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# DESCRIPTION

## FIELD OF THE INVENTION

**[0001]** The present invention generally relates to antibodies and other agents that bind VEGF, DLL4, or both VEGF and DLL4, particularly anti-VEGF/anti-DLL4 bispecific antibodies, as well as to methods of using the antibodies or other agents for the treatment of diseases such as cancer.

## BACKGROUND OF THE INVENTION

**[0002]** Angiogenesis plays an important role in the pathogenesis of a number of disorders, including solid tumors and metastasis. The production of new blood vessels is essential for providing oxygen and nutrients for the growth and spread of a tumor, and therefore angiogenesis is a good target for cancer therapeutics.

**[0003]** Angiogenesis involves a family of proteins acting as angiogenic activators, including vascular endothelial growth factor (VEGF-A), VEGF-B, VEGF-C, VEGF-E, and their respective receptors (VEGFR-1, VEGFR-2, and VEGFR-3). VEGF-A, also referred to as VEGF or vascular permeability factor (VPF), exists in several isoforms that arise from alternative splicing of mRNA of a single VEGF gene, with VEGF<sub>165</sub> being the most biologically relevant isoform.

**[0004]** Anti-VEGF antibodies have been shown to suppress the growth of tumor cells *in vitro* and *in vivo*. A humanized anti-VEGF monoclonal antibody, bevacizumab (AVASTIN) has been developed and approved in the United States as a cancer therapeutic.

**[0005]** The Notch signaling pathway is a universally conserved signal transduction system. It is involved in cell fate determination during development including embryonic pattern formation and post-embryonic tissue maintenance. In addition, Notch signaling has been identified as a critical factor in the maintenance of hematopoietic stem cells.

**[0006]** The Notch pathway has been linked to the pathogenesis of both hematologic and solid tumors and cancers. Numerous cellular functions and microenvironmental cues associated with tumorigenesis have been shown to be modulated by Notch pathway signaling, including cell proliferation, apoptosis, adhesion, and angiogenesis (Leong et al., 2006, Blood, 107:2223-2233). In addition, Notch receptors and/or Notch ligands have been shown to play potential oncogenic roles in a number of human cancers, including acute myelogenous leukemia, B cell chronic lymphocytic leukemia, Hodgkin lymphoma, multiple myeloma, T-cell acute lymphoblastic leukemia, brain cancer, breast cancer, cervical cancer, colon cancer, lung cancer, pancreatic cancer, prostate cancer, and skin cancer. (Leong et al., 2006, Blood, 107:2223-2233).

**[0007]** Delta-like 4 ligand (DLL4) is an important component of the Notch pathway and has been identified as a target for cancer therapy. DLL4 is a Notch ligand, characterized by an N-terminal

domain, a Delta/Serrate/Lag-2 (DSL) domain and tandem EGF-like repeats within the extracellular domain. It has been reported that DLL4 is induced by VEGF and that DLL4 may act as a negative feedback regulator for vascular proliferation.

**[0008]** Anti-DLL4 antibodies have been shown to enhance angiogenic sprouting and branching which leads to non-productive angiogenesis and decreased tumor growth (Noguera-Troise et al., 2006, *Nature*, 444:1032-1037). In addition, an anti-DLL4 antibody, 21M18, has been shown to inhibit tumor growth and reduce the frequency of cancer stem cells in xenograft tumor models (Hoey et al., 2009, *Cell Stem Cell*, 5:168-177; U.S. Patent No. 7,750,124 ; US2011/165162; WO2008042236) and in clinical trials (WO2012068098; Smith et al (2010) *European Journal of Cancer* 8 7 73).

**[0009]** Although there have been significant strides in development of monoclonal antibodies for use in cancer treatments, there is still great potential for further improvements. One class of antibody molecules with the promise of enhanced potency and/or reduced side effects (e.g., toxicity) is bispecific antibodies (WO2010129304; US2010/076178).

**[0010]** Early bispecific molecules were mainly generated using chemical cross-linking of two antibodies, or were hybrid hybridomas or "quadromas". One success of the quadroma format is triomabs, which are mouse/rat combinations that demonstrate a preferential species-specific heavy/light chain pairing. More recently, advances in antibody engineering have provided a wide variety of new antibody formats, including, but not limited to, tandem scFv (bi-scFv), diabodies, tandem diabodies (tetra-bodies), single chain diabodies, and dual variable domain antibodies.

**[0011]** It is one of the objectives of the present invention to provide improved molecules for cancer treatment, particularly bispecific antibodies that specifically bind human VEGF and human DLL4.

