00164] Exemestane has the structure:



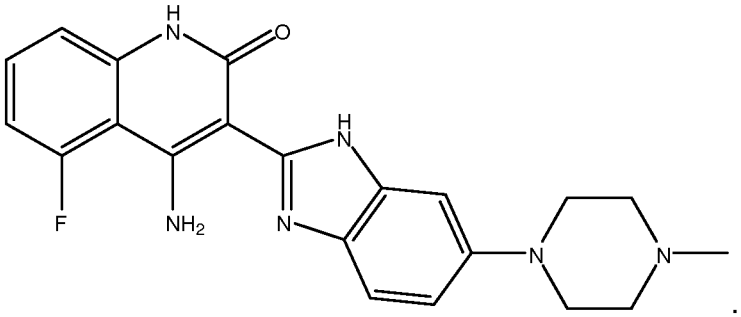
[00165] Letrozole has the structure:



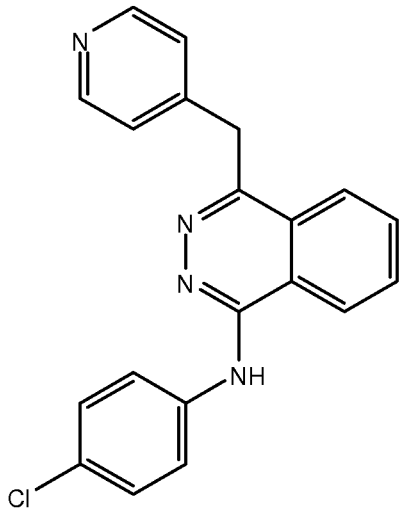
[00166] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with an estrogen such as diethylstilbestrol (DES), estradiol (sold as Estrol® by Warner Chilcott, Inc.; Rockaway, NJ), or conjugated estrogens (sold as Premarin® by Wyeth Pharmaceuticals Inc., Philadelphia, PA). DES has the structure:



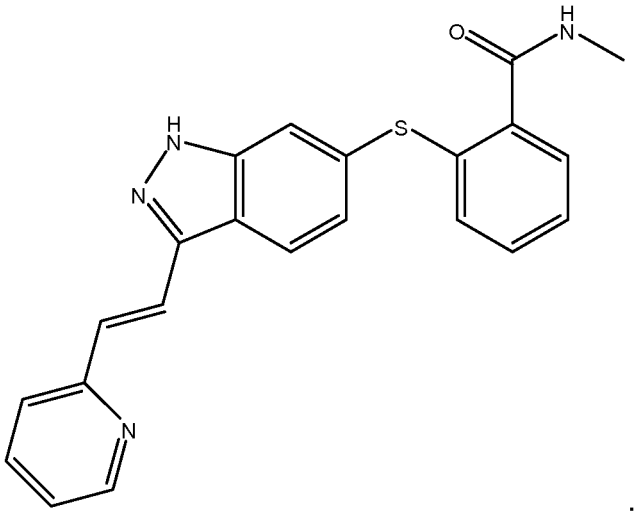
[00167] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with an anti-angiogenic agent such as Bevacizumab, VEGFR-2 antibody IMC-1C11, other VEGF-R inhibitors such as CHIR-258, Vatalanib (PTK/ZK; CGP-79787; ZK-222584), AG-013736, 3-[5-(methylsulfonylpiperadinemethyl)-indolyl]-quinolone, or the VEGF trap (AVE-0005), a soluble decoy receptor comprising portions of VEGF receptors 1 and 2. CHIR-258 has the structure:



[00168] Vatalanib has the structure:

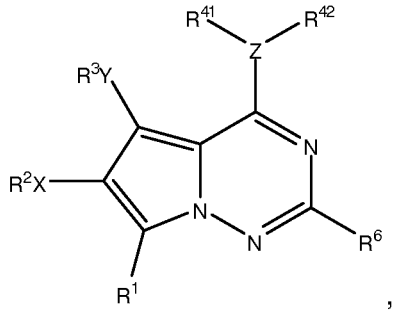


[00169] AG-013736 has the structure:



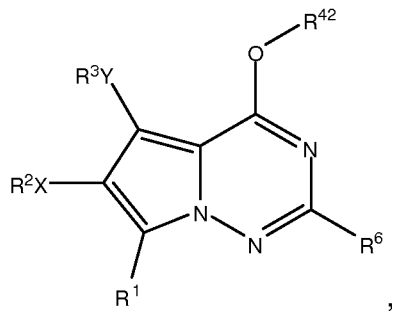
.

[00170] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with an anti-angiogenic agent having the core structure set forth in WO2004/13145:



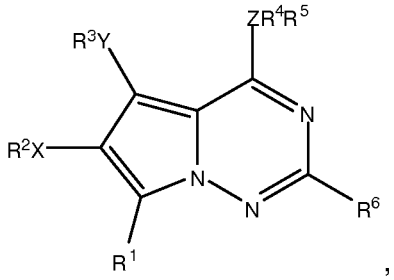
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WO2004/09542:



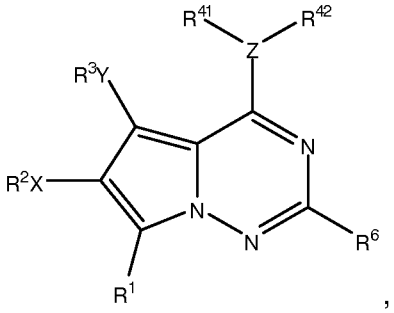
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WO00/71129:



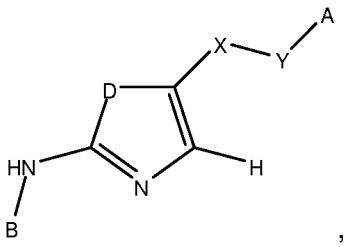
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WO2004/09601:



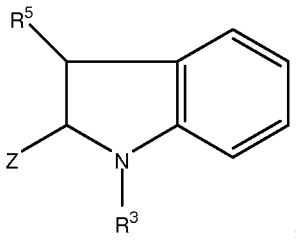
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WO2004/0105:



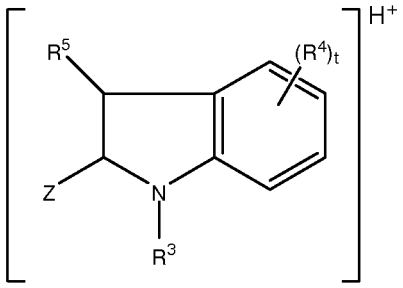
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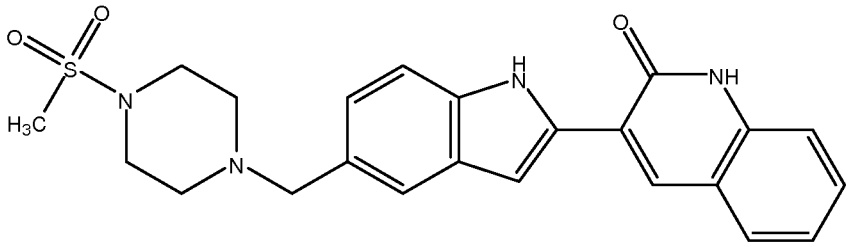
WO02/32861:



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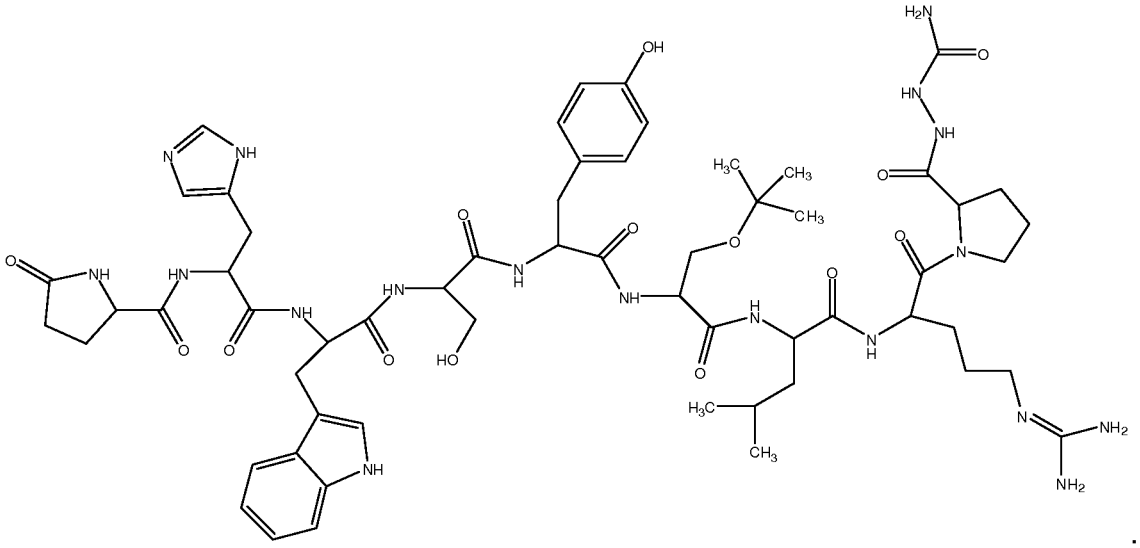
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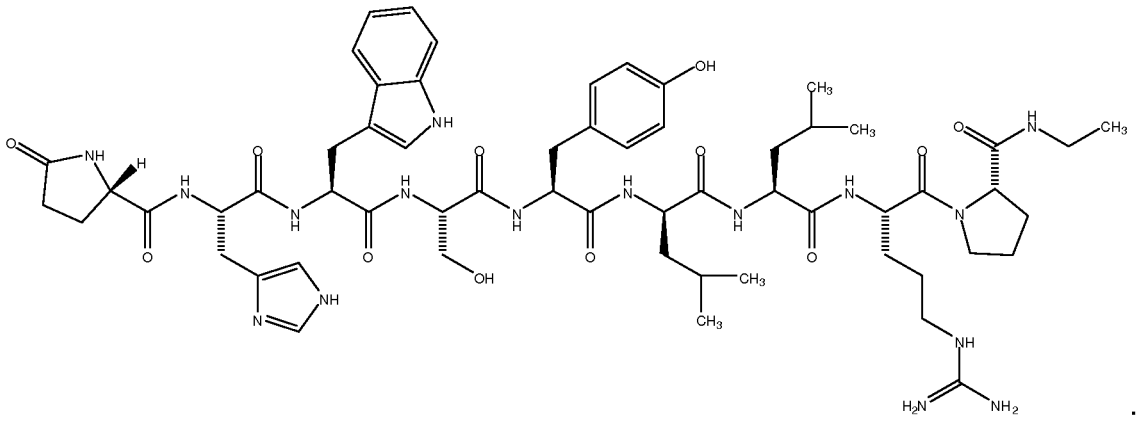
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[00171] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with a luteinizing hormone-releasing hormone (LHRH) or gonadotrophin releasing hormone (GnRH) agonist such as an goserelin acetate (sold as Zoladex® by AstraZeneca UK Limited; Macclesfield, England), leuprolide acetate (sold as Eligard® by Sanofi-Synthelabo Inc.; New York, NY), or triptorelin pamoate (sold as Trelstar® by Pharmacia Company, Kalamazoo, MI).

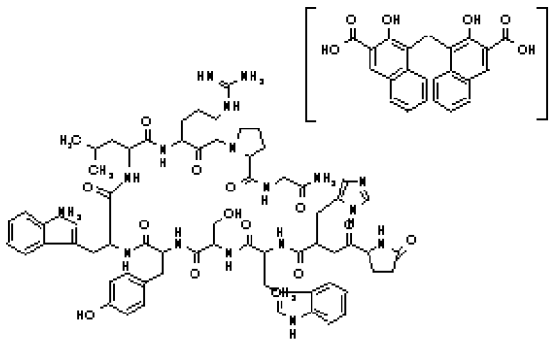
[00172] Goserelin acetate is an acetate salt of [D-Ser(Bu^t)^6,Azgly^{10}]LHRH with the chemical name pyro-Glu-His-Trp-Ser-Tyr-D-Ser(Bu^t)-Leu-Arg-Pro-Azgly-NH_2 acetate [$C_{59}H_{84}N_{18}O_{14}$ $C_2H_4O_2)_x$, where x = 1 to 2.4]). The structure of goserelin acetate is:



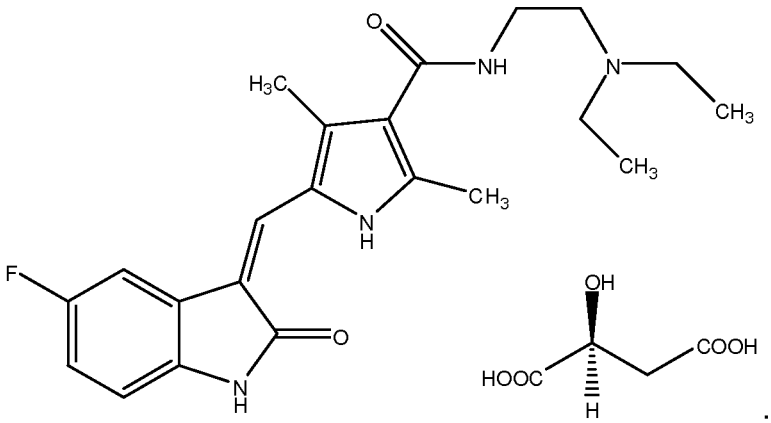
[00173] Leuprolide acetate is a synthetic nonapeptide of LHRH with the chemical name 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate (salt). The structure of leuprolide acetate is:



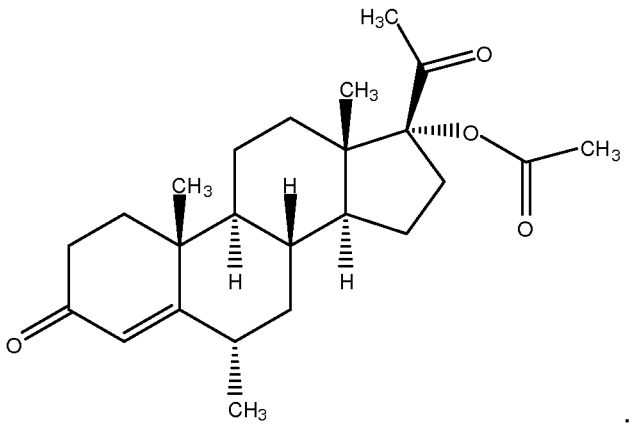
[00174] Triptorelin pamoate has the structure:



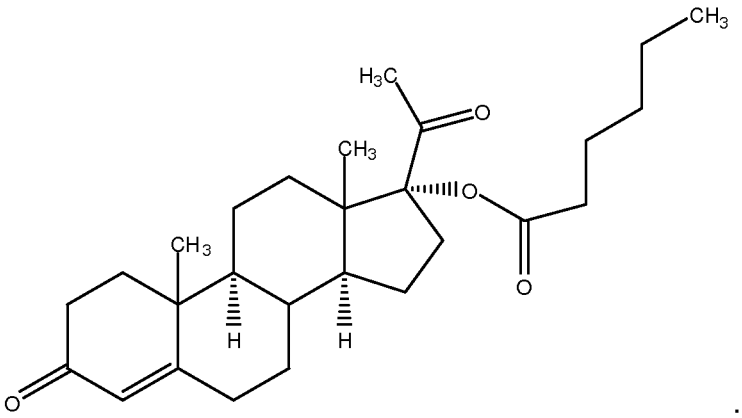
[00175] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with sunitinib or sunitinib malate. Sunitinib has the structure:



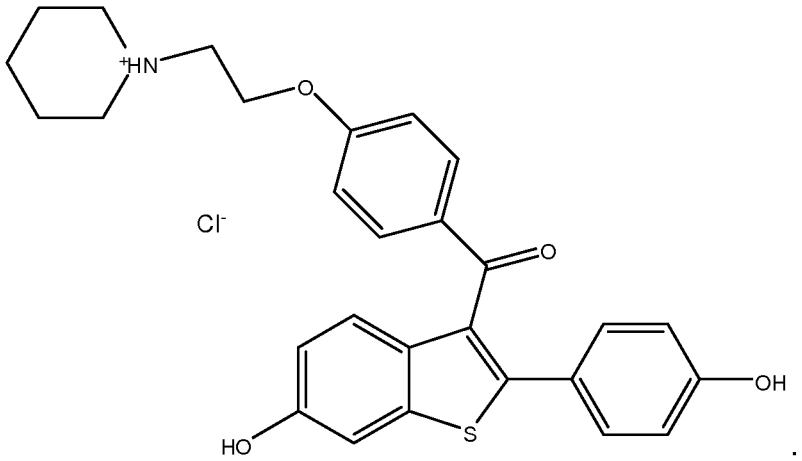
[00176] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with a progestational agent such as medroxyprogesterone acetate (sold as Provera® by Pharmacia & Upjohn Co.; Kalamazoo, MI), hydroxyprogesterone caproate (17-((1-Oxohexyl)oxy)pregn-4-ene-3,20-dione)), megestrol acetate, or progestins. Medroxyprogesterone acetate has the structure:



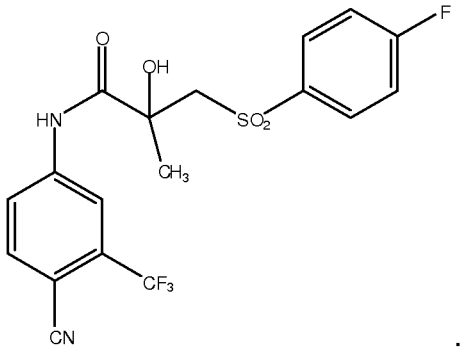
[00177] Hydroxyprogesterone caproate has the structure:



[00178] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with a selective estrogen receptor modulator (SERM) such as raloxifene (sold as Evista® by Eli Lilly and Company; Indianapolis, IN), which has the structure:

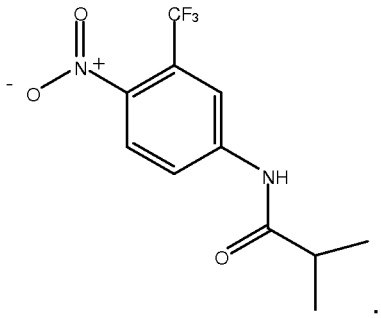


[00179] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with an anti-androgen such as bicalutamide (sold at CASODEX ® by AstraZeneca Pharmaceuticals LP; Wilmington, DE), flutamide (2-methyl-N-[4-nitro-3 (trifluoromethyl) phenyl] propanamide; sold as Eulexin® by Schering Corporation; Kenilworth, NJ), nilutamide (sold as Nilandron® by Aventis Pharmaceuticals Inc.; Kansas City, MO), or megestrol acetate (sold as Megace® by Bristol-Myers Squibb). Bicalutamide has the structure:



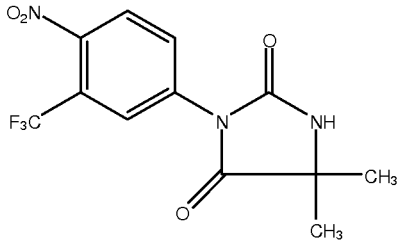
.

[00180] Flutamide has the structure:



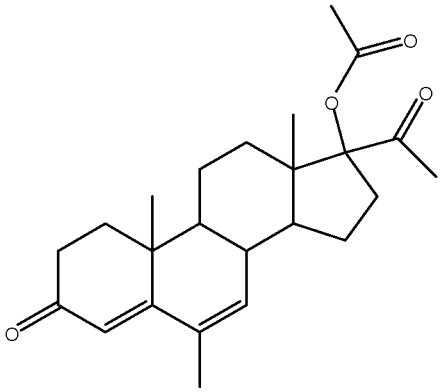
.

[00181] Nilutamide has the structure:



.

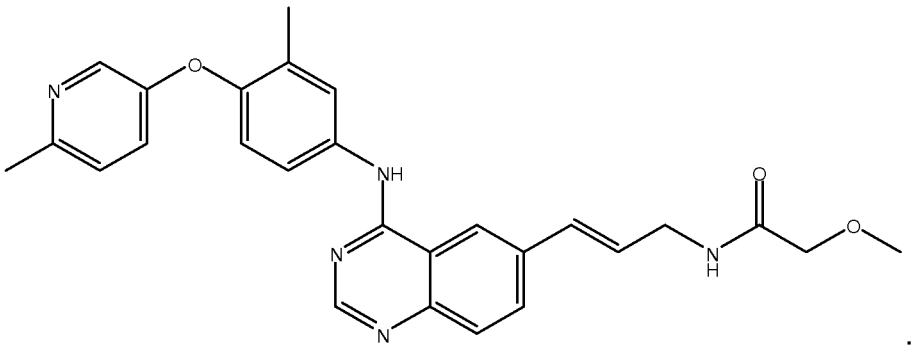
[00182] Megestrol acetate has the structure:



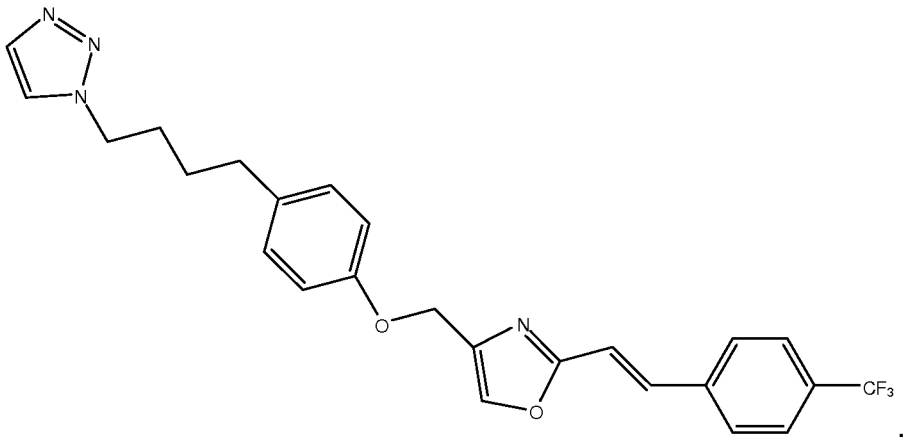
.

[00183] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with one or more inhibitors that antagonize the action of the EGF Receptor or HER2, such as CP-724714, TAK-165 (mubritinib), HKI-272, OSI-774 (erlotinib; Hidalgo 2001), lapatinib (GW2016; Rusnak 2001; N-{3-Chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furyl]-4-quinazolinamine; PCT Application No. WO99/35146), canertinib (CI-1033; Erlichman 2001; Smaill 2000), EKB-569 (Wissner 2003), PKI-166 (CGP-75166), ABX-EGF antibody (Abgenix, Inc., Freemont, CA; Yang 1999; Yang 2001), erbitux (IMC-C225, cetuximab; U.S. Patent No. 6,217,866; Imclone, New York, NY), GW-572016, or any anti-EGFR or anti-HER2 antibody.