## SUMMARY OF THE INVENTION

**[0012]** The present invention is defined by the claims.

**[0013]** The present disclosure provides binding agents, such as antibodies, that bind VEGF, DLL4, or both VEGF and DLL4 (VEGF/DLL4-binding agents), as well as compositions, such as pharmaceutical compositions, comprising the binding agents. Binding agents that bind VEGF or DLL4, as well as at least one additional antigen or target, and pharmaceutical compositions of such binding agents, are also provided. In certain disclosure herein, the binding agents are novel polypeptides, such as antibodies, antibody fragments, and other polypeptides related to such antibodies. In certain disclosure herein, the binding agents are antibodies that specifically bind human VEGF. In some disclosure herein, the binding agents are antibodies that specifically bind human DLL4. In some disclosure herein, the binding agents are bispecific antibodies that specifically bind human VEGF and human DLL4. The disclosure further provides methods of inhibiting the growth of a tumor by administering the binding agents to a subject with a tumor. The disclosure further provides methods of treating cancer by administering the binding agents to a

subject in need thereof. In some disclosure herein, the methods of treating cancer or inhibiting tumor growth comprise targeting cancer stem cells with the binding agents. In certain disclosure herein, the methods comprise reducing the frequency of cancer stem cells in a tumor, reducing the number of cancer stem cells in a tumor, reducing the tumorigenicity of a tumor, and/or reducing the tumorigenicity of a tumor by reducing the number or frequency of cancer stem cells in the tumor.

**[0014]** The disclosure provides a binding agent, such as an antibody, that specifically binds human VEGF. In some disclosure herein, the binding agent inhibits binding of VEGF to at least one VEGF receptor. In some disclosure herein, the binding agent inhibits binding of VEGF to VEGFR-1 and/or VEGFR-2. In some disclosure herein, the binding agent modulates angiogenesis. In certain disclosure herein, the antibody or other binding agent further specifically binds to and/or inhibits human DLL4 in addition to human VEGF.

**[0015]** In some disclosure herein, the binding agent is an antibody which comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0016]** In certain disclosure herein, the binding agent is an antibody that comprises a heavy chain variable region having at least 80% sequence identity to SEQ ID NO:11; and/or a light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In certain disclosure herein, the binding agent comprises a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:11; and/or a light chain variable region having at least 90% sequence identity to SEQ ID NO:12. In certain disclosure herein, the binding agent comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:11; and/or a light chain variable region having at least 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the binding agent is an antibody that comprises a heavy chain variable region of SEQ ID NO:11; and/or a light chain variable region of SEQ ID NO:12.

**[0017]** In some disclosure herein, the binding agent is antibody 219R45, 219R45-MB-21M18, 219R45-MB-21R79, 219R45-MB-21R75, or 219R45-MB-21R83.

**[0018]** The disclosure also provides a binding agent, such as an antibody, that specifically binds human DLL4. In some disclosure herein the binding agent inhibits binding of DLL4 to at least one Notch receptor. In some disclosure herein the binding agent inhibits binding of DLL4 to Notch1, Notch2, Notch3, and/or Notch4. In some disclosure herein, the binding agent inhibits Notch signaling. In some disclosure herein, the binding agent promotes unproductive angiogenesis. In certain disclosure herein the antibody or other binding agent further specifically binds to and/or inhibits human VEGF in addition to human DLL4.

**[0019]** In some disclosure herein, the binding agent is an antibody that binds human DLL4 and comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13) or AYYIH (SEQ ID NO:79), a

heavy chain CDR2 comprising YIX<sub>1</sub>X<sub>2</sub>YX<sub>3</sub>X<sub>4</sub>ATNYNQKFKG (SEQ ID NO:80), wherein X<sub>1</sub> is serine or alanine, X<sub>2</sub> is serine, asparagine, or glycine, X<sub>3</sub> is asparagine or lysine, and X<sub>4</sub> is glycine, arginine, or aspartic acid, and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the antibody comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0020]** In certain disclosure herein, the binding agent is an antibody that comprises a heavy chain variable region having at least 90% or at least 95% sequence identity to SEQ ID NO:10; and/or a light chain variable region having at least 90% or at least 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the binding agent is an antibody that comprises a heavy chain variable region of SEQ ID NO:10; and a light chain variable region of SEQ ID NO:12.

**[0021]** In some disclosure herein, the binding agent is antibody 21R79 or antibody 219R45-MB-21R79.

**[0022]** In some disclosure herein, the binding agent is an antibody which comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising YIAGYKDATNYNQKFKG (SEQ ID NO:59), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0023]** In certain disclosure herein, the binding agent is an antibody that comprises a heavy chain variable region having at least 90% or at least 95% sequence identity to SEQ ID NO:58; and/or a light chain variable region having at least 90% or at least 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the binding agent is an antibody that comprises a heavy chain variable region of SEQ ID NO:58; and a light chain variable region of SEQ ID NO:12.