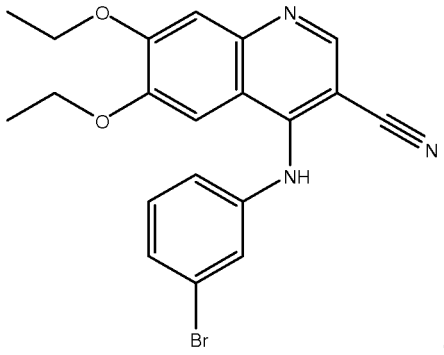
[00184] CP-724714 has the structure:



[00185] TAK-165 (mubritinib) has the structure:

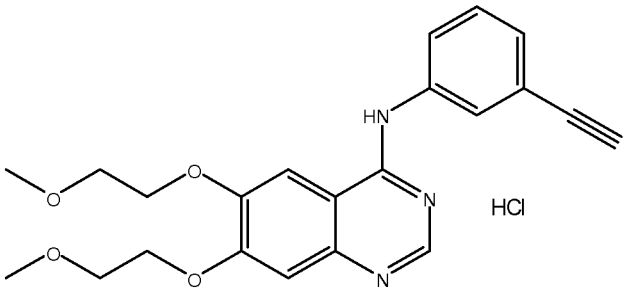


[00186] HKI-272 has the structure:

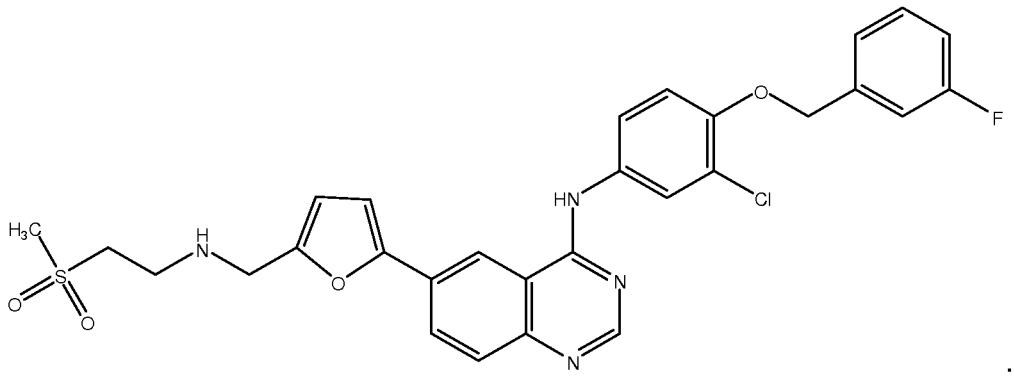


.

[00187] OSI-774 (erlotinib) has the structure:

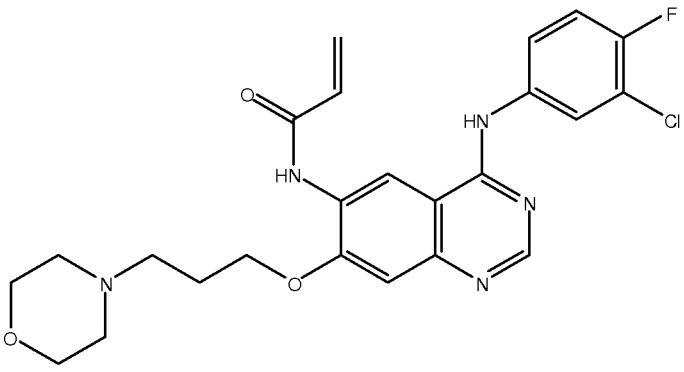


[00188] Lapatinib has the structure:



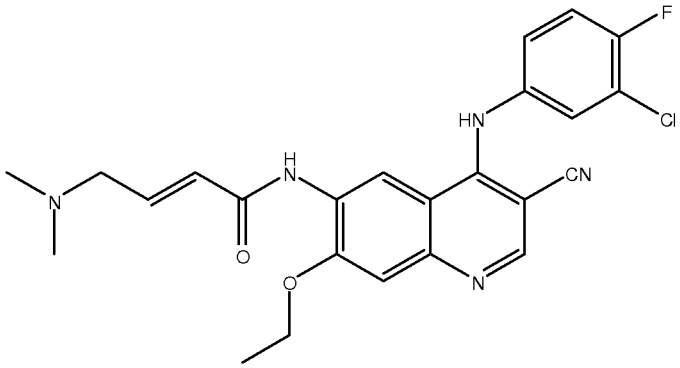
.

[00189] Canertinib has the structure:

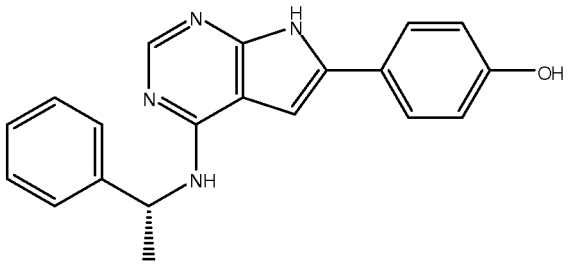


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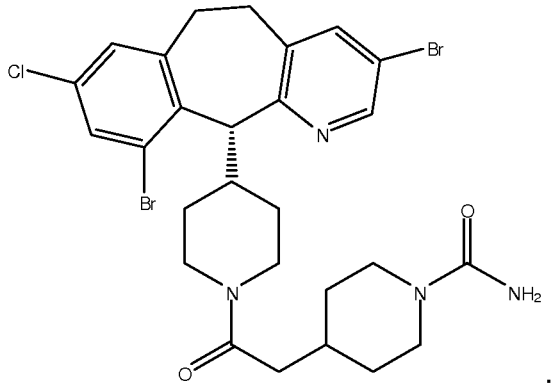
[00190] EKB-569 has the structure:



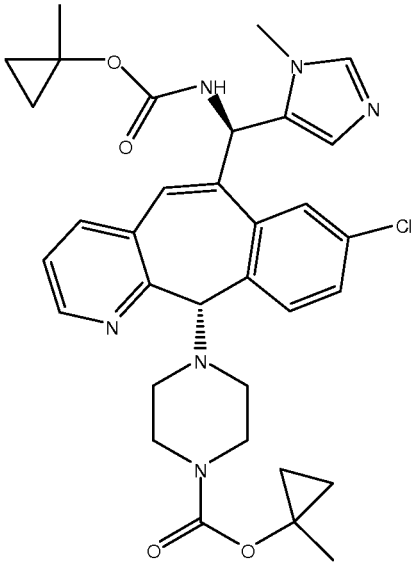
[00191] PKI-166 has the structure:



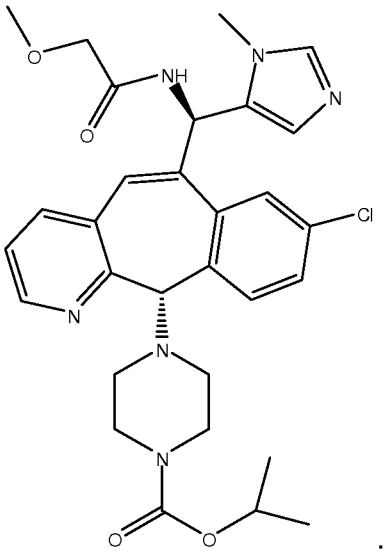
[00192] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with lonafarnib (sold as Sarasar® by Schering-Plough, Kenilworth, NJ), which has the structure:



[00193] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with an FPT inhibitor having the structure:

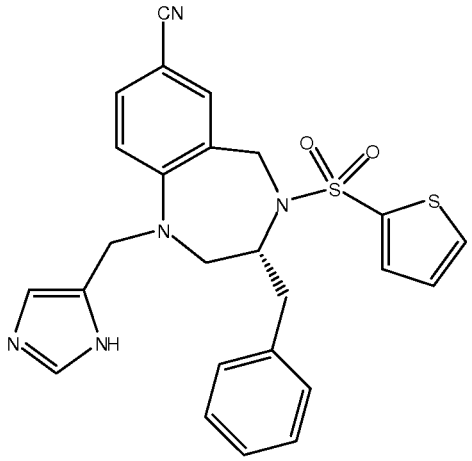


or

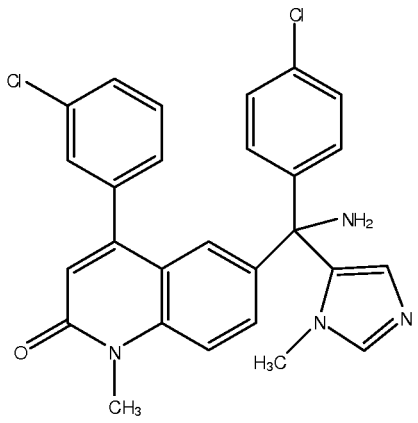


.

[00194] In other embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with an FPT inhibitor such as BMS-214662 (Hunt 2000; Dancey 2002; (R)-7-cyano-2,3,4,5-tetrahydro-1-(1H-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1H-1,4-benzodiazepine) or R155777 (tipifarnib; Garner 2002; Dancey 2002; (B)-6-[amino(4-chlorophenyl)(1-methyl-1H-imidazol-5-yl)-methyl]-4-(3-chlorophenyl)-1-methyl-2(1H)-quinolinone]; sold as Zarnestra® by Johnson & Johnson, New Brunswick, NJ). BMS-214662 has the structure:



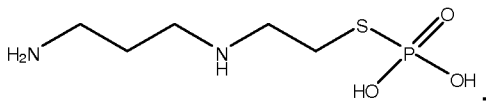
[00195] R155777 has the structure:



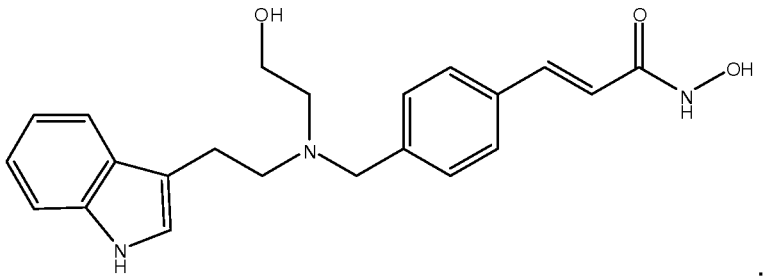
[00196] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with amifostine, NVP-LAQ824 (Atadja 2004), suberoyl analide hydroxamic acid, valproic acid (Michaelis 2004), trichostatin A, FK-228 (Furumai 2002), SU11248 (Mendel 2003), BAY43-9006 (sorafenib), KRN951, aminoglutethimide, amsacrine, anagrelide, anastrozole (sold as Arimidex® by AstraZeneca Pharmaceuticals LP, Wilmington, DE), asparaginase, bacillus Calmette-Guerin (BCG) vaccine (Garrido 1997), bleomycin, buserelin, busulfan (1,4-butanediol dimethanesulfonate; sold as Busulfex® by ESP Pharma, Inc., Edison, New Jersey), satraplatin, carboplatin (sold as Paraplatin® by Bristol-Myers Squibb, Princeton, NJ), carmustine, chlorambucil, cisplatin, cladribine, clodronate, cyclophosphamide, cyproterone, cytarabine, dacarbazine,

dactinomycin, daunorubicin, diethylstilbestrol, epirubicin, fludarabine, fludrocortisone, fluoxymesterone, flutamide, hydroxyurea, idarubicin, ifosfamide, imatinib (sold as Gleevec® by Novartis Pharmaceuticals Corporation, East Hanover, NJ), leucovorin, leuprolide, levamisole, lomustine, mechlorethamine, melphalan (sold as Alkeran® by Celgene Corporation, Warren, NJ), mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, octreotide (Katz 1989; sold as Sandostatin LAR® Depot by Novartis Pharm Corp., E. Hanover, NJ), edotreotide (yttrium-90 labeled or unlabeled), oxaliplatin (sold as Eloxatin® by Sanofi-Synthelabo Inc., New York, NY), pamidronate (sold as Aredia® by Novartis Pharmaceuticals Corp., East Hanover, NJ), Pentostatin (sold as Nipent® by Supergen, Dublin, CA), plicamycin, porfimer (sold as Photofrin® by Axcan Scandipharm Inc., Birmingham, AL), procarbazine, raltitrexed, rituximab (sold as Rituxan® by Genentech, Inc.; South San Francisco, CA), streptozocin, teniposide, testosterone, thalidomide, thalidomide combined with dexamethasone, thioguanine, thiotepa, tretinoin, vindesine, all trans-retinoic acid, or 13-cis-retinoic acid.

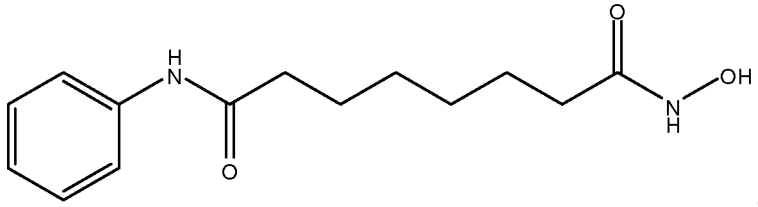
Amifostine has the structure:



[00197] NVP-LAQ824 has the structure:

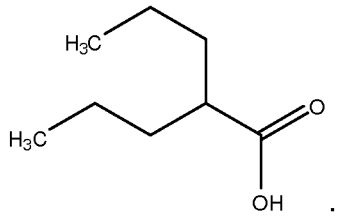


[00198] Suberoyl analide hydroxamic acid has the structure:



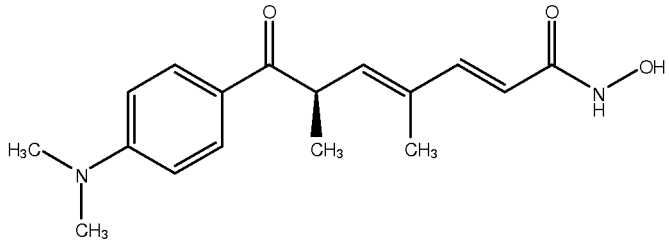
.

[00199] Valproic acid has the structure:



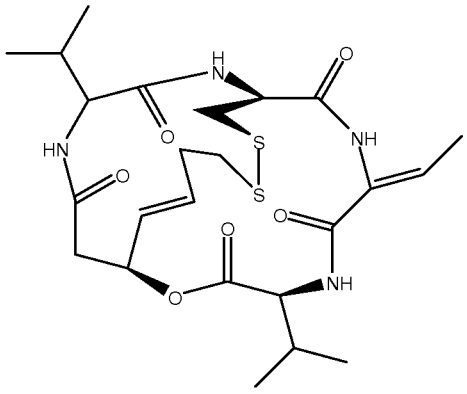
.

[00200] Trichostatin A has the structure:



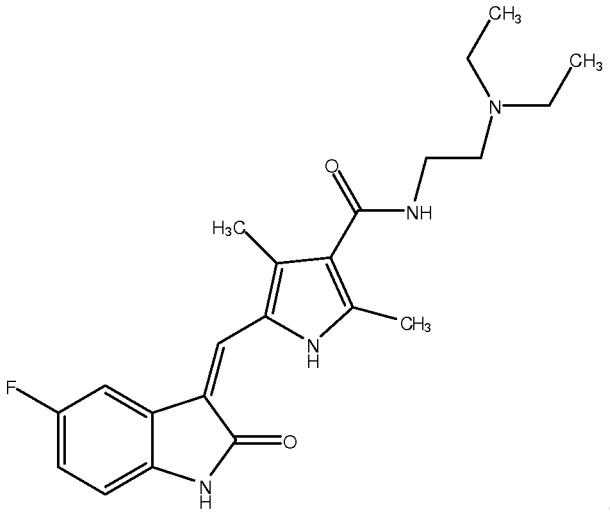
.

[00201] FK-228 has the structure:



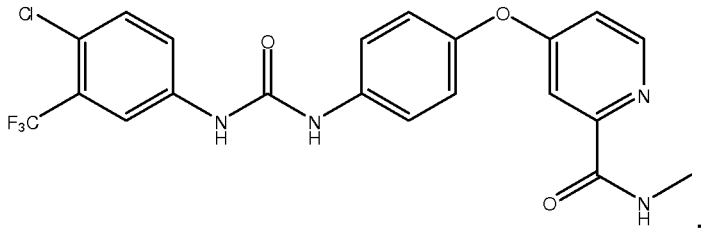
.

[00202] SU11248 has the structure:



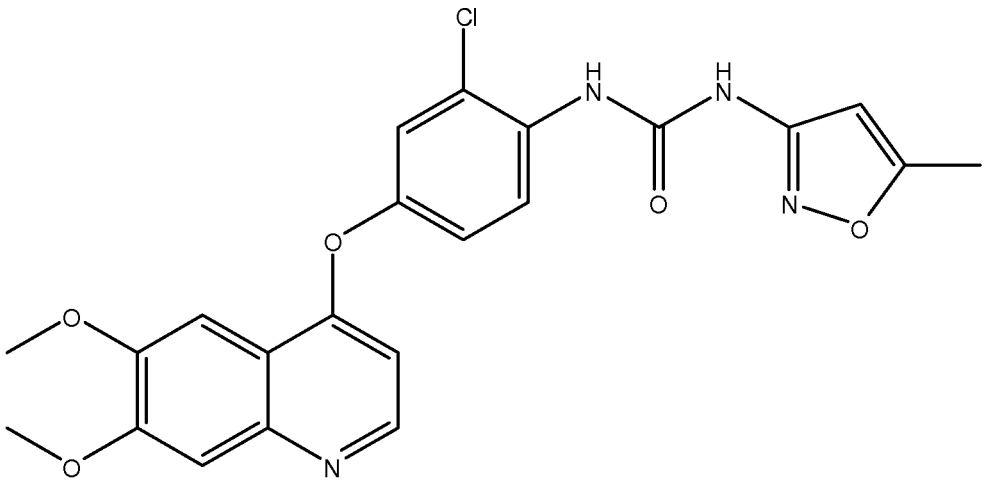
.

[00203] BAY43-9006 has the structure:



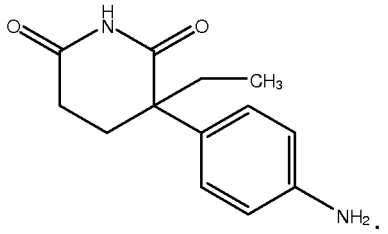
.

[00204] KRN951 has the structure:

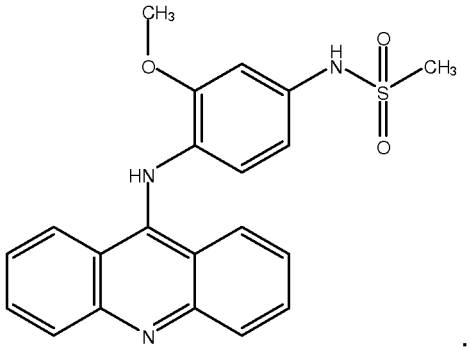


.

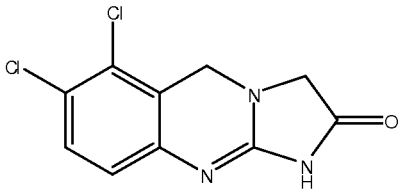
[00205] Aminoglutethimide has the structure:



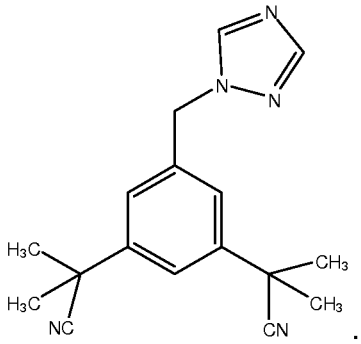
[00206] Amsacrine has the structure:



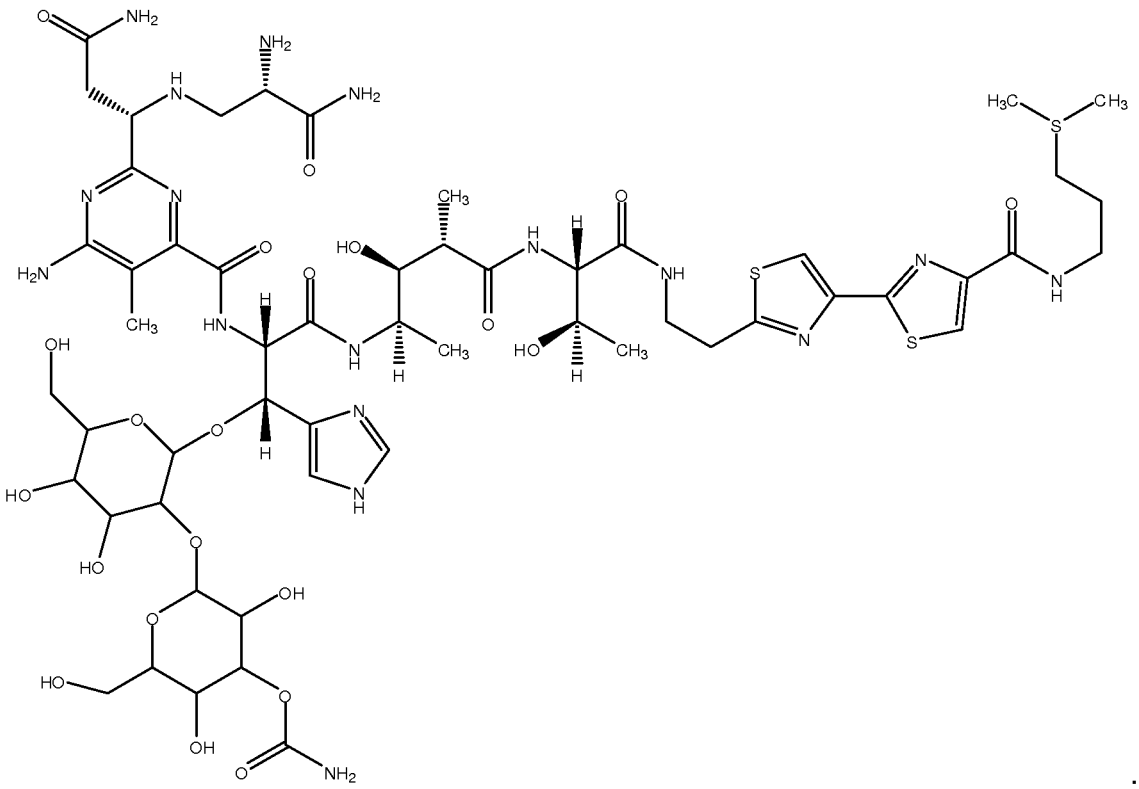
[00207] Anagrelide has the structure:



[00208] Anastrozole has the structure:

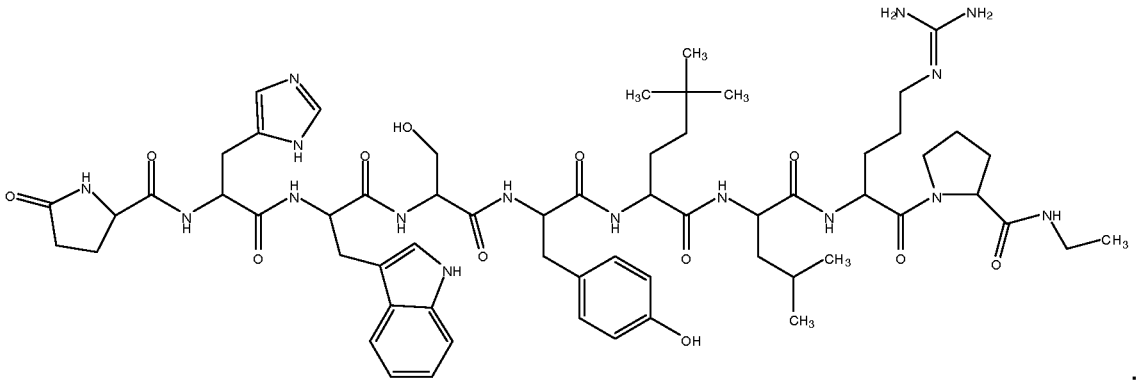


[00209] Bleomycin has the structure:



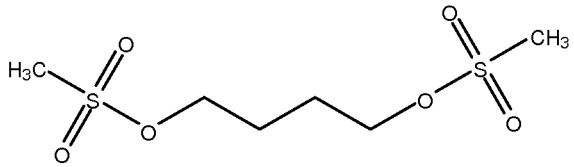
.

[00210] Buserelin has the structure:



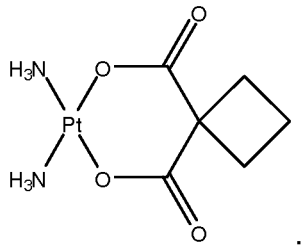
.

[00211] Busulfan has the structure:



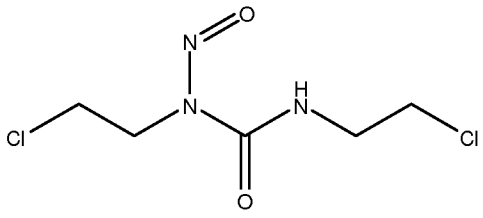
.

[00212] Carboplatin has the structure:



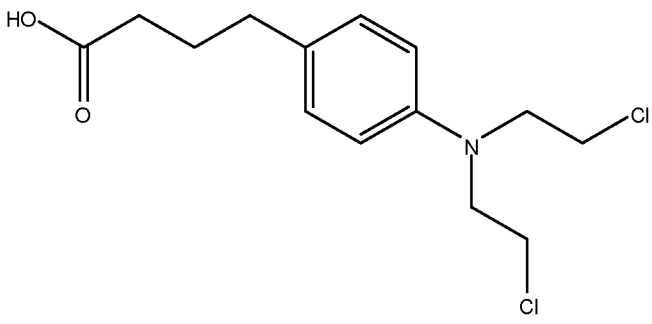
.

[00213] Carmustine has the structure:



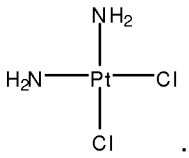
.

[00214] Chlorambucil has the structure:



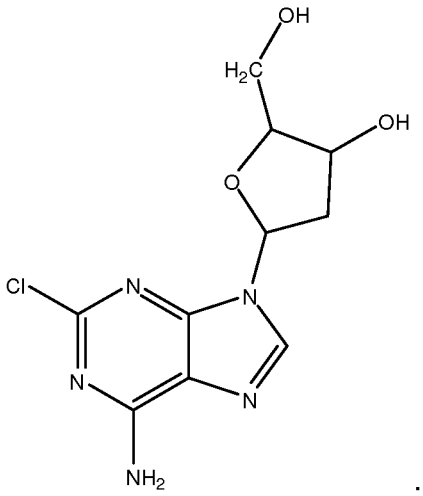
.

[00215] Cisplatin has the structure:



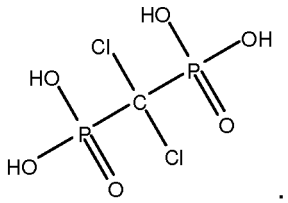
.

[00216] Cladribine has the structure:



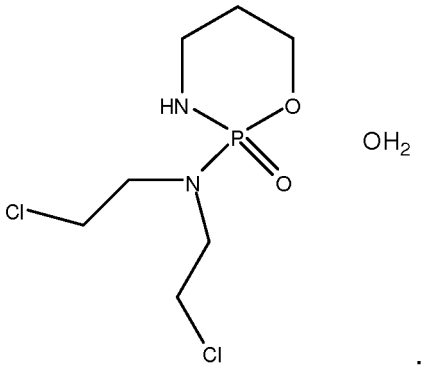
.

[00217] Clodronate has the structure:



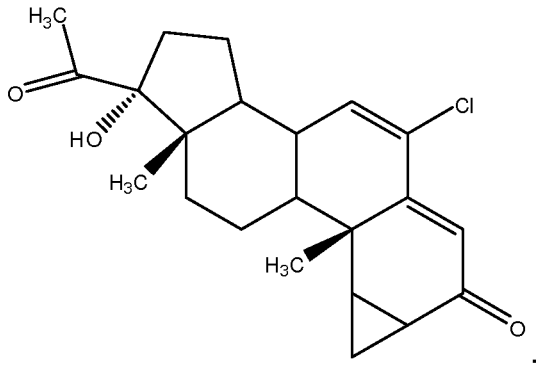
.

[00218] Cyclophosphamide has the structure:



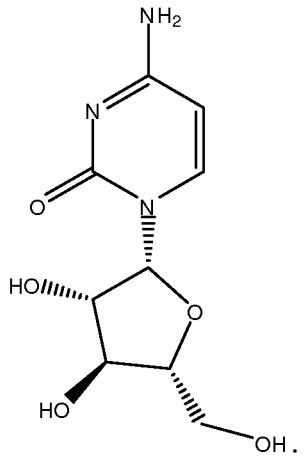
.

[00219] Cyproterone has the structure:



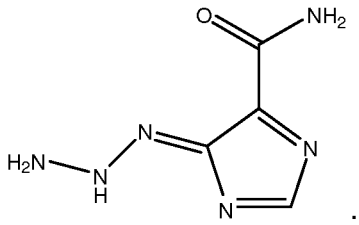
.

[00220] Cytarabine has the structure:



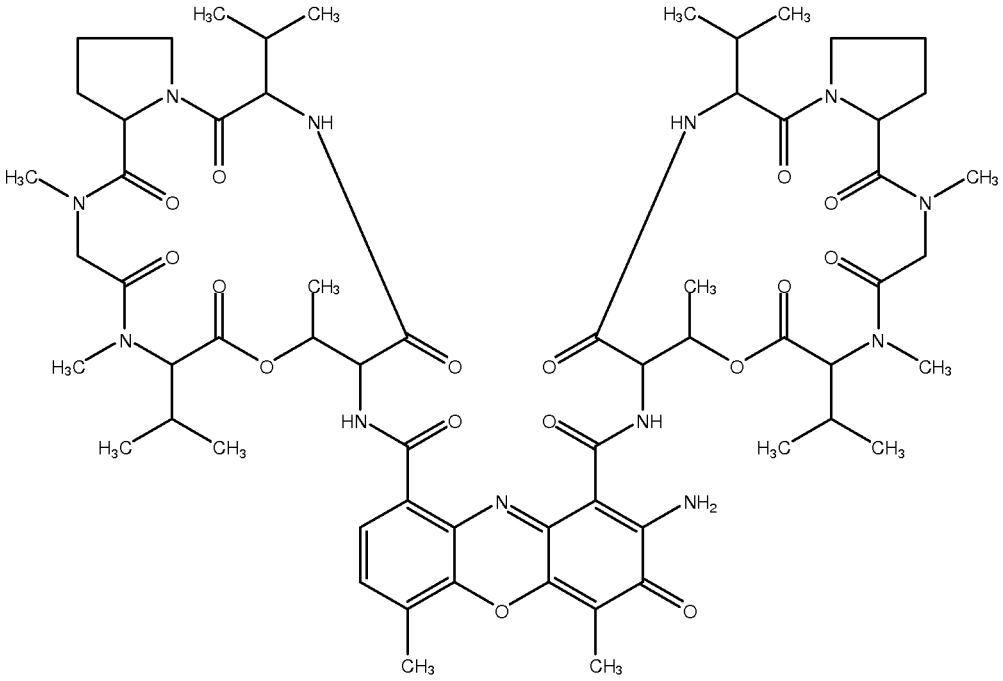
.

[00221] Dacarbazine has the structure:

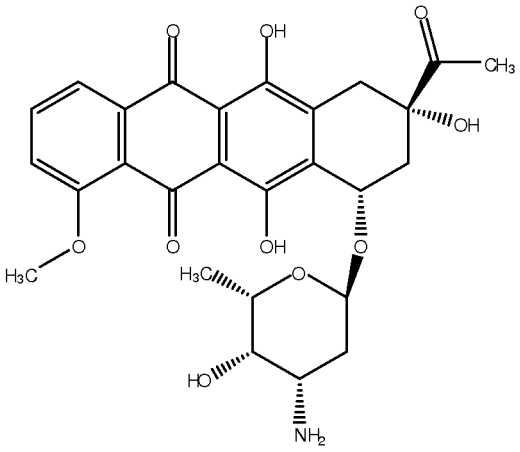


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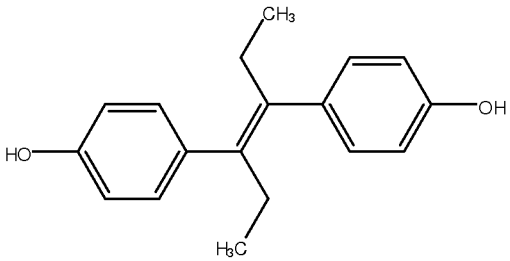
[00222] Dactinomycin has the structure:



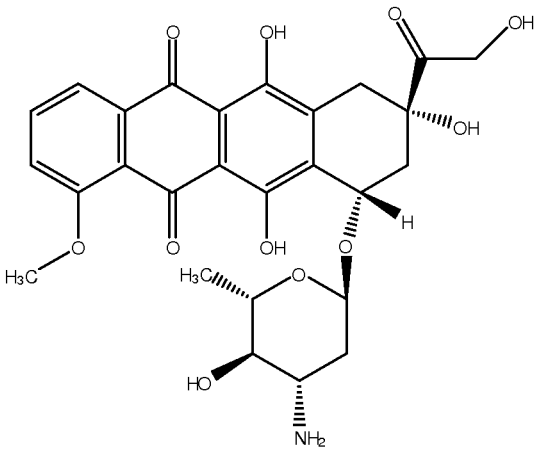
[00223] Daunorubicin has the structure:



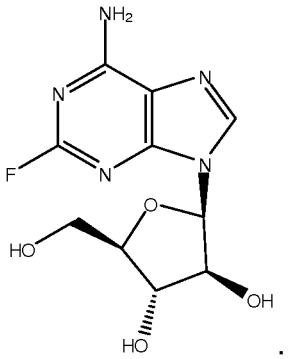
[00224] Diethylstilbestrol has the structure:



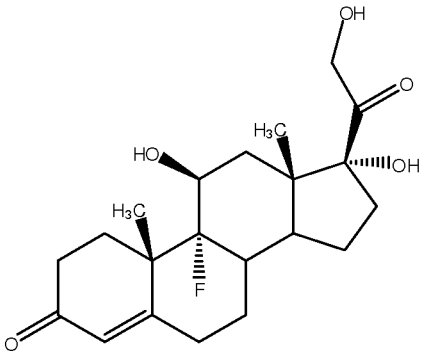
[00225] Epirubicin has the structure:



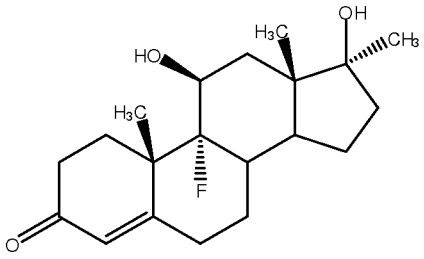
[00226] Fludarabine has the structure:



[00227] Fludrocortisone has the structure:

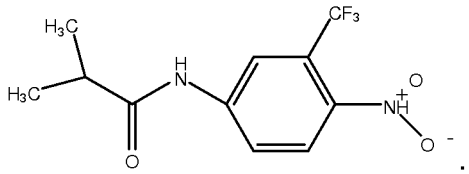


[00228] Fluoxymesterone has the structure:



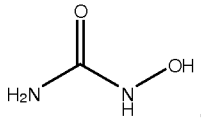
.

[00229] Flutamide has the structure:



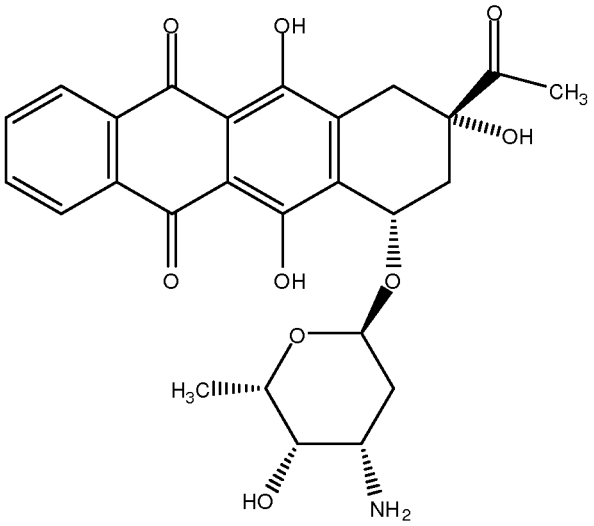
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[00230] Hydroxyurea has the structure:



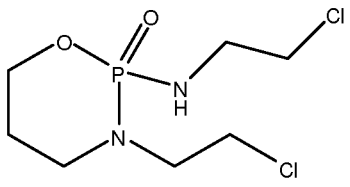
.

[00231] Idarubicin has the structure:



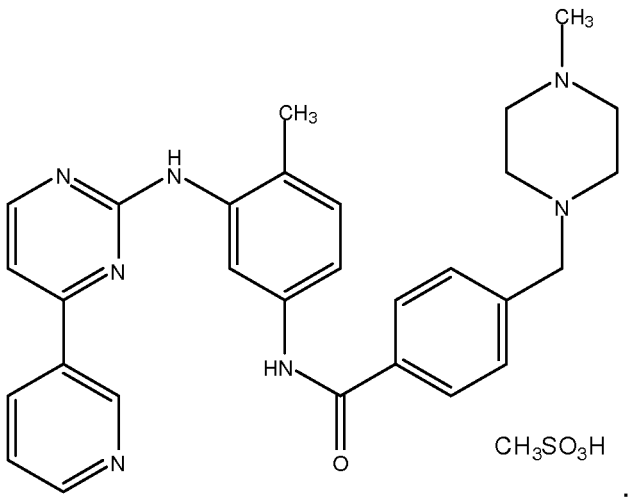
.

[00232] Ifosfamide has the structure:



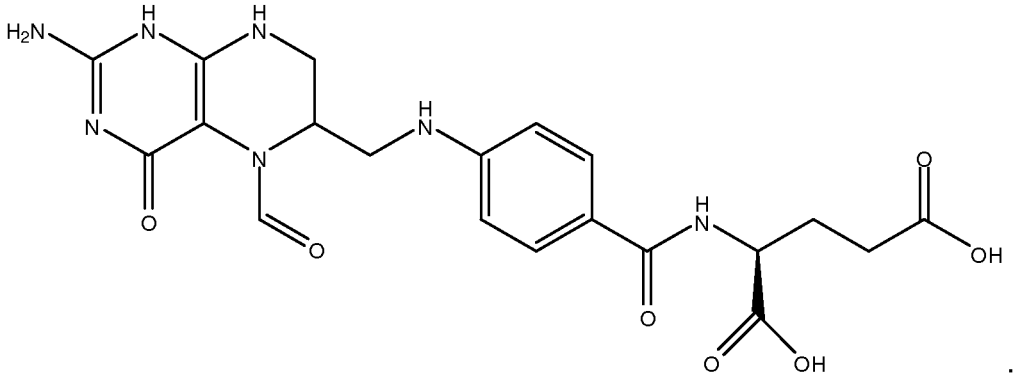
.

[00233] Imatinib has the structure:



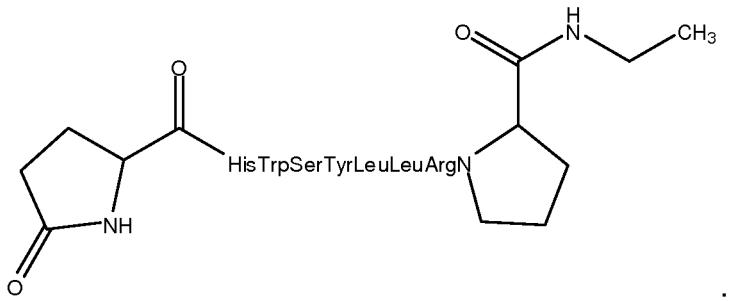
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[00234] Leucovorin has the structure:



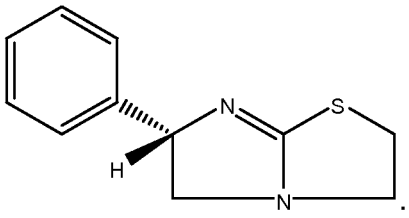
.

[00235] Leuprolide has the structure:

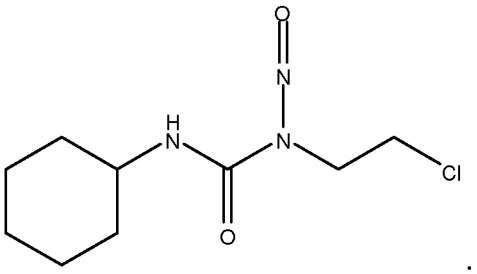


.

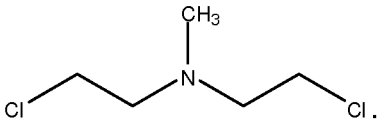
[00236] Levamisole has the structure:



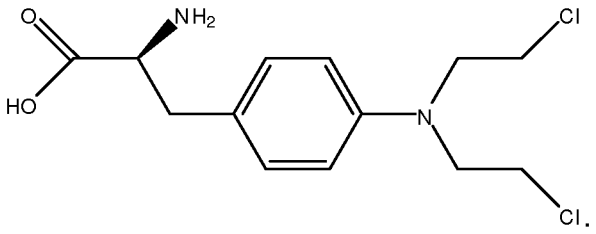
[00237] Lomustine has the structure:



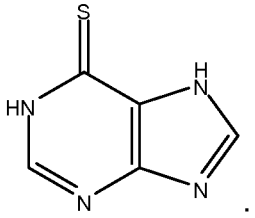
[00238] Mechlorethamine has the structure:



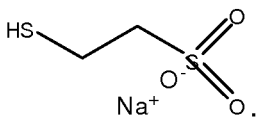
[00239] Melphalan has the structure:



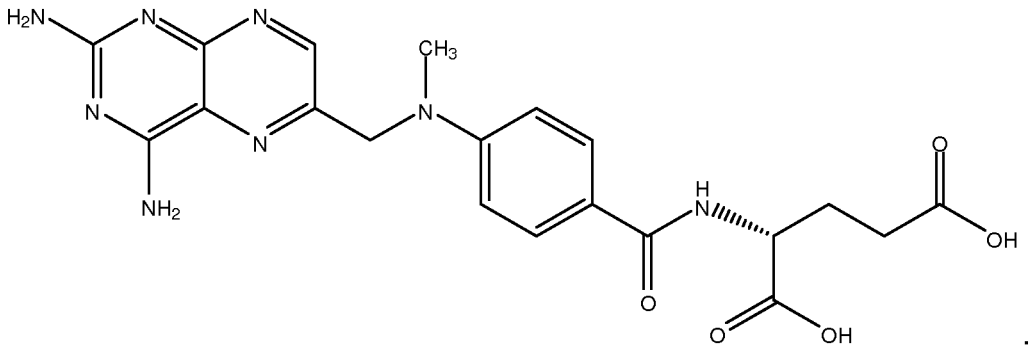
[00240] Mercaptopurine has the structure:



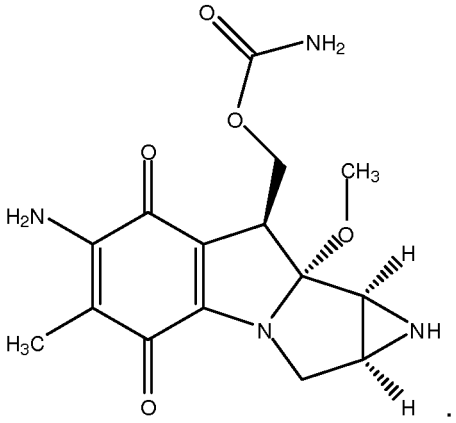
[00241] Mesna has the structure:



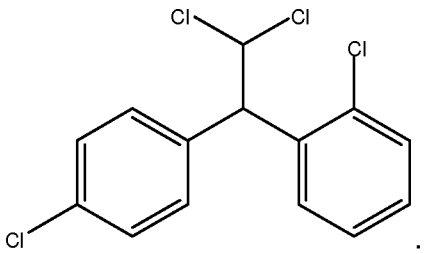
[00242] Methotrexate has the structure:



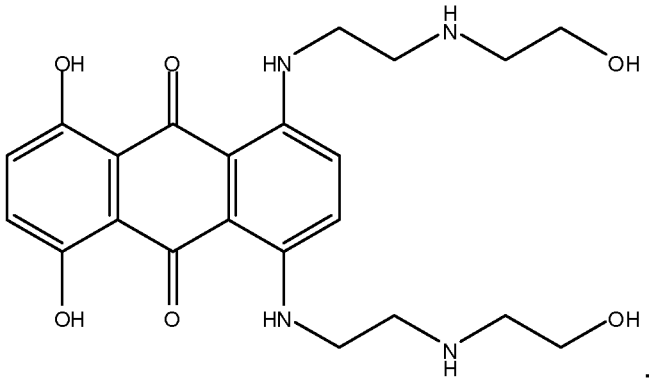
[00243] Mitomycin has the structure:



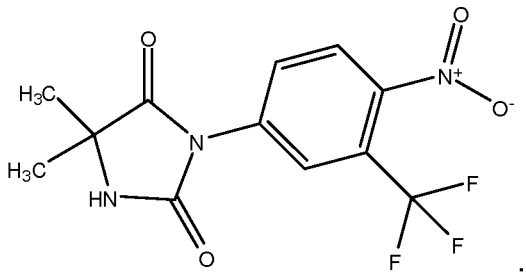
[00244] Mitotane has the structure:



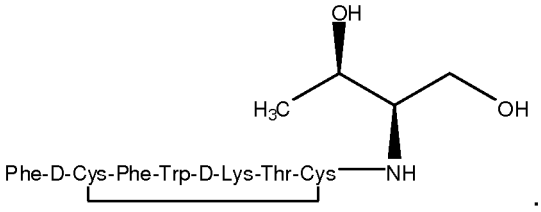
[00245] Mitoxantrone has the structure:



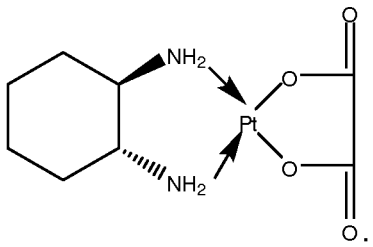
[00246] Nilutamide has the structure:



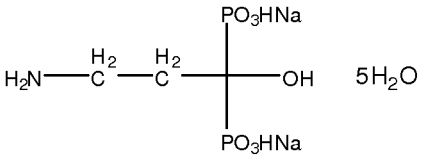
[00247] Octreotide has the structure:



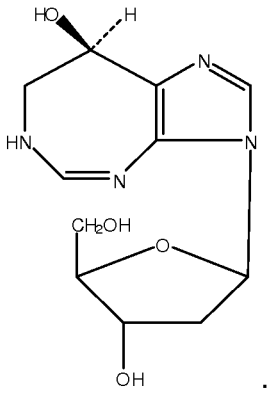
[00248] Oxaliplatin has the structure:



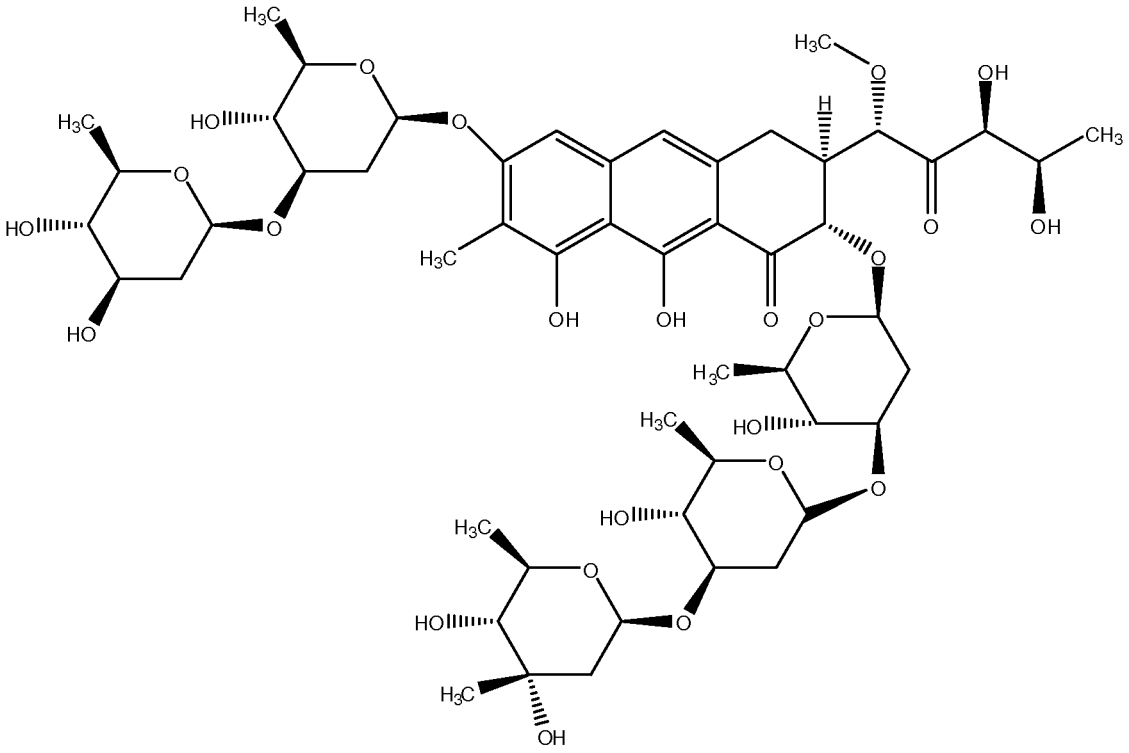
[00249] Pamidronate has the structure:



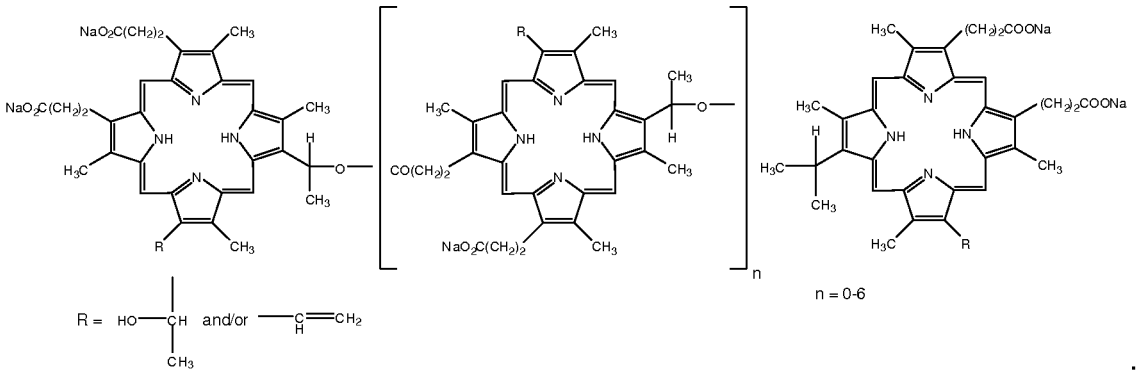
[00250] Pentostatin has the structure:



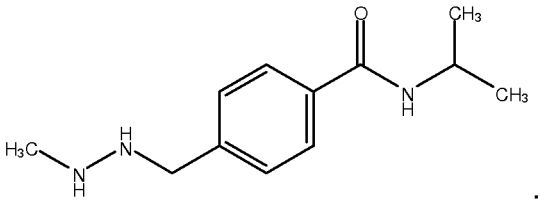
[00251] Plicamycin has the strcture:



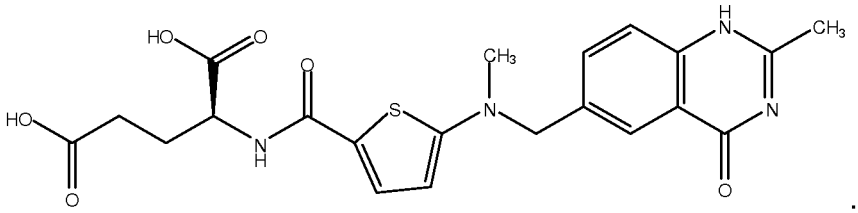
[00252] Porfimer has the structure:



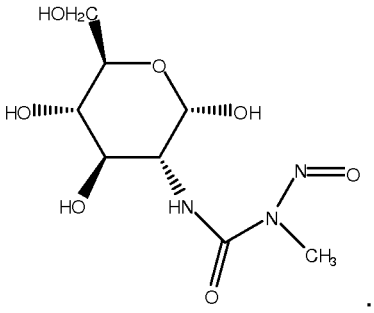
[00253] Procarbazine has the structure:



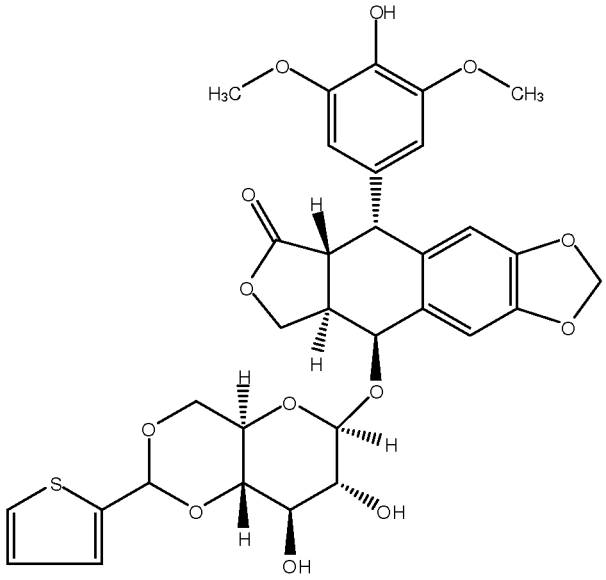
[00254] Raltitrexed has the structure:



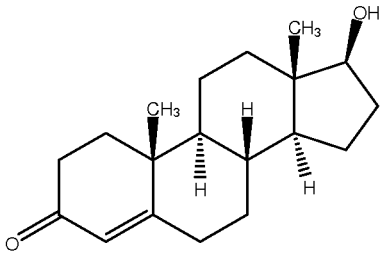
[00255] Streptozocin has the structure:



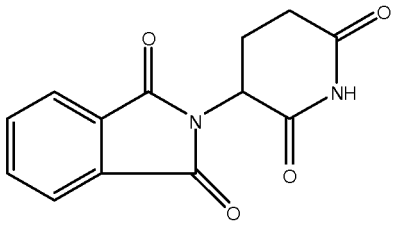
[00256] Teniposide has the structure:



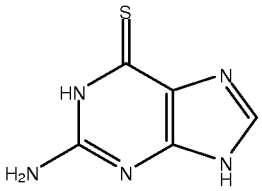
[00257] Testosterone has the structure:



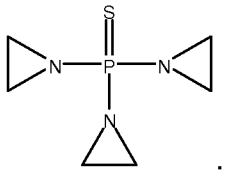
[00258] Thalidomide has the structure:



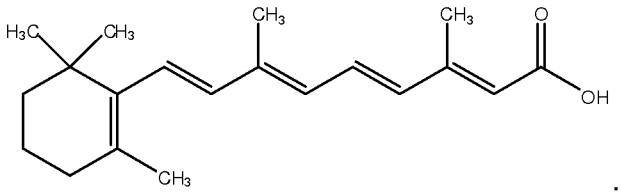
[00259] Thioguanine has the structure:



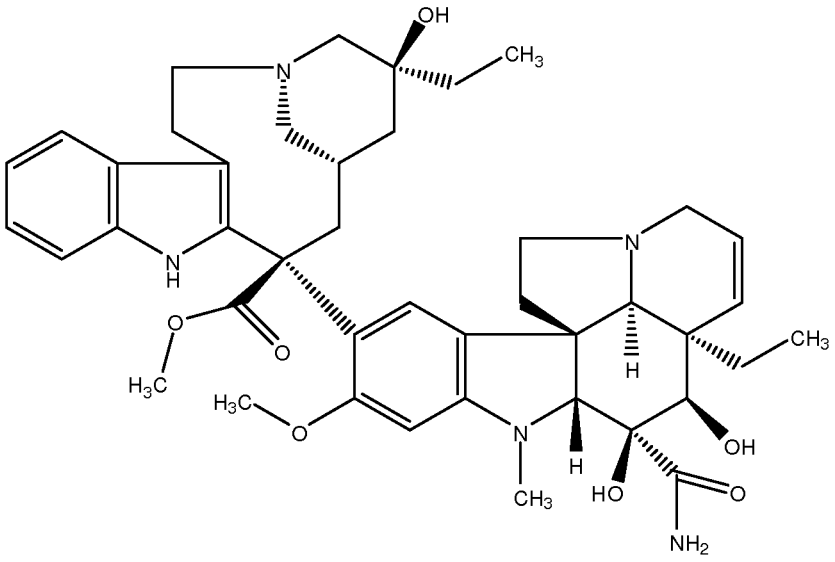
[00260] Thiotepa has the structure:



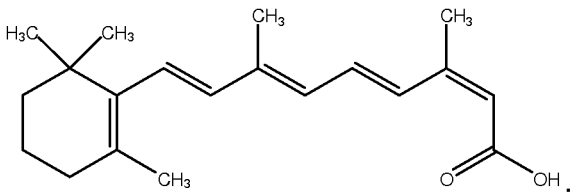
[00261] Tretinoin has the structure:



[00262] Vindesine has the structure:



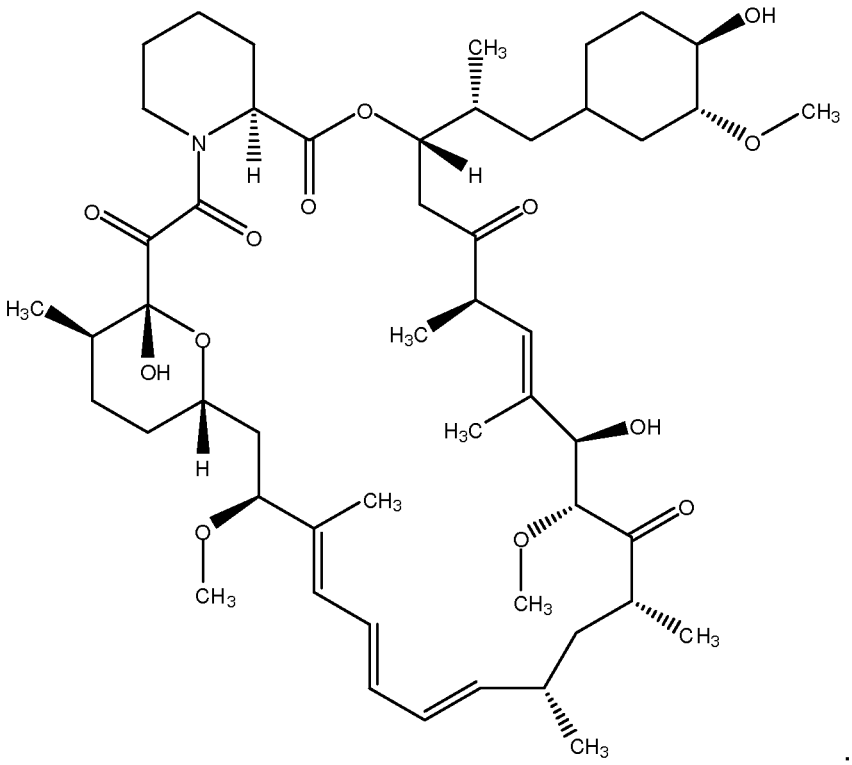
[00263] 13-cis-retinoic acid has the structure:



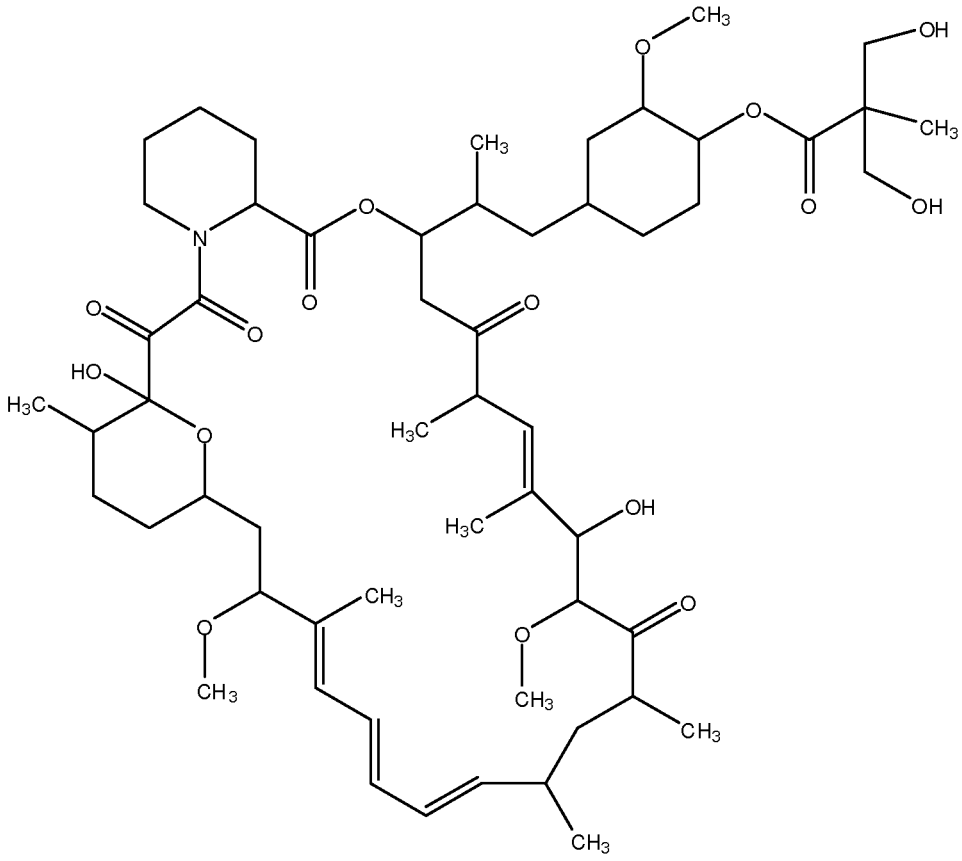
[00264] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or used in combination with abraxane. Abraxane is an injectable suspension of paclitaxel protein-bound particles comprising an albumin-bound form of paclitaxel with a mean particle size of approximately 130 nanometers. Abraxane is

supplied as a white to yellow, sterile, lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection, USP prior to intravenous infusion. Each single-use vial contains 100 mg of paclitaxel and approximately 900 mg of human albumin. Each milliliter (mL) of reconstituted suspension contains 5 mg paclitaxel. Abraxane is free of solvents and cremophor (polyoxyethylated castor oil).

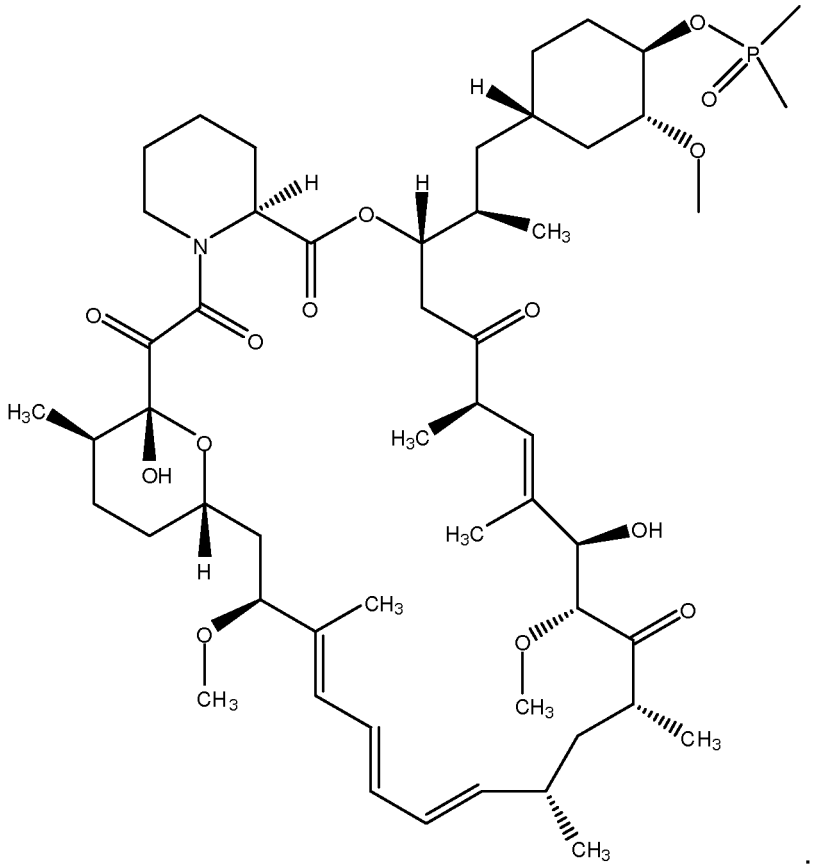
[00265] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with one or more of phenylalanine mustard, uracil mustard, estramustine, altretamine, floxuridine, 5-deooxyuridine, cytosine arabinoside, 6-mecaptopurine, deoxycoformycin, calcitriol, valrubicin, mithramycin, vinblastine, vinorelbine, topotecan, razoxin, marimastat, COL-3, neovastat, BMS-275291, squalamine, endostatin, SU5416, SU6668, EMD121974, interleukin-12, IM862, angiostatin, vitaxin, droloxifene, idoxyfene, spironolactone, finasteride, cimitidine, trastuzumab, denileukin, diftitox, gefitinib, bortezimib, paclitaxel, docetaxel, epithilone B, BMS-247550 (Lee 2001), BMS-310705, droloxifene (3-hydroxytamoxifen), 4-hydroxytamoxifen, pipendoxifene, ERA-923, arzoxifene, fulvestrant, acolbifene, lasofoxifene (CP-336156), idoxifene, TSE-424, HMR-3339, ZK186619, topotecan, PTK787/ZK 222584 (Thomas 2003), VX-745 (Haddad 2001), PD 184352 (Sebolt-Leopold 1999), LY294002, LY292223, LY292696, LY293684, LY293646 (Vlahos 1994), wortmannin, BAY-43-9006, (Wilhelm 2002), ZM336372, L-779,450, a Raf inhibitor (Lowinger 2002), flavopiridol (L86-8275/HMR 1275; Senderowicz 2000), UCN-01 (7-hydroxy staurosporine; Senderowicz 2000), any mTOR inhibitor, rapamycin (sirolimus), everolimus (40-*O*-(2-hydroxyethyl) derivative of rapamycin), CCI-779 (temsirolimus; Sehgal 1994; Elit 2002), AP-23573, RAD001, ABT-578, or BC-210. Rapamycin (sirolimus) has the structure:



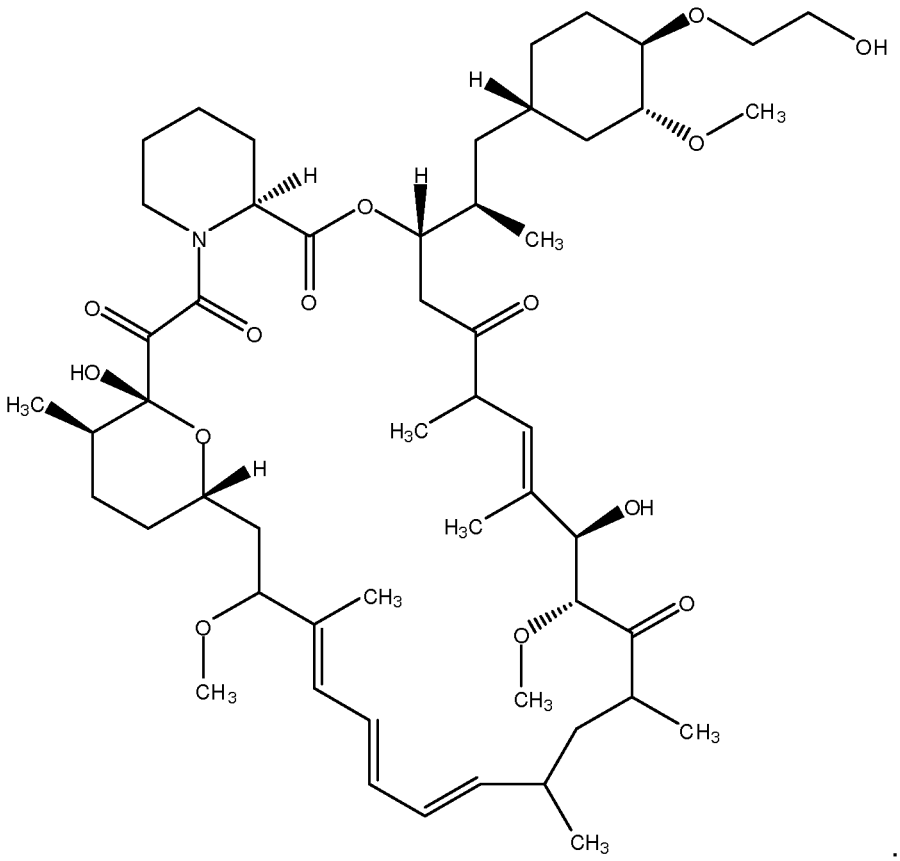
[00266] CCI-779 has the structure:



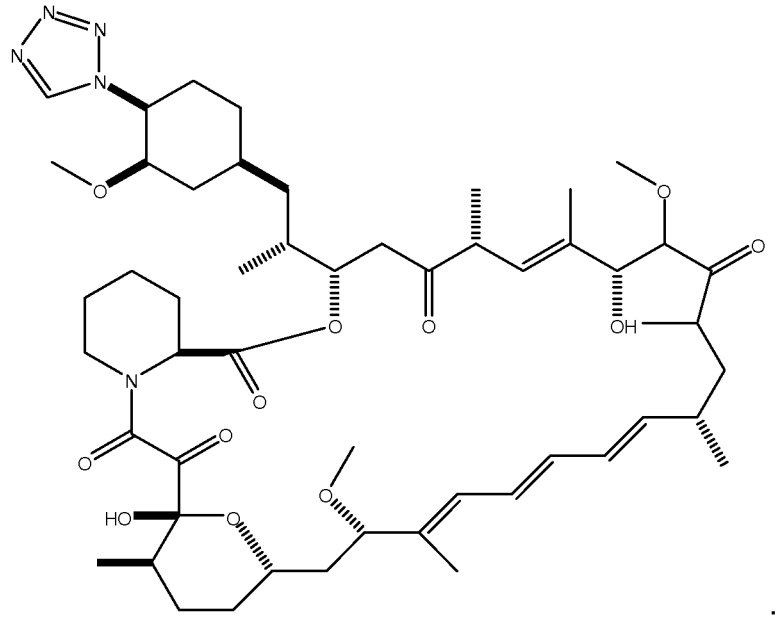
[00267] AP-23573 has the structure:



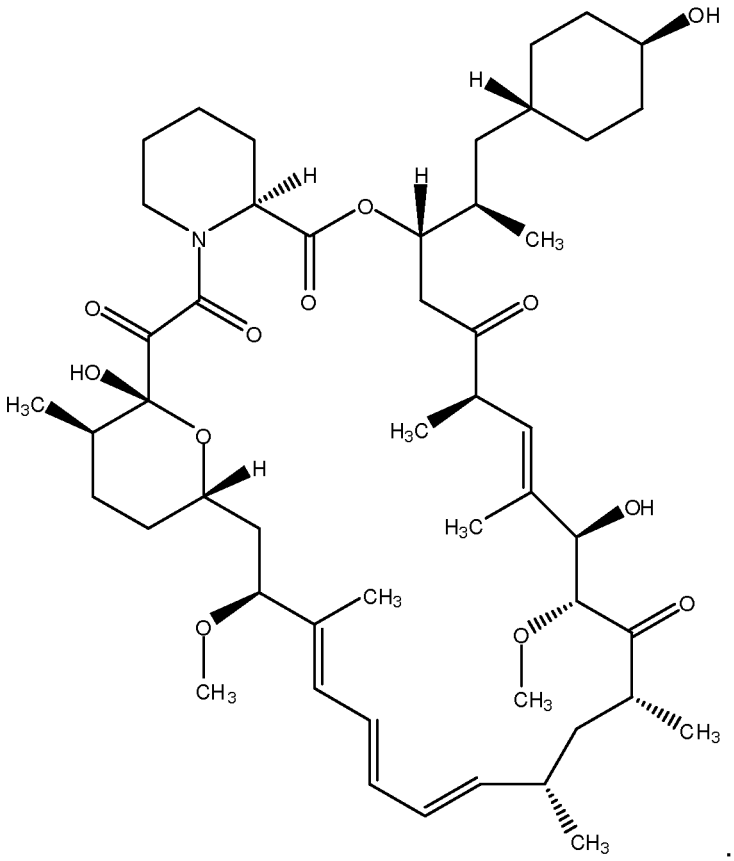
[00268] RAD001 has the structure:



[00269] ABT-578 has the structure:



[00270] BC-210 has the structure:



[00271] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with one or more of the compounds set forth in U.S. Patent No. 5,656,655, which discloses styryl substituted heteroaryl EGFR inhibitors; U.S. Patent No. 5,646,153, which discloses bis mono and/or bicyclic aryl heteroaryl carbocyclic and heterocarbocyclic EGFR and PDGFR inhibitors; U.S. Patent No. 5,679,683, which discloses tricyclic pyrimidine compounds that inhibit the EGFR; U.S. Patent No. 5,616,582, which discloses quinazoline derivatives that have receptor tyrosine kinase inhibitory activity; Fry 1994, which discloses a compound having a structure that inhibits EGFR (see Figure 1 of Fry 1994); U.S. Patent No. 5,196,446, which discloses heteroarylethenediyl or heteroarylethenediylaryl compounds that inhibit EGFR; and Panek 1997, which discloses a compound identified as PD166285 (6-(2,6-dichlorophenyl)-2-(4-(2-

diethylaminoethoxy)phenylarnino)-8-methyl-8H- pyrido(2,3-d)pyrimidin-7-one) that inhibits the EGFR, PDGFR, and FGFR families of receptors.

[00272] In certain embodiments, an antibody or antigen-binding fragment provided herein is linked to or in combination with one or more of pegylated or unpegylated interferon alfa-2a, pegylated or unpegylated interferon alfa-2b, pegylated or unpegylated interferon alfa-2c, pegylated or unpegylated interferon alfa n-1, pegylated or unpegylated interferon alfa n-3, and pegylated, unpegylated consensus interferon or albumin-interferon-alpha.

[00273] Other interferon alpha conjugates can be prepared by coupling an interferon alpha to a water-soluble polymer. A non-limiting list of such polymers includes other polyalkylene oxide homopolymers such as polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof. As an alternative to polyalkylene oxide-based polymers, effectively non-antigenic materials such as dextran, polyvinylpyrrolidones, polyacrylamides, polyvinyl alcohols, carbohydrate- based polymers and the like can be used. Such interferon alpha-polymer conjugates are described, for example, in U.S. Patent No. 4,766,106, U.S. Patent No. 4,917, 888, EP Application No. 0236987 or 0593868, and International Publication No. WO95/13090. A PEG12000-IFN alfa 2b can be prepared by attaching in a PEG polymer to a histidine residue in the interferon alfa-2b molecule.

[00274] Pharmaceutical compositions of pegylated interferon alpha suitable for parenteral administration can be formulated with a suitable buffer, *e.g.*, Tris-HCl, acetate or phosphate such as dibasic sodium phosphate/monobasic sodium phosphate buffer, and pharmaceutically acceptable excipients (*e.g.*, sucrose), carriers (e.g. human plasma albumin), toxicity agents (*e.g.*, NaCl), preservatives (*e.g.*, thimerosol, cresol or benzyl alcohol), and surfactants (*e.g.*, tween or polysorbates) in sterile water for injection. The

pegylated interferon alpha can be stored as lyophilized powder under refrigeration at 2°-8°C. The reconstituted aqueous solutions are stable when stored between 2° and 8°C and used within 24 hours of reconstitution (see, *e.g.*, U.S. Patent Nos. 4,492,537; 5,762,923 and 5,766,582. The reconstituted aqueous solutions may also be stored in pre-filled, multi-dose syringes such as those useful for delivery of drugs such as insulin. Typical, suitable syringes include systems comprising a prefilled vial attached to a pen-type syringe such as the NOVOLET® Novo Pen available from Novo Nordisk or the REDIPEN®, available from Schering Corporation, Kenilworth, NJ. Other syringe systems include a pen-type syringe comprising a glass cartridge containing a diluent and lyophilized pegylated interferon alpha powder in a separate compartment.

[00275] Compositions comprising an antiemetic are useful for preventing or treating nausea; a common side effect of chemotherapy. Accordingly, in certain embodiments compositions are provided that comprise an antibody or antigen-binding fragment provided herein linked to or in combination with one or more anti-cancer chemotherapeutic agents and one or more antiemetics, including but not limited to casopitant (GlaxoSmithKline), Netupitant (MGI-Helsinn) and other NK-1 receptor antagonists, palonosetron (sold as Aloxi by MGI Pharma), aprepitant (sold as Emend by Merck and Co.; Rahway, NJ), diphenhydramine (sold as Benadryl® by Pfizer; New York, NY), hydroxyzine (sold as Atarax® by Pfizer; New York, NY), metoclopramide (sold as Reglan® by AH Robins Co,; Richmond, VA), lorazepam (sold as Ativan® by Wyeth; Madison, NJ), alprazolam (sold as Xanax® by Pfizer; New York, NY), haloperidol (sold as Haldol® by Ortho-McNeil; Raritan, NJ), droperidol (Inapsine®), dronabinol (sold as Marinol® by Solvay Pharmaceuticals, Inc.; Marietta, GA), dexamethasone (sold as Decadron® by Merck and Co.; Rahway, NJ), prednisolone, methylprednisolone (sold as Medrol® by Pfizer; New York, NY), prochlorperazine (sold as Compazine® by Glaxosmithkline;

Research Triangle Park, NC), granisetron (sold as Kytril® by Hoffmann-La Roche Inc.; Nutley, NJ), ondansetron (sold as Zofran® by Glaxosmithkline; Research Triangle Park, NC), dolasetron (sold as Anzemet® by Sanofi-Aventis; New York, NY), tropisetron (sold as Navoban® by Novartis; East Hanover, NJ). Also provided herein are methods of treating cancer by administering such compositions to a subject (*e.g.*, a mammalian subject such as a human, primate, canine, rat, rabbit, or mouse) in need thereof.

[00276] Other side effects of cancer treatment include red and white blood cell deficiency. Accordingly, compositions are provided that comprise an antibody or antigen-binding fragment provided herein linked to or in combination with an agent that treats red and/or white blood cell deficiency such as pegfilgrastim, erythropoietin, epoetin alfa, or darbepoetin alfa.

[00277] In certain embodiments, compositions are provided that comprise an antibody or antigen-binding fragment thereof linked to or in combination with one or more anti-hypertensive agents such as a diuretic, an adrenergic receptor antagonist, an adrenergic receptor agonist, a calcium channel blockers, an ACE inhibitor, an angiotensin II receptor antagonist, an aldosterone antagonist, a vasodilator, or a centrally acting adrenergic drug.

[00278] In certain embodiments, the antibodies or antigen-binding fragments disclosed herein may be administered as part of a pharmaceutical composition that comprises one or more physiologically tolerable components. Therefore, in certain embodiments, such compositions and methods of formulating such compositions are provided herein. Compositions comprising one or more antibodies or antigen-binding fragments as disclosed herein and one or more physiologically tolerable components may be used in the treatment of diseases associated with high VEGF expression levels and/or signaling, and/or increased angiogenesis.

[00279] Physiologically tolerable components for use in the pharmaceutical compositions disclosed herein may include, for example, pharmaceutically acceptable liquid, gel, or solid carriers, diluents, adjuvants, excipients, or non-toxic auxiliary substances, other components known in the art, or various combinations thereof. Suitable components may include, for example, antioxidants, fillers, binders, disintegrants, buffers, preservatives, lubricants, flavorings, thickeners, coloring agents, or emulsifiers.

[00280] Suitable antioxidants may include, for example, methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxanisol, butylated hydroxytoluene, and/or propyl gallate. As disclosed herein, inclusion of one or more antioxidants such as methionine in a composition comprising an antibody or antigen-binding fragment as provided herein decreases oxidation of the antibody or antigen-binding fragment. This reduction in oxidation prevents or reduces loss of binding affinity, thereby improving antibody stability and maximizing shelf-life. Therefore, in certain embodiments compositions are provided that comprise one or more antibodies or antigen-binding fragments as disclosed herein and one or more antioxidants such as methionine. Further provided are methods for preventing oxidation of, extending the shelf-life of, and/or improving the efficacy of an antibody or antigen-binding fragment as provided herein by mixing the antibody or antigen-binding fragment with one or more antioxidants such as methionine.

[00281] Suitable carriers may include, for example, aqueous vehicles such as sodium chloride injection, Ringer's injection, isotonic dextrose injection, sterile water injection, or dextrose and lactated Ringer's injection, nonaqueous vehicles such as fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil, antimicrobial agents at bacteriostatic or fungistatic concentrations, isotonic agents such as sodium chloride or dextrose, buffers such as phosphate or citrate buffers, antioxidants such as

sodium bisulfate, local anesthetics such as procaine hydrochloride, suspending and dispersing agents such as sodium carboxymethylcelluose, hydroxypropyl methylcellulose, or polyvinylpyrrolidone, emulsifying agents such as Polysorbate 80 (TWEEN-80), sequestering or chelating agents such as EDTA (ethylenediaminetetraacetic acid) or EGTA (ethylene glycol tetraacetic acid), ethyl alcohol, polyethylene glycol, propylene glycol, sodium hydroxide, hydrochloric acid, citric acid, or lactic acid. Antimicrobial agents utilized as carriers may be added to pharmaceutical compositions in multiple-dose containers that include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Suitable excipients may include, for example, water, saline, dextrose, glycerol, or ethanol. Suitable non-toxic auxiliary substances may include, for example, wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, or agents such as sodium acetate, sorbitan monolaurate, triethanolamine oleate, or cyclodextrin.

[00282] The term "therapeutically effective amount" or "effective dosage" as used herein refers to the dosage or concentration of a drug effective to treat a disease or condition. For example, with regard to the use of the antibodies or antigen-binding fragments disclosed herein to treat cancer, a therapeutically effective amount is the dosage or concentration of the antibody or antigen-binding fragment capable of eradicating all or part of a tumor, inhibiting or slowing tumor growth, inhibiting growth or proliferation of cells mediating a cancerous condition, inhibiting tumor cell metastasis, ameliorating any symptom or marker associated with a tumor or cancerous condition, preventing or delaying the development of a tumor or cancerous condition, or some combination thereof.

[00283] The effective dosage of an antibody or antigen-binding fragment provided herein may be determined using methods well known in the art. For example, the effective dosage may be established by determining whether a tumor being treated in a subject shrinks, ceases to grow, or grows more slowly following administration at a particular dosage. The size and progress of a tumor can be determined using methods well known in the art, such as for example X-ray, magnetic resonance imaging (MRI), CT scan, or visual detection (*e.g.*, a surgical procedure). For example, where the cancer being treated is glioblastoma multiforme, a clinician may monitor treatment progress by imaging the tumor using a CT or MRI scan. Patient interviews regarding the appearance of symptoms of tumor growth (*e.g.*, headaches, behavioral or mood changes) are also informative. Depending on the findings of the clinician, the dosage regimen can be altered accordingly. In another example, where the cancer being treated is melanoma, a clinician can monitor treatment progress by visual inspection of the melanoma lesion. The clinician may evaluate a variety of visual parameters, such as for example size, thickness, changes in growth pattern, or changes in appearance. Depending on the findings of the clinician, the dosage regimen can be altered accordingly.

[00284] In general, tumor size and proliferation can be measured using a thymidine PET scan (see, *e.g.*, Wells 1996). The thymidine PET scan generally requires the injection of a radioactive tracer, such as [2-^{11}C]-thymidine, followed by a PET scan of the subject's body (Vander Borght 1991a; Vander Borght 1991b). Other tracers that can be used include [^{18}F]-FDG (18-fluorodeoxyglucose), [^{124}I]IUdR (5-[124I]iodo-2'-deoxyuridine), [^{76}Br]BrdUrd (Bromodeoxyuridine), [^{18}F]FLT (3'-deoxy-3'fluorothymidine) or [^{11}C]FMAU (2'-fluoro-5-methyl-1-ß-D-arabinofuranosyluracil).

[00285] The effective dosage of an antibody or antigen-binding fragment as provided herein will depend on various factors known in the art, such as for example body weight,

age, past medical history, present medications, state of health of the subject and potential for cross-reaction, allergies, sensitivities and adverse side-effects, as well as the administration route and extent of tumor development. Dosages may be proportionally reduced or increased by one of ordinary skill in the art (*e.g.*, physician or veterinarian) as indicated by these and other circumstances or requirements.

[00286] In certain embodiments, an antibody or antigen-binding fragment as provided herein may be administered at a therapeutically effective dosage of about 0.01 mg/kg to about 100 mg/kg (*e.g.*, about 0.01 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 65 mg/kg, about 70 mg/kg, about 75 mg/kg, about 80 mg/kg, about 85 mg/kg, about 90 mg/kg, about 95 mg/kg, or about 100 mg/kg). In certain of these embodiments, the antibody or antigen-binding fragment is administered at a dosage of about 50 mg/kg or less, and in certain of these embodiments the dosage is 10 mg/kg or less, 5 mg/kg or less, 1 mg/kg or less, 0.5 mg/kg or less, or 0.1 mg/kg or less. A given dosage may be administered at various intervals, such as for example once a day, two or more times per day, two or more times per week, once per week, once every two weeks, once every three weeks, once a month, or once every two or more months. In certain embodiments, the administration dosage may change over the course of treatment. For example, in certain embodiments the initial administration dosage may be higher than subsequent administration dosages. In certain embodiments, the administration dosage may vary over the course of treatment depending on the reaction of the subject.

[00287] Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single dose may be administered, or several divided doses may be administered over time.

[00288] Pharmaceutical compositions comprising the antibodies or antigen-binding fragments disclosed herein, and in certain embodiments various chemotherapeutic agents, may be prepared by methods well known in the art of pharmacy. See, *e.g.*, Gilman, *et al.*, (eds.) (1990), The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; A. Gennaro (ed.), Remington's Pharmaceutical Sciences, 18th Edition, (1990), Mack Publishing Co., Easton, Pennsylvania.; Avis, *et al.*, (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, New York; Lieberman, *et al.*, (eds.) (1990) Pharmaceutical Dosage Forms: Tablets Dekker, New York; and Lieberman, *et al.*, (eds.) (1990), Pharmaceutical Dosage Forms: Disperse Systems Dekker, New York.

[00289] The antibodies and antigen-binding fragments disclosed herein may be administered by any route known in the art, such as for example parenteral (*e.g.*, subcutaneous, intraperitoneal, intravenous, including intravenous infusion, intramuscular, or intradermal injection) or non-parenteral (*e.g.*, oral, intranasal, intraocular, sublingual, rectal, or topical) routes. In embodiments wherein the antibodies or antigen-binding fragments are administered via injection, injectable pharmaceutical compositions may be prepared in any conventional form, such as for example liquid solution, suspension, emulsion, or solid forms suitable for generating liquid solution, suspension, or emulsion. Preparations for injection may include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile

dry insoluble products ready to be combined with a vehicle just prior to use, and sterile emulsions. The solutions may be either aqueous or nonaqueous.

[00290] In certain embodiments, unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration should be sterile, as is known and practiced in the art.

[00291] In certain embodiments, a sterile, lyophilized powder is prepared by dissolving an antibody or antigen-binding fragment as disclosed herein in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological components of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, water, dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides a desirable formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial can contain a single dosage or multiple dosages of the anti-VEGF antibody or antigen-binding fragment thereof or composition thereof. Overfilling vials with a small amount above that needed for a dose or set of doses (*e.g.*, about 10%) is acceptable so as to facilitate accurate sample withdrawal and accurate dosing. The lyophilized powder can be stored under appropriate conditions, such as at about 4°C to room temperature.

[00292] Reconstitution of a lyophilized powder with water for injection provides a formulation for use in parenteral administration. In one embodiment, for reconstitution the lyophilized powder is added to sterile water or other liquid suitable carrier. The precise

amount depends upon the selected therapy being given, and can be empirically determined.

[00293] In certain embodiments, expression systems are provided for expressing the antibodies or antigen-binding fragments disclosed herein. These expression systems include polynucleotides encoding the antibodies or antigen-binding fragments, vectors comprising these polynucleotides, and host cells comprising these vectors. Polynucleotides encoding the antibodies or antigen-binding fragments disclosed herein may be isolated or synthesized using methods known in the art, and inserted into a replicable vector for amplification or cloning. Polynucleotides encoding variable light (V_L) and variable heavy (V_H) chains of the antibodies may be expressed from a single vector, or they may be expressed using two separate vectors, followed by *in vitro* assembly. In certain embodiments, they may be co-expressed from two separate vectors within the same cell and assembled intracellularly (see, *e.g.*, U.S. Patent Nos. 4,816,567 or 5,595,898). Suitable vectors may contain various configurations of one or more regulatory sequences, such as promoters, enhancers, or transcription initiation sequences, as well as genes encoding markers for phenotypic selection. Vectors having suitable backbones for expression of the antibodies or antigen-binding fragments disclosed herein are known in the art (see, *e.g.*, U.S. Patent No. 7,192,737). In certain embodiments, the vector may contain a polynucleotide sequence encoding the constant regions of the heavy chain (C_H) and light chain (C_L) of a human IgG2 immunoglobulin. Alternatively, the vector may express only the V_H and V_L chains of the antibody, with the expressed polypeptide comprising an Fv fragment rather than a whole antibody. Vectors may be inserted into a suitable host cell for amplification or expression of the polynucleotide sequence. The host cells may be cultured for antibody production in a variety of media known in the art, such as for example Minimal Essential Medium (MEM) (Sigma), RPMI-1640 (Sigma),

Dulbecco's Modified Eagle's Medium (DMEM) (Sigma), and Ham's F10 (Sigma). Media may be supplemented with a variety of agents, such as for example hormones, growth factors, salts, buffers, nucleotides, antibiotics, trace elements, glucose, or other energy sources. Culture conditions such as temperature and pH may be adjusted using parameters well known in the art. Following expression, one or more antibodies or antigen-binding fragments as provided herein may be purified using methods known in the art.

[00294] The antibodies or antigen-binding fragments disclosed herein may comprise conjugates for specific delivery to cancer cells. In addition, binding of the antibodies or antigen-binding fragments to tumor cells may be used to recruit host immune responses. This host immune response may be increased by utilizing bivalent antibodies, with one binding site corresponding to the fully human antibodies or antigen-binding fragments provided herein and another binding site that recognizes another antigen.

[00295] In certain embodiments, the antibodies or antigen-binding fragments disclosed herein may comprise oligosaccharides with high fucose content. In other embodiments, the antibodies or antigen-binding fragments disclosed herein may have reduced fucose content, such as for example fucose-free Fc antibodies. Reduced fucose antibodies may be generated using a cell line with reduced fucosylation activity, such as for example rat YB2/0 cells (Shinkawa 2003) or the CHO variant cell line Lec13 (Shields 2002).

[00296] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. All specific compositions, materials, and methods described below, in whole or in part, fall within the scope of the present invention. These specific compositions, materials, and methods are not intended to limit the invention, but merely to illustrate specific embodiments falling

within the scope of the invention. One skilled in the art may develop equivalent compositions, materials, and methods without the exercise of inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

Examples

Example 1: Generation of antibodies that bind $hVEGF_{165}$:

[00297] Human single-chain Fv (scFv) phage display libraries were panned against immobilized $hVEGF_{165}$ to identify a panel of antibody fragments with the ability to bind $hVEGF_{165}$. Panning was carried out using standard protocols (see, *e.g.*, Methods in Molecular Biology, vol. 178: Antibody Phage Display: Methods and Protocols Edited by: P.M. O'Brien and R. Aitken, Humana Press;, "Panning of Antibody Phage-Display Libraries," Coomber, D.W.J., pp. 133-145, and "Selection of Antibodies Against Biotinylated Antigens," Chames, P., et al., pp. 147-157).

[00298] Briefly, three wells of a NUNC® MAXISORP plate were coated with 50 µl of recombinant $hVEGF_{165}$ (R&D Systems, catalog no. 293-VE) at a concentration of 10 µg/ml in PBS. After overnight incubation at 4°C, free binding sites were blocked with 5% milk in PBS for one hour at room temperature. Approximately 200 µl of phage library in 5% milk/PBS was then added to the blocked wells and incubated at room temperature for approximately one to two hours. Wells were washed and bound phage was eluted using standard methods (see, *e.g.*, Sambrook and Russell, Molecule Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, 2001). Eluted phage was amplified via infection for one hour at 37°C into *E. coli* TG1 host cells in logarithmic growth phase. Infected TG1 cells were recovered by centrifugation at 2,500 RPM for five

minutes, plated onto 15 cm 2YT-ampicillin-2% glucose agar plates, and incubated at 30°C overnight. The panning process was then repeated using the amplified phage.

[00299] The cycle of panning, elution, and amplification was repeated for three rounds with decreasing concentration (*e.g.*, 50ug/ml $hVEGF_{165}$ at Round One, 10ug/ml at Round Two, and then 10ug/ml at Round Three), at which point single colonies from the plated TG1 cells were used to inoculate media in 96-well plates. Microcultures were grown to an OD_{600} of 0.6, at which point expression of soluble scFv was induced by addition of 1 mM IPTG and overnight incubation in a shaker at 30°C. Bacteria were spun down, and periplasmic extract was used to test scFv binding to immobilized $hVEGF_{165}$ using a standard ELISA assay.

Example 2: Blocking of $hVEGF_{165}$ binding to VEGF receptors by scFvs:

[00300] Phage clones from Example 1 exhibiting $hVEGF_{165}$ binding by ELISA were tested for their ability to block $hVEGF_{165}$ binding to VEGF-R1 and/or VEGF-R2 using the microplate-based competitive screening DELFIA® assay (Perkins Elmer, Waltham, MA).

[00301] Briefly, biotinylated $hVEGF_{165}$ solution was added 1:1 in volume to periplasmic extracts from Example 1 to a final concentration of 0.5 µg/ml. 100 µl of this mixture was added to a plate coated with VEGF-R1 or VEGF-R2 (R&D Systems: VEGF-R1/Flt-1, catalog no. 321-FL; VEGF-R2/KDR/Flk-1, catalog no. 357-KD) and incubated for 1.5 hours at room temperature. Plates were washed with PBST, and a 1:250 dilution of Europrium-Streptavidin in DELFIA Assay Buffer was added at 50 µl/well. Plates were incubated at room temperature for one hour, then washed with DELFIA Wash Buffer. DELFIA Enhancement Buffer was added at 50 µl/well, and plates were incubated for five minutes at room temperature. Plates were read on a Time-Resolved Fluorescence Gemini plate reader.

Example 3: Conversion of scFv to scFv-Fc and IgG:

[00302] Two scFvs from Example 2 that inhibited $hVEGF_{165}$ binding to VEGF-R1 or VEGF-R2 by more than 60%, XPA.10.064 and XPA.10.072, were selected for conversion to scFv-Fc and/or IgG. The heavy chain variable regions (including heavy chain CDRs) and light chain variable regions (including light chain CDRs) of XPA.10.064 and XPA.10.072 are set forth in Figure 1. The heavy chain CDRs (*e.g.*, HCDR1, HCDR2 and HCDR3) and the light chain CDRs (*e.g.*, LCDR1, LCDR2, and LCDR3) were determined by the Kabat numbering system (Kabat, E.A., et al. 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA). HCDR1, HCDR2 and HCDR3 amino acid sequences for XPA.10.064 and XPA.10.072 are set forth in SEQ ID NOs: 6, 7, and 8, respectively. LCDR1, LCDR2, and LCDR3 amino acid sequences for XPA.10.064 are set forth in SEQ ID NOs: 12, 13, and 14, respectively. LCDR1, LCDR2, and LCDR3 amino acid sequences for XPA.10.072 are set forth in SEQ ID NOs: 9, 10, and 11, respectively.

[00303] For conversion of XPA.10.064 and XPA.10.072 into scFv-Fc fusion proteins, scFv cDNAs were cloned into eukaryotic expression vectors that had been modified to encode the CH2 and CH3 domains of the gamma-2 (γ2) heavy chain constant region gene (U.S. Patent No. 7,192,737; WO 2004/033693).

[00304] For conversion of XPA.10.064 and XPA.10.072 into IgG, the variable regions of both heavy and light chains were cloned into eukaryotic expression vectors encoding the kappa (κ), lambda (λ), or gamma-2 (γ2) heavy and light chain constant region genes (US 2006/0121604).

[00305] XPA.10.064 and XPA.10.072 scFv-Fc and IgG antibodies were transiently expressed in 293E cells as described previously (US 2006/0121604). Supernatant from transfected cells was harvested at day six of culture, and IgG was purified by Protein-A chromatography.

Example 4: Biacore analysis of XPA.10.064 and XPA.10.072 scFv-Fc and IgG binding kinetics:

[00306] Binding affinity of XPA.10.064 and XPA.10.072 scFv-Fcs was assessed using a BIACORE 2000 and a CM5 sensor chip (Biacore) with Protein A/G (Piece) immobilized on all flow cells at high density. Dilution and running buffer for these experiments was HBS-EP (Biacore) with 1:50 dilution of Chemiblocker® (Chemicon). Antibody capture was performed by injecting diluted XPA.10.064 and XPA.10.072 scFv-Fcs over flow cell 2 (fc2) at 20 µl/minute for a variable volume to achieve roughly 50-70 RU of antibody capture. Antibody concentrations were approximately 0.5 µg/ml. $hVEGF_{165}$ expressed from sf21 cells was injected over five minutes at 30 µl/minute using the Kinject feature with 15 minute dissociation over fc1 and 2. Four dilutions of $hVEGF_{165}$ were prepared in a three-fold serial dilution, giving concentrations of 5 µg/ml (119 nM), 1.667 µg/ml (39.7 nM), 0.55 µg/ml (13.2 nM), and 0.185 µg/ml (4.4 nM). Regenerations were performed with two injections of 100 mM HCl at 50 µl/minutes for twelve seconds each. Data was processed in Scrubber2 and fit by a 1:1 Langmuir interaction model after double referencing of the control flow cell and blank injections. XPA.10.064 and XPA.10.072 scFv-Fcs exhibited high and nearly identical binding affinity for $hVEGF_{165}$.

[00307] A similar protocol was utilized to assess binding kinetics of XPA.10.064 and XPA.10.072 IgG2. Both XPA.10.064 and XPA.10.072 IgG2s bound specifically to $hVEGF_{165}$ with similar low single digit nanomolar affinity (Figures 2-4), and exhibited only weak binding (> 100 nM) to $mVEGF_{165}$. Both antibodies exhibited binding kinetics similar to those of Bevacizumab (Table 2).

Table 2: Binding affinity of XPA.10.064 and XPA.10.072 IgG2s to $hVEGF_{165}$

	k_a (1/Ms)	k_d (1/s)	R_{max} (RU)	K_D (M)	Chi^2
Bevacizumab	1.22×10^5	7.38×10^{-5}	20	6.05×10^{-10} (605 pM)	0.251
XPA.10.064	2.30×10^5	2.86×10^{-4}	30	1.24×10^{-9} (1.24 nM)	0.379
XPA.10.072	1.68×10^5	2.79×10^{-4}	24	1.66×10^{-9} (1.66 nM)	0.110

Example 5: Blocking of $hVEGF_{165}$ binding to VEGF receptors by XPA.10.064 and XPA.10.072 IgGs:

[00308] The ability of XPA.10.064 and XPA.10.072 IgG2s to block binding of $hVEGF_{165}$ to VEGF-R1 and/or VEGF-R2 was assessed using a Biacore 2000 with a CM5 chip.

[00309] VEGF receptor (R&D Systems) was immobilized on the CM5 chip at a density of approximately 15,000 via amine coupling (Biacore). VEGF-R2 was immobilized on fc2 and VEGF-R1 was immobilized on fc4. Flow cells 1 and 3 served as references, and were activated and blocked in the same manner as the receptor immobilized flow cells. 0.15 µg/ml $hVEGF_{165}$ in HBS-EP running buffer was mixed 1:1 with antibody sample or buffer. Final antibody concentrations were 15, 5, 1.667, 0.556, 0.185, 0.0617, 0.0206, and 0 µg/ml. Samples were incubated for at least one hour prior to initiation of the Biacore analysis run. All samples were injected in duplicate and each set of antibody replicates had its own positive and negative control (no antibody with VEGF and no VEGF, respectively). Samples were injected at 10 µl/minute for 1.5 minutes over all flow cells. Regeneration was performed with a twelve second injection of Glycene, pH 1.75, at 50 µl/minute.

[00310] For data analysis, the slope of a linear portion of association (30 seconds to one minute) of the association phase was determined with a linear fit. The signal from each point was subtracted by the nearest blank, then divided by the matched 100% signal

(no antibody) control to give the percent inhibition of that cycle. Data was plotted in GraphPad Prism and fit with a sigmoidal dose response curve to calculate the EC_{50}.

[00311] XPA.10.064 and XPA.10.072 IgG2s both blocked $hVEGF_{165}$ binding to VEGF-R1 and VEGF-R2 at levels similar to Bevacizumab (Figures 5-6, Table 3).

Table 3: Inhibition of $hVEGF_{165}$ binding to VEGF-R1/R2 by XPA.10.064 and XPA.10.072:

	Bevacizumab	**XPA.10.064**	**XPA.10.072**
$hVEGF_{165}$/VEGF-R1 (EC_{50})	0.1002 µg/ml	0.1589 µg/ml	0.1211 µg/ml
$hVEGF_{165}$/VEGF-R2 (EC_{50})	0.06328 µg/ml	0.1287 µg/ml	0.1730 µg/ml

Example 6: Analysis of $hVEGF_{165}$ epitopes bound by XPA.10.064 and XPA.10.072:

[00312] To determine whether the $hVEGF_{165}$ epitopes recognized by XPA.10.064 and XPA.10.072 were linear or conformational, three 200 ng samples of non-reduced or reduced and heat-denatured recombinant $hVEGF_{165}$ were subjected to electrophoresis on three separate SDS-PAGE gels. Electrophoresed proteins were transferred to Immulon-P membranes, and the blots were hybridized with XPA.10.064 IgG, XPA.10.072 IgG or Bevacizumab antibodies and incubated with 1 µg/ml secondary goat anti-human IgG HRP-conjugated antibody. Binding was detected with enhanced chemiluminescence (ECL) substrate (Pierce).

[00313] XPA.10.064, XPA.10.072, and Bevacizumab all bind to linear epitopes on $hVEGF_{165}$ (Figure 7).

Example 7: XPA.10.064 and XPA.10.072 $hVEGF_{121}$ epitope binding studies:

[00314] To determine whether XPA.10.064 and XPA.10.072 bind the same $hVEGF_{121}$ epitope as Bevacizumab, three ELISA comparative binding assays were performed using various $hVEGF_{121}$ mutants.

[00315] Previous mutation analysis has shown that VEGF residues M81, G88, Q89, and G92 are important for Bevacizumab binding to $hVEGF_{165}$ (Fuh 2006). To determine whether these residues are important for XPA.10.064 and XPA.10.072 binding, the following $hVEGF_{121}$ mutants were generated: $hVEGF_{121}$-M81A, $hVEGF_{121}$-Q89A, $hVEGF_{121}$-G92A, and $hVEGF_{121}$-G88S. Mutants were transiently expressed in CHO-K1 cells, and cell supernatants were collected for binding analysis by ELISA.

[00316] A microtiter plate was coated with XPA.10.064, XPA.10.072, Bevacizumab, or a control polyclonal goat anti-human VEGF (PAB) at a concentration of 1, 2, or 5 µg/ml and incubated overnight at 4℃. The plate was blocked with 30% ChemiBlock™ reagent (Millipore) in PBS for one hour at room temperature, and 30, 60, or 100 µl of CHO-K1 culture supernatant for each mutant, or 1 µg/ml of wild-type $hVEGF_{121}$, recombinant $hVEGF_{165}$, or recombinant $mVEGF_{165}$, was added to appropriate wells. After one hour of incubation, the plate was washed and incubated with a biotinylated goat polyclonal anti-VEGF antibody for one hour at room temperature. Detection was performed with HRP-conjugated streptavidin, followed by TMB chromogenic substrate (Calbiochem) using manufacturer protocol.

[00317] The binding pattern of XPA.10.064 and XPA.10.072 to $hVEGF_{121}$ mutants was similar to that of Bevacizumab (Tables 4-5), indicating that the antibodies bind overlapping or similar epitopes.

Table 4: XPA.10.064 and XPA.10.072 binding to hVEGF mutants:

Antibody	CHO	hVEGF $_{121}$	hVEGF $_{121}$-M81A	hVEGF $_{121}$-G88S	hVEGF $_{121}$-Q89A	hVEGF $_{121}$-G92A	rHu-VEGF $_{165}$	rMu-VEGF $_{165}$
PAB	1.0	28	18.8	8.4	10.8	23.9	26.9	1.0
	0.09	2.5575	1.7145	0.769	0.9852	2.1765	2.454	0.0939
Bevacizumab	0.9	39.4	13.1	0.9	5.3	35.6	36.7	0.9
	0.09	3.5953	1.1941	0.0828	0.4801	3.249	3.3501	0.0843
XPA.10.064	0.9	29.1	9.5	0.9	3.5	22.8	23	0.7
	0.085	2.6504	0.8678	0.0778	0.3194	2.0792	2.0946	0.0667
XPA.10.072	1.5	31.8	13.4	1.0	3.3	30.5	29.2	1.1
	0.142	2.902	1.2228	0.0951	0.2964	2.7809	2.6597	0.0982

Top row for each antibody: fold binding over CHO background
Bottom row for each antibody: Raw data

Table 5: Summary of epitope binding results

Antibody	hVEGF $_{21}$	hVEGF $_{21}$-M81A	hVEGF $_{21}$-G88S	hVEGF $_{21}$-Q89A	hVEGF $_{21}$-G92A	rHu-VEGF $_{65}$	rMu-VEGF $_{65}$
PAB	Yes	Yes	Yes	Yes	Yes	Yes	No
Bevacizumab	Yes	Reduced	No	Weak	Yes	Yes	No
XPA.10.064	Yes	Reduced	No	Weak	Yes	Yes	No
XPA.10.072	Yes	Reduced	No	Weak	Yes	Yes	No

Example 8: Tissue cross-reactivity of XPA.10.064 and XPA.10.072:

[00318] A frozen normal human tissue array (TMA) was used to evaluate immunohistochemical (IHC) reactivity of XPA.10.064 and XPA.10.072 using a single-color chromogenic technique. The TMA comprised 32 normal human tissue types, with each type consisting of tissues from two to three different donors. In addition to the TMA, larger sections of normal human liver, kidney, Fallopian tubes, pancreas, ureter, and adrenal gland were used to confirm staining results from the TMA or to replace missing tissues in the TMA. Positive controls included hVEGF proteins spotted on UV-resin slides and renal carcinoma tissue expressing high level hVEGF as assessed by strong staining with anti-hVEGF rabbit monoclonal antibody.

[00319] The TMA and normal human tissues and the hVEGF protein spot and renal carcinoma positive controls were stained with XPA.10.064, XPA.10.072 (human IgG2), or Bevacizumab at 20 µg/ml using a human-on-human IHC staining protocol. A human tonsillitis case was also included to monitor the effectiveness of the staining protocol. The final protocol did not have reactivity with tissue endogenous immunoglobulin in B-cell region of the tonsil tissue. Negative control antibodies were human IgG1 and IgG2 (Sigma, St. Louis, MO) and the human KLH antibody CHO.KLHG2.60 (IgG2).

[00320] XPA.10.064, XPA.10.072, and Bevacizumab were all reactive with hVEGF protein spots at 2-3+ on a scale of 0-4+, where 4+ indicates the highest staining intensity. For renal carcinoma tissue, Bevacizumab gave equivocal staining, while XPA.10.064 and XPA.10.072 stained cytoplasm of tumor cells.

[00321] Human IgG1 and IgG2 did not stain any tissue elements, giving only minimal background staining. CHO.KLHG2.60 had reactivity with cells in the

adrenal cortex, epithelial cells in esophagus, mammary gland, pancreas, prostate, stomach, thyroid gland, ureter cervix, and Fallopian tube. Due to this reactivity, human IgG1 and IgG2 were used as reference negative controls.

[00322] XPA.10.064 had the broadest tissue reactivity spectrum of the test antibodies. XPA.10.064 stained strongly with smooth muscle cells in bladder, GI track, Fallopian tube, mammary gland, prostate, ureter, and uterus, and with epithelial cells in Fallopian tube, prostate, skin, small intestine, stomach, thyroid gland, ureter, endometrial glands of the uterus, and uterus cervix. In addition, XPA.10.064 stained some neurons and nerve fibers in cerebellum, cerebral cortex, and spinal cord, as well as cardiac and skeletal muscles, cells in pituitary glands, renal glomeruli, liver sinusoid endothelium, stromal cells of thymus, macrophages in lung, and cells in the adrenal cortex. XPA.10.072 stained strongly with nerve fibers in cerebellum, cerebral cortex, and spinal cord. XPA.10.072 also stained smooth muscles of the GI track, Fallopian tube, prostate, ureter, and uterus, epithelial cells in esophagus, Fallopian tube, mammary gland, prostate, stomach, small intestine, thyroid gland, and ureter, macrophages in lung, and fibroblast/histiocytes in placenta. Bevacizumab stained negative with all normal human tissues.

[00323] To determine whether the immunohistochemical reactivity of XPA.10.064 and XPA.10.072 represented on- or off-target binding, a multicolor immunofluorescence-based approach was utilized. This approach is based on the simultaneous comparison of immunoreactivity of a known "gold standard" anti-VEGF antibody to test antibodies. On-target reactivity of test antibodies manifests as co-localization with the "gold standard" antibody, while lack of co-localization indicates off-target reactivity.

[00324] Frozen sections of cell pellets from positive (Du145) and negative (Hek 293) control cells were stained with commercial mouse anti-human anti-VEGF antibody (BD Pharmingen, clone G153-694) using the same chromogenic IHC methodology employed in the initial experiments. This antibody stained Du145 cells, but failed to stain Hek 293 cells. In addition, frozen sections of colon carcinoma stained with this antibody demonstrated a characteristic pattern of reactivity in the epithelial and tumor associated matrix components with good internal negative controls. Therefore, G153-694 was designated the "gold standard" positive control antibody.

[00325] Using a protocol from the Zenon IgG Labeling Kit (Molecular Probes), primary antibody was pre-incubated with a fluorochrome conjugated Fc-targeted anti-human Fab, followed by neutralization of non-reacted Fab with molar excess of the appropriate normal serum. Fluorescent antibody-Fab complex used as the staining reagent, followed by nuclear counterstaining with DAPI. Frozen sections from an adenocarcinoma of the colon (Tissue ID 4558) were used as control VEGF-positive tissue for these co-localization studies. XPA.10.064 and XPA.10.072 antibodies were labeled with red (Alexa Fluor 594), and "gold standard" antibody was labeled with green (Alexa Fluor 488). The assay was repeated using the reverse color combination, which gave essentially the same results. Images were captured using a Leica TCS-SP, model DM RXE laser scanning confocal microscope and Leica Confocal software, version 2.0 (Leica Microsystems, Wetzler, Germany). Multiple fields were imaged at 400X (at least three), and representative fields were analyzed for colocalization using Image Pro software (Media Cybernetics, Silver Spring, MD). Since Bevacizumab

was essentially negative in all of the initial IHC studies on both colon carcinoma and frozen pellet sections, it was not included in the co-localization studies.

[00326] XPA.10.064 and XPA.10.072 demonstrated similar high degree of colocalization, with XPA.10.064 showing the greatest intensity of tissue staining (Figures 8-9).

Example 9: Inhibition of HUVEC proliferation by XPA.10.064 and XPA.10.072:

[00327] XPA.10.064 and XPA.10.072 scFv-Fcs and IgG2s were tested for their ability to block proliferation of HUVECs.

[00328] Pooled HUVECs (Clonetics #CC-2519) were grown in ECGM Complete Media (Clonetics #CC-3024) plus BulletKit-2 (supplemented with rhEGF, rhFGF, rhVGEF, ascorbic acid, hydrocortisone, IGF, heparin, gentamycin/amphotericin, and 2% FBS). Cells were seeded at 2-3x10^5 cells per T-75 flask, and reached confluence at 3-4 days. The sub-confluent monolayer was washed with PBS, trypsinized, and neutralized with complete media containing PBS.

[00329] To measure HUVEC proliferation in the presence of $hVEGF_{165}$, a 16-point dose titration of $hVEGF_{165}$ expressed from HEK 293 cells was set up by diluting in basal growth medium (0-200 ng/ml final, 2x dilutions, 2x concentration, 50 µl/well). HUVEC cells were re-suspended at 2x10^5 cells/ml in cold basal medium/0.1% BSA, and 50 µl of cells (1x10^4 c/w) were added to each well of the $hVEGF_{165}$ titration plate for a final volume of 100 µl/well. Outer wells were flooded with PBS, and plates were sealed with parafilm to prevent dehydration. Plates were incubated for 96 hours in 5% CO_2 at 37°C, then brought to room temperature over approximately 15-20 minutes. Cell TiterGlo (TTG, Promega) was thawed and brought to substrate temperature, and 100 µl of substrate/buffer

mixture was added to each well. The plate was shaken on an orbital plate shaker for 1-2 minutes, and 150 µl from each well was transferred to white bottom, white walled plates and incubated in the dark for 5-10 minutes. The plate was read on a luminometer with a one second integration.

[00330] For the proliferation inhibition assay, titrations of XPA.10.064, XPA.10.072, and Bevacizumab were generated (0-50 µg/ml final, 3x dilutions, 4x concentrations, 25 µl/well final volume). Antibodies were pre-incubated 1:1 with $hVEGF_{165}$ for two hours. After pre-incubation, 50 µl/well of VEGF/antibody complex was added to 50 µl/well of re-suspended HUVEC cells, and the plates were incubated for 96 hours and treated with TiterGlo buffer as described above.

[00331] XPA.10.064 and XPA.10.072 scFvs and IgG2s inhibited HUVEC proliferation. IgG2 results are set forth in Figure 10 and Table 6).

Table 6: Inhibition of HUVEC proliferation by XPA.10.064, XPA.10.072, and Bevacizumab.

	EC_{50} (µg/ml)		
	XPA.10.064	XPA.10.072	BM-1
HEK 293 $hVEGF_{165}$	0.66	0.68	0.04

Example 10: Inhibition of VEGF-R2 phosphorylation by XPA.10.064 and XPA.10.072:

[00332] The ability of XPA.10.064 and XPA.10.072 to inhibit VEGF-R2 phosphorylation by $hVEGF_{165}$ was analyzed by ELISA.

[00333] To generate lysate plates, HUVEC cells between passages two and six were thawed and plated into TC flasks in EGM2 complete media (Lonza), and allowed to grow for one to two passages. Sub-confluent cells were trypsinized,

neutralized with complete media, washed twice with PBS, and counted. Cells were plated at $1x10^5$ cells/well in complete media in 24w format (triplicate wells) and incubated at 37°C for 24 hours. After incubation, cells were washed twice with room temperature PBS and starved in EBM2 medium (Lonza) plus 0.1% BSA for five hours. PBS was decanted and cells were incubated with a dose titration of $hVEGF_{165}$ (stimulation) or pre-complexed VPA.10.064+$hVEGF_{165}$, VPA.10.072+$hVEGF_{165}$, or Bevacizumab+$hVEGF_{165}$ (inhibition) for five minutes. VPA.10.064+$hVEGF_{165}$ and VPA.10.072+$hVEGF_{165}$ were generated by mixing a 2X dose titration of antibody 1:1 with 2X $hVEGF_{165}$ (final concentration: 20 ng/ml) and incubating at 37°C for 24 hours. $hVEGF_{165}$, VPA.10.064+$hVEGF_{165}$, and VPA.10.072+$hVEGF_{165}$ were decanted and cells were washed twice with ice cold PBS. 65 µl/well of lysis buffer/well (1% NP-40, 20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate, 10 µg/ml Leupeptin) was added, and cells were rocked at 4°C for 30 minutes until needed.

[00334] Capture antibody specific for VEGF-R2 (R&D Systems, VEGF-R2/KDR/Flk-1, catalog no. 357-KD) was diluted to a working concentration of 8.0 g/ml in PBS and coated onto a 96 well microplate at 100 µl/well. VEGF-R2/KDR/Flk-1 binds both phosphorylated and non-phosphorylated VEGF-R2. The plate was sealed and incubated overnight. Each well was aspirated and washed with wash buffer five times, and the plate was blocked by adding 300 µl/well of block buffer and incubating at room temperature for one to two hours. Each well was aspirated and washed with wash buffer five more times, and 100 µl of HUVEC lysate was added to each. The plate was incubated for two hours at room temperature, and wells were aspirated and washed five times with wash

buffer. 100 µl of HRP-conjugated detection antibody specific for phosphorylated tyrosine was added to each well, and the plate was covered and incubated for two hours at room temperature out of direct light. Wells were aspirated and washed five times with wash buffer, and 100 µl of substrate solution was added to each well. The plate was incubated for 20 minutes at room temperature out of direct light, and 50 µl of stop solution was added to each well. The optical density of each well was read on a microplate reader at 450 nm.

[00335] HUVECs treated with a dose titration of $hVEGF_{165}$ exhibited an increase in phosphorylated VEGF-R2 (Figure 11). HUVECs treated with a dose titration of Bevacizumab+$hVEGF_{165}$ exhibited a decrease in VEGF-R2 phosphorylation (Figure 12). HUVECs treated with a dose titration of XPA.10.064+$hVEGF_{165}$ or XPA.10.072+$hVEGF_{165}$ exhibited a decrease in VEGF-R2 phosphorylation. Results for each antibody are summarized in Figure 13.

Example 11: Inhibition of angiogenesis by XPA.10.064 and XPA.10.072:

[00336] A Matrigel® plug assay was used to measure the ability of XPA.10.064 and XPA.10.072 to inhibit angiogenesis *in vivo*.

[00337] Female NU/NU mice age 6-7 weeks were injected s.c. in the abdomen with 0.5 ml Matrigel® (BD Biosciences, San Jose, CA) containing $2x10^6$ DU145 cells, which produce human VEGF to induce angiogenesis. Mice were injected i.p. on days 0 and 3 with vehicle control or 0.1, 1, or 5 mg/kg XPA.10.064, XPA.10.072, or Bevacizumab. On day 7, mice were sacrificed and Matrigel® plugs were excised, weighed, and photographed. Plugs were given a visual score of 0 to 3 based on the following scheme: 0, no color or obvious vessels; 1, hint of color and few vessels; 2, yellow-red with distinct vessels; and 3, homogenous red or pink with dark vessels (Figure 14). Plugs were evaluated

by blinded scorers who received photographs in which the plug order was scrambled.

[00338] Administration of XPA.10.064 resulted in a significant decrease in angiogenesis at all dosages tested, while administration of XPA.10.072 resulted in a significant decrease at 1 mg/kg and 5 mg/kg (Figures 15 &16). The level of angiogenesis inhibition was similar to that observed in the presence of Bevacizumab.

[00339] XPA.10.064, XPA.10.072, and Bevacizumab concentrations in mouse serum were measured by ELISA at four days after the last antibody dose. There was no significant difference in antibody levels between the three antibodies at any of the dosages tested.

Example 12: Inhibition of tumor growth by XPA.10.064 and XPA.10.072:

[00340] The ability of XPA.10.064 and XPA.10.072 to inhibit tumor growth was tested with the A673 Rhabdomyosarcoma tumor growth model using a previously disclosed protocol (Liang 2006). A673 cells maintained in culture were grown until confluent, then harvested and re-suspended in sterile 50% Matrigel®. Xenografts were established by s.c. injection of $5x10^6$ cells in Matrigel® into the flanks of six-week-old female nude mice. When tumor size reached about 100 mm^3, mice were randomized into eight groups of ten and injected i.p. with vehicle only (Group 1), 0.5 mg/kg XPA.10.064 IgG2 (Group 2), 5 mg/kg XPA.10.064 IgG2 (Group 3), 0.5 mg/kg XPA.10.072 IgG2 (Group 4), 5 mg/kg XPA.10.072 IgG2 (Group 5), 5 mg/kg isotype control anti-KLH IgG2 (Group 6), 0.5 mg/kg Bevacizumab (Group 7), or 5 mg/kg Bevacizumab (Group 8) twice a week for 18 days (six total doses). Blood and tissue samples were collected at 24, 72, and 168 hours after the last dose.

[00341] Administration of XPA.10.064 and XPA.10.072 resulted in significant *in vivo* tumor growth inhibition at both dosages tested (Figure 17). The level of growth inhibition for both antibodies was slightly higher than that observed with Bevacizumab at all dosages tested. Serum levels were similar for all antibodies administered at a given dosage (0.5 mg/kg, serum level approximately 5-7 µg/ml; 5 mg/kg, serum level approximately 75-100 µg/ml).

Example 13: Affinity maturation:

[00342] Affinity maturation was carried out for XPA.10.064 to optimize affinity. scFv libraries were generated by random mutagenesis of HCDR3 using standard molecular biology techniques (see, *e.g.*, Clackson & Lowman, Phage Display – A Practical Approach (Oxford University Press, 2004)). HCDR3 was randomized in two blocks of five amino acids in order to cover the entire 10 amino acid CDR, resulting in libraries H3B1 (N-terminal five amino acid block of HCDR3) and H3B2 (C-terminal five amino acid block of HCDR3). Phage selections were performed on both libraries using techniques similar to those described in Example 1. The coating concentration of target antigen was reduced with each successive round of panning.

[00343] Five scFv clones exhibiting improved k_{off} rates for binding to $hVEGF_{165}$ versus the parental antibody were converted to IgGs using techniques similar to those described in Example 3. The VEGF binding affinity of these IgGs was tested on a Biacore 2000® by injecting the antibodies over a very low density antigen surface. Human or murine $VEGF_{165}$ was immobilized via standard amine coupling methods to flow cells on a C1 (planar carboxy surface) chip. Approximately 85 RU of each antigen was immobilized and a reference flow cell was activated and blocked. Antibodies were injected over the chip

surface for 400 seconds at two different concentrations, after which dissociation was monitored. Data was analyzed by Scrubber® using double referencing and fitting with a 1:1 interaction. Regenerations were performed with 90mM HCl with 500mM NaCl.

[00344] The affinity matured clones XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 each bound $hVEGF_{165}$ with greater affinity than parental XPA.10.064 or Bevacizumab (Table 7). The HCDR3 amino sequence for XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 are set forth in SEQ ID NOs:15-19, respectively. The complete heavy chain sequences of XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 are set forth in SEQ ID NOs:20-24, respectively. The light chain sequences of XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 are identical to those of XPA.10.064 and XPA.10.072 (SEQ ID NO:5). All five of these affinity matured antibodies contain a methionine residue in HCDR3.

Table 7: Binding affinity of parental and affinity matured XPA.10.064 to $hVEGF_{165}$:

IgG2 clone	k_a (1/Ms)	k_d (1/s)	K_D
Bevacizumab	1.92×10^5	8.08×10^{-5}	4.25×10^{-10} M (425 pM)
XPA.10.064	1.93×10^5	3.35×10^{-4}	1.71×10^{-9} M (1.71 nM)
XPA.10.064.03	1.96×10^5	1.80×10^{-5}	9.17×10^{-11} M (92 pM)
XPA.10.064.04	1.50×10^5	2.94×10^{-5}	1.966×10^{-10} M (197 pM)
XPA.10.064.06	1.78×10^5	2.07×10^{-5}	1.21×10^{-10} M (121 pM)
XPA.10.064.07	2.07×10^5	6.65×10^{-6}	3.49×10^{-11} M

			(35 pM)
XPA.10.064.10	2.16 x 10^{5}	1.16 x 10^{-5}	5.56 x 10^{-11} M (56 pM)

[00345] Parental XPA.10.064 and the affinity matured clones XPA.10.064.06 and XPA.10.064.07 each exhibited only weak binding (> 100 nM) binding to $mVEGF_{165}$ (Table 8).

Table 8: Binding affinity of parental and affinity matured XPA.064 to $mVEGF_{165}$:

IgG2 clone	**K_D**
Bevacizumab	No binding
XPA.10.064	197 nM
XPA.10.064.06	208 nM
XPA.10.064.07	130 nM

Example 14: Inhibition of HUVEC proliferation by affinity matured XPA.10.064:

[00346] Affinity matured XPA.10.064 IgG2s were tested for their ability to block proliferation of HUVECs using a protocol similar to that described above in Example 9. Titrations of XPA.10.064, XPA.10.064.03, XPA.10.064.06, XPA.10.064.07, XPA.10.064.10, and Bevacizumab were generated, and the antibodies were pre-incubated with $hVEGF_{165}$ for two hours. After pre-incubation, the VEGF/antibody complex was added to the suspended HUVEC cells. The experiment was repeated four times for each antibody.

[00347] Individual results for each antibody are set forth in Figures 18-23, and the results are summarized in Figure 24. The affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 each inhibited HUVEC proliferation to a greater degree than parental XPA.10.064. The geometric mean IC_{50} for each antibody is set forth in Table 9. The P-values presented in Table 9 are based on a paired within experiment one-tailed t-test on log(IC_{50}) for each antibody relative to that of

Bevacizumab. In this analysis, the affinity matured XPA.10.064.03, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 antibodies were not statistically different from one another.

Table 9: Affinity matured XPA.10.064 HUVEC assay summary:

Antibody	Geometric mean IC_{50} (SE)	P-value (power to detect 2X change in IC_{50})
Bevacizumab	275 pM (29)	---
XPA.10.064	1320 pM (984)	--- (10%)
XPA.10.064.03	174 pM (29)	0.0394 (90%)
XPA.10.064.06	173 pM (22)	0.0132 (98%)
XPA.10.064.07	129 pM (40)	0.0525 (40%)
XPA.10.064.10	139 pM (38)	0.04625 (50%)

Example 15: Inhibition of tumor growth by XPA.10.064.06:

[00348] The ability of XPA.10.064.06 to inhibit tumor growth was tested using the same A673 Rhabdomyosarcoma tumor growth model discussed above in Example 12. $2.5x10^6$ tumor cells were injected s.c. in 50% Matrigel® into the midline thoracic vertebral region of six-week old nude mice starting at day 0. At day 3, when tumor size had reached about 200 mm^3, mice were randomized into seven groups of 20 mice and injected i.p. twice a week with isotype control anti-KLH IgG2, XPA.10.064.06 at 0.1, 0.5, or 5 mg/kg, or Bevacizumab at 0.1, 0.5, or 5 mg/kg. Tumor size was measured on days 3, 7, 10, 14, 17, 21, 24, 28, and 31. Mice were sacrificed if and when tumor size reached 2000 mm^3. Tumors and serum samples were collected following the final tumor measurement for each mouse.

[00349] XPA.10.064.06 and Bevacizumab both inhibited tumor growth in a dose-dependent manner (Figures 25-27). Treatment with 0.5 mg/kg XPA.10.064.06 resulted in tumor regression through day 17, with tumor size remaining below 200 mm^3 (Figures 25-27). Mice treated with 0.1 or 0.5 mg/kg XPA.10.064.06 exhibited significantly smaller tumors at days 17, 21, 24, 28, and 31 than mice treated with Bevacizumab at the same dosages (Figures 25-27). Three mice treated with Bevacizumab at 0.1 mg/kg and three treated with control antibody had to be sacrificed on day 24 because they had tumor volumes exceeding 2000 mm^3, whereas none of the mice treated with XPA.10.064.06 reached this threshold over the course of the study.

[00350] The percent tumor growth inhibition (%TGI) at day 24 (the last measurement day on which all animals were alive) in mice treated with 0.1 mg/kg XPA.10.064.06 (55%) was similar to that obtained for Bevacizumab at five times the dosage (50% at 0.5 mg/kg). Similarly, the %TGI in mice treated with XPA.10.064.06 at 0.5 mg/kg (85%) was similar to that observed in mice treated with ten times the dosage of Bevacizumab (95% at 5 mg/kg). These results show that XPA.10.064.06 is at least five times more effective at reducing tumor growth *in vivo* than Bevacizumab. Percent tumor growth inhibition results for each antibody at day 24 are summarized in Table 10.

Table 10: Inhibition of tumor growth by XPA.10.064.06

Antibody comparison	% tumor growth inhibition	P-value (determined by Anova followed by Tukey test)
Control vs. BM-1 (0.1 mg/kg)	No effect	--
Control vs. XPA.10.064.06 (0.1 mg/kg)	55%	p<0.01
Control vs. BM-1 (0.5 mg/kg)	50%	p<0.01
Control vs. XPA.10.064.06 (0.5 mg/kg)	85%	p<0.01
Control vs. BM-1 (5 mg/kg)	95%	p<0.01
Control vs. XPA.10.064.06 (5 mg/kg)	90%	p<0.01

[00351] Tumor growth delay by each antibody was calculated as the difference in the number of days it took for a tumor to reach 500 mm^3 following treatment with the antibody versus control. Tumor growth delay was significantly longer for XPA.10.064.06 than for BM-1 at the 0.5 mg/kg dosage (14 days versus 5 days, respectively) and at the 0.1 mg/kg dosage (7 days versus 2 days, respectively). These results show that XPA.10.064.06 delays tumor growth to a specified size for a duration at least two to three times longer than Bevacizumab when the antibodies are administered at the same dosage. Results are summarized in Table 11.

Table 11: Tumor growth delay by XPA.10.064.06

Antibody (dosage)	Tumor growth delay (days)
Control huIgG (0.5 mg/kg)	0
XPA.10.064.06 (0.1 mg/kg)	7
BM-1 (0.1 mg/kg)	2
XPA.10.064.06 (0.5 mg/kg)	14
BM-1 (0.5 mg/kg)	5

[00352] Area under the curve (AUC) calculations based on tumor volume versus time were performed at day 28 for each antibody/dosage. The AUC for BM-1 at 0.5 mg/kg was significantly higher than that of XPA.10.064.06 at the same dosage (8865 versus 5517, respectively). Although the difference was less marked, the AUC for BM-1 was also higher than that of XPA.10.064.06 at 0.1 mg/kg (9638 versus 8703, respectively). Results are summarized in Table 12.

Table 12: Area under the curve for XPA.10.064

Antibody (dosage)	**AUC (tumor volume x days)**
Control huIgG (0.5 mg/kg)	11015
XPA.10.064.06 (0.1 mg/kg)	8703
BM-1 (0.1 mg/kg)	9638
XPA.10.064.06 (0.5 mg/kg)	5517
BM-1 (0.5 mg/kg)	8865
XPA.10.064.06 (5 mg/kg)	5069
BM-1 (5 mg/kg)	4418

[00353] The ability of XPA.10.064.06 to inhibit tumor growth was further tested in the HT-29 human colorectal adenocarcinoma model. Preliminary results indicated that twice-weekly administration of XPA.10.064.06 at 0.1, 0.5, or 5 mg/kg did not result in a statistically significant reduction in tumor size over 35 days. Similar results were obtained with Bevacizumab at the same concentrations.

Example 16: Inhibition of affinity-matured antibody oxidation:

[00354] As discussed above in Example 13, the affinity matured antibodies XPA.10.064.03, XPA.10.064.04, XPA.10.064.06, XPA.10.064.07, and XPA.10.064.10 each contain a methionine residue in HCDR3 (specifically, at residue 101) that is not present in the parental antibody. Oxidation of methionine residues is a common degradation pathway for protein products during storage.

[00355] To test the effect of methionine oxidation on the affinity matured antibodies, XPA.10.064.06 and parental XPA.10.064 were both exposed to the oxidizing agent tert-butylhydroperoxide (tBHP). The degree of resultant oxidation at each methionine residue was analyzed by tryptic mapping followed by liquid chromatography-mass spectrometry (LC/MS). Results are summarized in Table 13. Both antibodies exhibited oxidation at methionine residues 48 and 81, and no measurable oxidation at methionine residue 70. The extra methionine residue in XPA.10.064.06 at position 101 exhibited a significant degree of oxidation.

Table 13: Oxidation of methionine residues in XPA.10.064 and XPA.10.064.06

	Degree of oxidation (%)	
Methionine residue	**XPA.10.064**	**XPA.10.064.06**
Met-48	39	20
Met-70	0	0
Met-81	28	14
Met-101	N/A	66

[00356] The binding kinetics of the oxidized antibodies for $hVEGF_{165}$ were analyzed by Biacore. Oxidation of XPA.10.064 had little effect on $hVEGF_{165}$ binding kinetics (Figure 28A), while oxidation of XPA.10.064.06 resulted in a significant decrease in *in vitro* VEGF binding affinity (Figure 28B).

[00357] To overcome issues related to oxidation and increase stability of the affinity matured antibodies for long-term storage, formulations comprising XPA.10.064.06 plus an antioxidant agent were tested. XPA.10.064 and XPA.10.064.06 at 1 mg/ml in 10 mM L-histidine and 140 mM L-arginine (pH 6.0) were subjected to oxidation via chemical or thermal stress. For chemical stress, the antibodies were incubated overnight at room temperature with 0.1% tBHP in the presence or absence of 5 mM L-methionine. For thermal stress, the antibodies were incubated in polysorbate for four weeks at 40°C in the presence

or absence of 5 mM L-methionine. The ability of the antibodies to bind $hVEGF_{165}$ was analyzed by Biacore and ELISA. The presence of methionine in the antibody formulation conserved $hVEGF_{165}$ binding affinity in the presence of thermal stress (Figures 29A, 29B, and 30A), and reduced the negative effects on binding affinity of chemical stress (Figure 30B). Results are summarized in Table 14.

Table 14:

	Binding affinity (% control)	
Sample	**Biacore**	**ELISA**
Thermal stress w/o Met	36%	42%
Thermal stress w/ Met	100%	95%
Chemical stress w/o Met	14%	13%
Chemical stress w/ Met	28%	31%

Example 17: Further affinity maturation of XPA.10.064:

[00358] Additional mutations may be introduced into the heavy chain variable region of XPA.10.064. Specifically, the methionine residue in XPA.10.064.06 HCDR3 may be mutated to one or more of alanine, lysine, proline, threonine, and leucine. The HCDR3 amino sequences of these mutants are set forth in SEQ ID NOs:25-29 respectively. Binding analysis will be conducted on antibodies containing the resulting HCDR3 sequences to determine binding affinity for $hVEGF_{165}$. Antibodies exhibiting high affinity for $hVEGF_{165}$ and an off rate faster than 10^{-5} will be reformatted to IgGs and subjected to functional analysis to determine their ability to inhibit HUVEC proliferation, angiogenesis, tumor growth, and/or $hVEGF_{165}$-induced phosphorylation of VEGF-R2.

Example 18: Expression of XPA.10.064.06:

[00359] A first vector was constructed for expressing XPA.10.064.06 IgG_2 in CHO-K1 cells. This vector, pMXSP117, contains XPA.10.064.06 heavy and light

chain variable regions fused to the Gamma-2 and Kappa constant regions, respectively, with each region under the control of the human CMV promoter and the mouse light chain 3' untranslated region. The vector contains the *neo* gene for selection of G418-resistant transfectants. The structure of pMXSP117 in circular and linearized form is set forth in Figures 31A and 31B, respectively. Prior to construction of pMXSP117, the XPA.10.064.06 heavy and light chain variable regions were cloned into modular expression vectors containing the heavy chain (Gamma-2) and light chain (Kappa) constant regions as shown in Figures 32A and 32B, respectively. The heavy chain variable region containing an antibody signal sequence was cloned as a SalI/BlpI fragment into the heavy chain modular vector. The light chain variable region containing an antibody signal sequence was cloned as a SalI/BsiWI fragment into the light chain modular vector.

[00360] A second vector, pMXSP119, which had the same structure as pMXSP117 but with the *his*D gene (histidinol dehydrogenase) encoding resistance to histidinol in place of the *neo* gene, was constructed in two steps. The first step involved combining the XPA.10.064.006 heavy chain transcriptional unit with a vector segment containing the *his*D gene to generate the vector pMXSP118. The second step involved combining a restriction fragment consisting of the XPA.10.064.06 light chain transcriptional unit with a vector segment from pMXSP118 containing the XPA.10.064.06 heavy chain transcriptional unit and the *his*D gene to generate pMXSP119.

[00361] CHO-K1 cell lines were developed which express XPA.10.064.06 IgG_2. Prior to transfection, cells from a research cell bank of untransfected cells were thawed and grown in EX-CELL® 302 serum-free medium (SAFC

Biosciences, Lenexa, KS). The genes encoding the light and heavy chains of XPA.10.064.06 were introduced into the animal product-free medium-adapted CHO-K1 cells by transfection using linear polyethylenimine (PEI; 25,000 MW; Polysciences, Warrington, PA) with pMXSP117 that had been linearized by digestion with *Xba*I (Figure 31B). After incubation for three days in an animal product-free medium, the cells were suspended in EX-CELL® 302 medium supplemented with 0.8 mg/ml Geneticin® and 1% fetal bovine serum (FBS) and plated into 96-well plates. Wells containing single colonies were transferred to EX-CELL® 302 medium without FBS in deep well 96-well plates. After an additional screen by ELISA, the top producing clones were tested for expression in 50 ml vented tubes containing 10 ml of culture and 125 ml shake flasks containing 25 ml of culture.

[00362] Several of the top producers from this initial transfection with pMXSP117, selected based on their production capabilities, were adapted to a production medium for the purpose of producing XPA.10.064.06 IgG_2. To further increase production levels, the top Geneticin-resistant producer will be re-transfected with an additional vector expressing the same heavy and light chain sequences, but with a different selectable marker (*e.g.*, the vector, pMXSP119 encoding resistance to histidinol). The pMXSP119 vector will be linearized by digestion with *Xba*I. After incubation for three days in an animal product-free medium, the cells are suspended in EX-CELL® 302 medium supplemented with 0.4 mg/ml Geneticin, 8 mM histidinol and 1% FBS and plated into 96-well plates. Wells containing single colonies identified as high producers by ELISA will be transferred to EX-CELL® 302 medium without FBS in deep well 96-well plates. After an additional screen by ELISA, the top producing clones will be tested for

expression in shake flasks. Several of the top producers from this re-transfection, selected based on their growth and production capabilities, will be adapted to an animal product-free production medium for the purpose of evaluating growth and production in shake flask cultures and bioreactors. Research cell banks will be prepared for these top clones that have been adapted to a production medium. Based on the results of these tests, one clone will be chosen for preparation of a Master Cell Bank (MCB) which will serve as the starting point for manufacture of XPA.10.064.06 IgG_2 under Good Manufacturing Practice (GMP).

Example 19: Purification of XPA.10.064.06:

[00363] Cells expressing XPA.10.064.06 or fragments thereof, such as the CHO-K1 cells described in Example 18, may be purified using the following procedure. After completion of fermentation, the cell culture is clarified and harvested by filtration through a filtration train comprising a depth filter (CUNO, Meriden, CT) followed by a charged membrane filter and then a sterile 0.2 µm filter. The cell-free clarified culture fluid is loaded onto a Protein A affinity column, which is then washed with equilibration buffer. The antibody is eluted with a low pH buffer in the pH 3-4 range, then subjected to viral inactivation at pH 3.8 +/- 0.2 for a maximum of 60 minutes. The viral-inactivated pool is adjusted for pH and conductivity, then loaded onto an anion exchange column in flow-through mode, whereby the antibody flows through the column while impurities bind. Flow-through from the anion exchange column is further purified over a hydrophobic interaction chromatography (HIC) column, which removes impurities such as aggregates, DNA, and host cell proteins, followed by filtration through a nano filter such as a Viresolve® Normal Flow Parvovirus (NFP) filter (Millipore) to

remove viral particles. Using ultrafiltration and diafiltration (UF/DF), the nano filtered antibody pool is formulated to a target concentration and buffer exchanged into formulation buffer to yield bulk drug substance (BDS).

[00364] The foregoing is merely intended to illustrate various embodiments of the present invention. The specific modifications discussed above are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein. All references cited herein are incorporated by reference in their entirety as if fully set forth herein.

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What is claimed is:

1. An isolated antibody or antigen-binding fragment thereof comprising one or more members selected from the group consisting of:

a) a heavy chain variable region comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8;

b) a light chain variable region comprising SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11;

c) a light chain variable region comprising SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14;

d) a heavy chain variable region comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:15;

e) a heavy chain variable region comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:16;

f) a heavy chain variable region comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:17;

g) a heavy chain variable region comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:18;

h) a heavy chain variable region comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:19;

i) a light chain comprising LCDR1, LCDR2, and LCDR3 of the XPA.10.072 light chain as set forth in SEQ ID NO:3;

j) a light chain comprising LCDR1, LCDR2, and LCDR3 of the XPA.10.064 light chain as set forth in SEQ ID NO:5;

k) a heavy chain comprising HCDR1, HCDR2, and HCDR3 of the XPA.10.072 heavy chain as set forth in SEQ ID NO:2;

l) a heavy chain comprising HCDR1, HCDR2, and HCDR3 of the XPA.10.064 heavy chain as set forth in SEQ ID NO:4;

m) a heavy chain comprising HCDR1, HCDR2, and HCDR3 of the XPA.10.064.03 heavy chain as set forth in SEQ ID NO:20;

n) a heavy chain comprising HCDR1, HCDR2, and HCDR3 of the XPA.10.064.04 heavy chain as set forth in SEQ ID NO:21;

o) a heavy chain comprising HCDR1, HCDR2, and HCDR3 of the XPA.10.064.06 heavy chain as set forth in SEQ ID NO:22;

p) a heavy chain comprising HCDR1, HCDR2, and HCDR3 of the XPA.10.064.07 heavy chain as set forth in SEQ ID NO:23; and

q) a heavy chain comprising HCDR1, HCDR2, and HCDR3 of the XPA.10.064.10 heavy chain as set forth in SEQ ID NO:24.

2. An isolated antibody or antigen-binding fragment thereof comprising one or more members selected from the group consisting of:

a) a heavy chain comprising SEQ ID NO:2;

b) a light chain comprising SEQ ID NO:3;

c) a heavy chain comprising SEQ ID NO:4;

d) a light chain comprising SEQ ID NO:5;

e) a heavy chain comprising SEQ ID NO:20;

f) a heavy chain comprising SEQ ID NO:21;

g) a heavy chain comprising SEQ ID NO:22;

h) a heavy chain comprising SEQ ID NO:23; and

i) a heavy chain comprising SEQ ID NO:24.

3. An isolated antibody or antigen-binding fragment thereof that specifically binds VEGF and comprises one or more of the following properties:

a) K_D for binding to human $VEGF_{165}$ of about 10^{-10} M;

b) k_a for binding to human $VEGF_{165}$ of about 1.89 X 10^5;

c) k_d for binding to human $VEGF_{165}$ of about 1.73 X 10^{-5};

d) binding affinity for human $VEGF_{165}$ that is about 4.25 times greater than the binding affinity of Bevacizumab for human $VEGF_{165}$;

e) inhibits HUVEC cell proliferation with an IC_{50} of about 154 pM;

f) inhibits HUVEC cell proliferation with an IC_{50} about 56% that of Bevacizumab;

g) K_D for binding to human $VEGF_{165}$ in the range of about 1.97 X 10^{-10} M to about 3.49 X 10^{-11} M;

h) k_a for binding to human $VEGF_{165}$ in the range of about 1.5 X 10^5 to about 2.16 X 10^5 M;

i) k_d for binding to human $VEGF_{165}$ in the range of about 6.65 X 10^{-6} to about 2.94 X 10^{-5} M;

j) inhibits HUVEC cell proliferation with an IC_{50} in the range of about 129 pM to about 174 pM;

k) binding affinity for human VEGF that is at least about 10 times greater than the affinity of the antibody or antigen-binding fragment thereof for murine VEGF;

l) competes with Bevacizumab for binding to human $VEGF_{165}$;

m) inhibits tumor growth as a function of percent tumor growth inhibition to the same or a greater degree than Bevacizumab when administered at the same or a lower dosage; and

n) delays tumor growth to a specified size for a greater duration than Bevacizumab when administered at the same or a lower dosage as Bevacizumab.

4. An isolated antibody or antigen-binding fragment thereof comprising:

a) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:20; and

b) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5.

5. An isolated antibody or antigen-binding fragment thereof comprising:

a) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:21; and

b) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5.

6. An isolated antibody or antigen-binding fragment thereof comprising:

a) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22; and

b) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5.

7. An isolated antibody or antigen-binding fragment thereof comprising:

a) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:23; and

b) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5.

8. An isolated antibody or antigen-binding fragment thereof comprising:

a) a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:24; and

b) a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5.

9. The antibody or antigen-binding fragment thereof as recited in any of claims 1-8, which is a monoclonal antibody.

10. The antibody or antigen-binding fragment thereof as recited in any of claims 1-8, which is a polyclonal antibody, a bispecific antibody, a chimeric antibody, a humanized antibody, a recombinant antibody, fully human antibody, a labeled antibody, a bivalent antibody, or an anti-idiotypic antibody.

11. The antibody or antigen-binding fragment thereof as recited in any of claims 1-8, which is a camelized single domain antibody, a diabody, a scFv, an scFv dimer, a dsFv, a $(dsFv)_2$, a dsFv-dsFv', an Fv fragment, a Fab, a Fab', a $F(ab')_2$, a ds diabody, a nanobody, a domain antibody, or a bivalent domain antibody.

12. The antibody or antigen-binding fragment thereof as recited in any of claims 1-8, further comprising an immunoglobulin constant region.

13. The antibody or antigen-binding fragment thereof of claim 12, wherein said immunoglobulin constant region is a κ light chain, γ1 heavy chain, γ2 heavy chain, γ3 heavy chain, or γ4 heavy chain constant region.

14. The antibody or antigen-binding fragment thereof as recited in any of claims 1-13, wherein said antibody or antigen-binding fragment inhibits tumor growth as a function of percent tumor growth inhibition to the same or a greater degree than Bevacizumab when administered at a two-fold lower dosage than Bevacizumab.

15. The antibody or antigen-binding fragment of claim 14, wherein said antibody or antigen-binding fragment inhibits tumor growth as a function of percent tumor growth inhibition to the same or a greater degree than Bevacizumab when administered at a three-fold lower dosage than Bevacizumab.

16. The antibody or antigen-binding fragment of claim 15, wherein said antibody or antigen-binding fragment inhibits tumor growth as a function of percent tumor growth inhibition to the same or a greater degree than Bevacizumab when administered at a four-fold lower dosage than Bevacizumab.

17. The antibody or antigen-binding fragment of claim 16, wherein said antibody or antigen-binding fragment inhibits tumor growth as a function of percent tumor growth inhibition to the same or a greater degree than Bevacizumab when administered at a five-fold lower dosage than Bevacizumab.

18. The antibody or antigen-binding fragment thereof as recited in any of claims 1-13, wherein said antibody or antigen-binding fragment inhibits tumor growth to a specified size for at least twice the duration of Bevacizumab when administered at the same or a lower dosage than Bevacizumab.

19. The antibody or antigen-binding fragment thereof as recited in any of claims 1-13, wherein said antibody or antigen-binding fragment inhibits tumor

growth to a specified size for at least three times the duration of Bevacizumab when administered at the same or a lower dosage than Bevacizumab.

20. The antibody or antigen-binding fragment thereof as recited in any of claims 1-13, wherein said antibody or antigen-binding fragment binds human $VEGF_{165}$ with a K_D of ≤ 200 pM.

21. The antibody or antigen-binding fragment thereof as recited in any of claims 1-13, wherein said antibody or antigen-binding fragment binds to an epitope on human $VEGF_{165}$ that overlaps at least partially with the epitope bound by Bevacizumab.

22. The antibody or antigen-binding fragment thereof as recited in any of claims 1-13, wherein said antibody or antigen-binding fragment blocks binding of human $VEGF_{165}$ to VEGF-R1 and VEGF-R2.

23. The antibody or antigen-binding fragment thereof as recited in any of claims 1-13, wherein said antibody or antigen-binding fragment binds human $VEGF_{165}$ with a K_D that is at least 10-fold lower in number than the K_D of said antibody or antigen-binding fragment for binding to murine $VEGF_{165}$.

24. The antibody or antigen-binding fragment thereof of claim 23, wherein said antibody or antigen-binding fragment binds human $VEGF_{165}$ with a K_D that is at least 50-fold lower in number than the K_D of said antibody or antigen-binding fragment for binding to murine $VEGF_{165}$.

25. The antibody or antigen-binding fragment thereof of claim 24, wherein said antibody or antigen-binding fragment binds human $VEGF_{165}$ with a K_D that is at least 100-fold lower in number than the K_D of said antibody or antigen-binding fragment for binding to murine $VEGF_{165}$.

26. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof as recited in any of claims 1-25 and one or more physiologically tolerable components.

27. The pharmaceutical composition of claim 26, wherein said one or more physiologically tolerable components comprise one or more pharmaceutically acceptable carriers.

28. The pharmaceutical composition of claim 27, wherein said one or more physiologically tolerable components comprise one or more antioxidants.

29. The pharmaceutical composition of claim 28, wherein said one or more antioxidants are selected from the group consisting of methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxanisol, butylated hydroxytoluene, and/or propyl gallate.

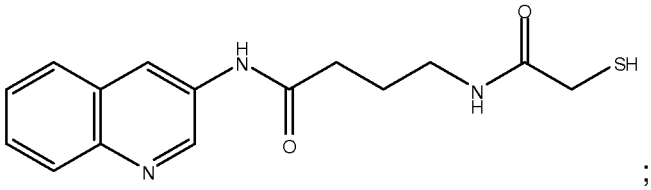
30. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof as recited in any of claims 1-25 linked to or in combination with one or more additional chemotherapeutic agents.

31. The composition of claim 30, wherein said additional chemotherapeutic agents are selected from the group consisting of everolimus, trabectedin, abraxane, TLK 286, AV-299, DN-101, pazopanib, GSK690693, RTA 744, ON 0910.Na, AZD 6244 (ARRY-142886), AMN-107, TKI-258, GSK461364, AZD 1152, enzastaurin, vandetanib, ARQ-197, MK-0457, MLN8054, PHA-739358, R-763, a FLT-3 inhibitor, a VEGF-R inhibitor, an EGFR TK inhibitor, an aurora kinase inhibitor, a PIK-1 modulator, a Bcl-2 inhibitor, an HDAC inhibitor, a c-MET inhibitor, a PARP inhibitor, a Cdk inhibitor, an EGFR TK inhibitor, an IGFR-TK inhibitor, an anti-HGF antibody, a PI3 kinase inhibitors, an AKT

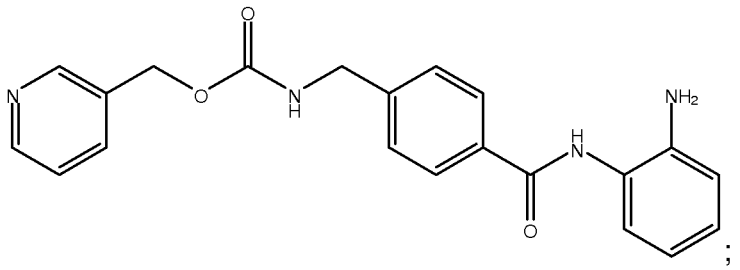
inhibitor, a JAK/STAT inhibitor, a checkpoint-1 or 2 inhibitor, a focal adhesion kinase inhibitor, a MAP kinase kinase (MEK) inhibitor, a VEGF trap antibody, pemetrexed, erlotinib, dasatinib, decatanib, panitumumab, amrubicin, oregovomab, Lep-etu, nolatrexed, azd2171, batabulin, ofatumumab, zanolimumab, edotecarin, tetrandrine, rubitecan, tesmilifene, oblimersen, ticilimumab, ipilimumab, gossypol, gossypol acetate, Bio 111, 131-I-TM-601, ALT-110, BIO 140, CC 8490, cilengitide, gimatecan, IL13-PE38QQR, INO 1001, IPdR, KRX-0402, lucanthone, LY 317615, neuradiab, vitespan, Rta 744, Sdx 102, talampanel, atrasentan, Xr 311, romidepsin, ADS-100380, CG-781, CG-1521, SB-556629, chlamydocin, JNJ-16241199, vorinostat, etoposide, gemcitabine, doxorubicin, liposomal doxorubicin, 5'-deoxy-5-fluorouridine, vincristine, temozolomide, ZK-304709, seliciclib; PD0325901, AZD-6244, capecitabine, L-Glutamic acid, N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate, camptothecin, irinotecan; a combination of irinotecan, 5-fluorouracil and leucovorin, PEG-labeled irinotecan, FOLFOX regimen, tamoxifen, toremifene citrate, anastrazole, exemestane, letrozole, DES(diethylstilbestrol), estradiol, estrogen, conjugated estrogen, Bevacizumab, an isolated antibody that binds specifically to human IGF1R, IMC-1C11, CHIR-258, 3-[5-(methylsulfonylpiperadinemethyl)-indolyl]-quinolone, vatalanib, AG-013736, AVE-0005, goserelin acetate, leuprolide acetate, triptorelin pamoate, sunitinib, sunitinib malate, medroxyprogesterone acetate, hydroxyprogesterone caproate, megestrol acetate, raloxifene, bicalutamide, flutamide, nilutamide, megestrol acetate, CP-724714; TAK-165, HKI-272, erlotinib, lapatanib, canertinib, ABX-EGF antibody, erbitux, EKB-569, PKI-166, GW-572016, lonafarnib, BMS-214662, tipifarnib; amifostine, NVP-LAQ824,

suberoyl analide hydroxamic acid, valproic acid, trichostatin A, FK-228, SU11248, sorafenib, KRN951, aminoglutethimide, amsacrine, anagrelide, L-asparaginase, Bacillus Calmette-Guerin (BCG) vaccine, bleomycin, buserelin, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, diethylstilbestrol, epirubicin, fludarabine, fludrocortisone, fluoxymesterone, flutamide, hydroxyurea, idarubicin, ifosfamide, imatinib, leucovorin, leuprolide, levamisole, lomustine, mechlorethamine, melphalan, 6-mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, octreotide, oxaliplatin, pamidronate, pentostatin, plicamycin, porfimer, procarbazine, raltitrexed, rituximab, streptozocin, teniposide, testosterone, thalidomide, thalidomide combined with dexamethasone, thioguanine, thiotepa, tretinoin, vindesine, 13-cis-retinoic acid, phenylalanine mustard, uracil mustard, estramustine, altretamine, floxuridine, 5-deooxyuridine, cytosine arabinoside, 6-mecaptopurine, deoxycoformycin, calcitriol, valrubicin, mithramycin, vinblastine, vinorelbine, topotecan, razoxin, marimastat, COL-3, neovastat, BMS-275291, squalamine, endostatin, SU5416, SU6668, EMD121974, interleukin-12, IM862, angiostatin, vitaxin, droloxifene, idoxyfene, spironolactone, finasteride, cimitidine, trastuzumab, denileukin diftitox, gefitinib, bortezimib, paclitaxel, cremophor-free paclitaxel, docetaxel, epithilone B, BMS-247550, BMS-310705, droloxifene, 4-hydroxytamoxifen, pipendoxifene, ERA-923, arzoxifene, fulvestrant, acolbifene, lasofoxifene, idoxifene, TSE-424, HMR-3339, ZK186619, topotecan, PTK787/ZK 222584, VX-745, PD 184352, rapamycin, 40-O-(2-hydroxyethyl)-rapamycin, temsirolimus, AP-23573, RAD001, ABT-578, BC-210, LY294002, LY292223, LY292696, LY293684, LY293646, wortmannin, ZM336372, L-779,450, PEG-

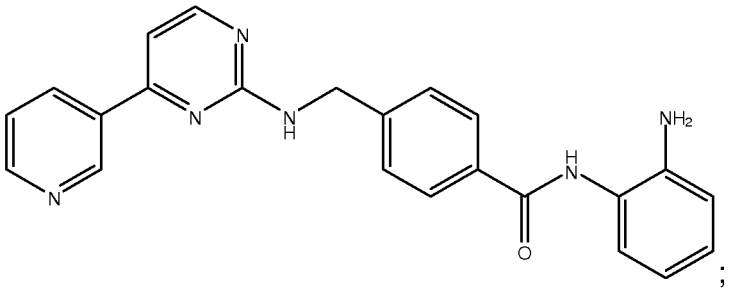
filgrastim, darbepoetin, 5-fluorouracil, erythropoietin, granulocyte colony-stimulating factor, zolendronate, prednisone, cetuximab, granulocyte macrophage colony-stimulating factor, histrelin, pegylated interferon alfa-2a, interferon alfa-2a, pegylated interferon alfa-2b, interferon alfa-2b, azacitidine, PEG-L-asparaginase, lenalidomide, gemtuzumab, hydrocortisone, interleukin-11, dexrazoxane, alemtuzumab, all-transretinoic acid, ketoconazole, interleukin-2, megestrol, nitrogen mustard, methylprednisolone, ibritgumomab tiuxetan, androgens, decitabine, hexamethylmelamine, bexarotene, tositumomab, arsenic trioxide, cortisone, editronate, mitotane, cyclosporine, liposomal daunorubicin, Edwina-asparaginase, strontium 89, casopitant, netupitant, an NK-1 receptor antagonists, palonosetron, aprepitant, , diphenhydramine, hydroxyzine, metoclopramide, lorazepam, alprazolam, haloperidol, droperidol, dronabinol, dexamethasone, methylprednisolone, prochlorperazine, granisetron, ondansetron, dolasetron, tropisetron, pegfilgrastim, erythropoietin, epoetin alfa and darbepoetin alfa,

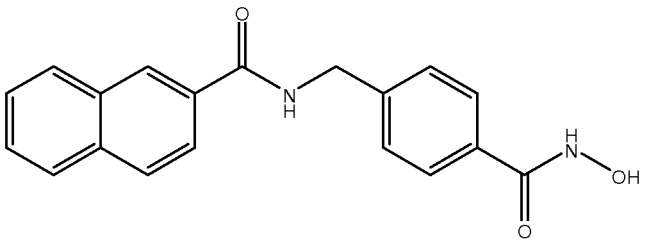


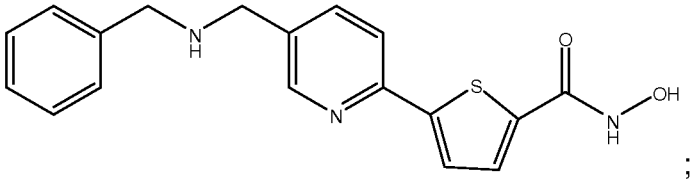
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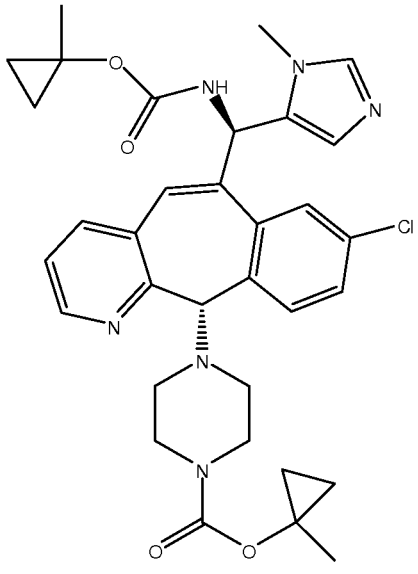


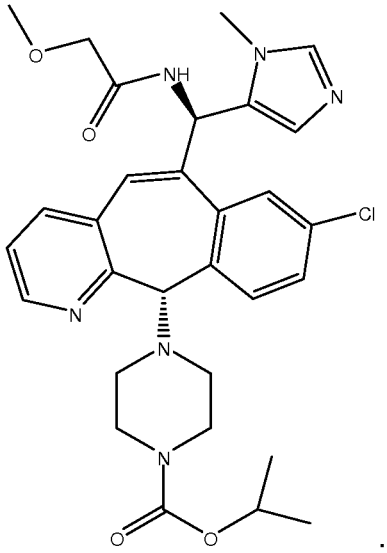
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32. A complex comprising an antibody or antigen-binding fragment thereof as recited in any of claims 1-25 bound to human VEGF or a fragment of human VEGF.

33. An isolated polypeptide comprising one or more amino acid sequences selected from the group consisting of SEQ ID NOs:2-5 and 20-24.

34. An isolated polynucleotide encoding the polypeptide of claim 33.

35. An isolated vector comprising the polynucleotide of claim 34.

36. An isolated host cell comprising the vector of claim 35.

37. A method of expressing a polypeptide comprising one or more amino acid sequences selected from the group consisting of SEQ ID NOs:2-5 and 20-24 comprising culturing the isolated host cell of claim 36 under conditions in which the polynucleotide of claim 34 is expressed.

38. The method of claim 37, wherein said polynucleotide is operably associated with a CMV promoter and wherein said host cell is a Chinese hamster ovary cell.

39. A method of inhibiting angiogenesis in a mammalian subject in need thereof comprising administering to said subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof as recited in any of claims 1-25, optionally linked to or in combination with one or more additional chemotherapeutic agents.

40. A method of treating a disease associated with aberrant angiogenesis in a mammalian subject in need thereof comprising administering to said subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof as recited in any of claims 1-25, optionally linked to or in combination with one or more additional chemotherapeutic agents.

41. A method of treating an inflammatory disease associated with VEGF signaling in a mammalian subject in need thereof comprising administering to said subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof as recited in any of claim 1-25, optionally linked to or in combination with one or more additional chemotherapeutic agents.

42. The method of claim 41, wherein said disease is selected from the group consisting of rheumatoid arthritis, psoriasis, scleroderma, chronic obstructive pulmonary disease, and asthma.

43. A method of treating wet acute macular degeneration or diabetic retinopathy in a mammalian subject in need thereof comprising administering to said subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof as recited in any of claims 1-25.

44. A method of treating a cancer associated with increased VEGF signaling in a mammalian subject in need thereof comprising administering to said subject a therapeutically effective amount of the antibody or antigen-binding

fragment thereof as recited in any of claims 1-25, optionally linked to or in combination with one or more additional chemotherapeutic agents.

45. The method of claim 44, wherein said cancer is selected from the group consisting of carcinoma, blastoma, sarcoma, germ cell tumor, hematological or lymphoid malignancy such as leukemia, lymphoma, or multiple myeloma, squamous cell cancer, lung cancer such as small cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, or squamous cell carcinoma of the lung, cancer of the peritoneum, liver cancer such as hepatocellular carcinoma/hepatoma, gastric or stomach cancer such as gastrointestinal cancer, pancreatic cancer, brain tumor such as glioblastoma/glioblastoma multiforme, non-glioblastoma brain tumor, or meningioma, glioma such as ependymoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, or mixed glioma such as oligoastrocytoma, cervical cancer, ovarian cancer, liver cancer such as hepatoblastoma, hepatocellular carcinoma/hepatoma, or hepatic carcinoma, bladder cancer such as urothelial cancer, breast cancer, colon cancer, colorectal cancer, rectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer such as rhabdoid tumor of the kidney, prostate cancer, vulval cancer, penile cancer, anal cancer such as anal squamous cell carcinoma, thyroid cancer, head and neck cancer such as nasopharyngeal cancer, skin cancer such as melanoma or squamous cell carcinoma, osteosarcoma, Ewing's sarcoma, chondrosarcoma, soft tissue sarcoma such as rhabdomyosarcoma, fibrosarcoma, Kaposi's sarcoma, carcinoid cancer, eye cancer such as retinoblastoma, mesothelioma, lymphocytic/lymphoblastic leukemia such as acute lymphocytic/lymphoblastic leukemia, chronic lymphoblastic/lymphocytic leukemia, acute

myelogenous/myeloblastic leukemia, including mast cell leukemia, chronic myelogenous/myelocytic/myeloblastic leukemia, hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, chronic myelomonocytic leukemia, follicular lymphoma, diffuse large B cell lymphoma, mantle cell lymphoma, Burkitt's lymphoma, mycosis fungoides, Sezary syndrome, cutaneous T-cell lymphoma, mast cell neoplasm, medulloblastoma, nephroblastoma, solitary plasmacytoma, myelodysplastic syndrome, chronic and non-chronic myeloproliferative disorder, central nervous system tumor, pituitary adenoma, vestibular schwannoma, primitive neuroectodermal tumor, ependymoma, choroid plexus papilloma, polycythemia vera, thrombocythemia, and idiopathic myelofibrosis.

46. A kit comprising the antibody or antigen-binding fragment thereof as recited in any of claims 1-25 and instructions for the use of said antibody or antigen-binding fragment.

47. An isolated antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19.