**[0024]** In some disclosure herein, the binding agent is antibody 21R75 or antibody 219R45-MB-21R75.

**[0025]** In some disclosure herein, the binding agent is an antibody which comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising YISNYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0026]** In certain disclosure herein, the binding agent is an antibody that comprises a heavy chain

variable region having at least 90% or at least 95% sequence identity to SEQ ID NO:64; and/or a light chain variable region having at least 90% or at least 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the binding agent is an antibody that comprises a heavy chain variable region of SEQ ID NO:64; and a light chain variable region of SEQ ID NO:12.

**[0027]** In some disclosure herein, the binding agent is antibody 21R83 or antibody 219R45-MB-21R83.

**[0028]** In certain disclosure herein, the binding agent is a bispecific antibody. An aspect of the invention provides a bispecific antibody that specifically binds both human VEGF and human DLL4 as set out in claim 1. In some disclosure herein, the bispecific antibody modulates angiogenesis. In certain disclosure herein, the bispecific antibody inhibits Notch signaling. In somedisclosure herein, the bispecific antibody modulates angiogenesis and inhibits N otch signaling. In some disclosure herein, the bispecific antibody reduces the number of frequency of cancer stem cells. In certain disclosure herein, the bispecific antibody comprises two identical light chains. In certain disclosure herein the bispecific antibody is an IgG antibody (e.g., IgG2).

**[0029]** In somedisclosure herein, the bispecific antibody comprises: a first antigen-binding site that specifically binds human VEGF, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19). In some disclosure herein, the bispecific antibody further comprises: a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In somedisclosure herein, the bispecific antibody comprises: a first antigen-binding site that specifically binds human VEGF, wherein the first antigen-binding site comprises (a) a heavy chain CDR1 comprising NYWMH (SEQ ID NO: 17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and (b) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0030]** In certain disclosure herein, the bispecific antibody comprises: a first antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising YIX<sub>1</sub>X<sub>2</sub>YX<sub>3</sub>X<sub>4</sub>ATNYNQKFKG (SEQ ID NO:80), wherein X<sub>1</sub> is serine or alanine, X<sub>2</sub> is serine, asparagine, or glycine, X<sub>3</sub> is asparagine or lysine, and X<sub>4</sub> is glycine, arginine,or aspartic acid, and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In somedisclosure herein, the bispecific antibody comprises: a first antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO: 14), YISSYNGATNYNQKFKG (SEQ ID NO:15), YIAGYKDATNYNQKFKG (SEQ ID NO:59), or YISNYNRATNYNQKFKG (SEQ ID NO:65), and a

heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein, the bispecific antibody further comprises: a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the the bispecific antibody comprises: a first antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises (a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO: 14), YISSYNGATNYNQKFKG (SEQ ID NO:15), YIAGYKDATNYNQKFKG (SEQ ID NO:59), or YISNYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16), and (b) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0031]** In some disclosure herein, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human VEGF, and b) a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO: 17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO: 18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19); wherein the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO: 13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising YIX<sub>1</sub>X<sub>2</sub>YX<sub>3</sub>X<sub>4</sub>ATNYNQKFKG (SEQ ID NO:80), wherein X<sub>1</sub> is serine or alanine, X<sub>2</sub> is serine, asparagine, or glycine, X<sub>3</sub> is asparagine or lysine, and X<sub>4</sub> is glycine, arginine, or aspartic acid, and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO: 16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human VEGF, and b) a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO: 19); wherein the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO: 14), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO: 16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human VEGF, and b) a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19); wherein the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISSYNGATNYNQKFKG (SEQ ID NO:15), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3

comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the a bispecific antibody comprises: a) a first antigen-binding site that specifically binds human VEGF, and b) a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19); wherein the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIAGYKDATNYNQKFKG (SEQ ID NO:59), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO: 16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human VEGF, and b) a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19); wherein the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0032]** In some disclosure herein, the bispecific antibody that specifically binds human VEGF, and comprises: a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:11, and/or a light chain variable region having at least 90% sequence identity to SEQ ID NO:12. In some disclosure herein, the bispecific antibody specifically binds human VEGF, and comprises: a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:11, and/or a light chain variable region having at least 95% sequence identity to SEQ ID NO:12.

**[0033]** In some disclosure herein, the bispecific antibody specifically binds human DLL4, and comprises: a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:58, or SEQ ID NO:64; and/or a light chain variable region having at least 90% sequence identity to SEQ ID NO:12. In some disclosure herein, the bispecific antibody specifically binds human DLL4, and comprises: a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:58, or SEQ ID NO:64; and/or a light chain variable region having at least 95% sequence identity to SEQ ID NO:12.

**[0034]** In some disclosure herein, the bispecific antibody specifically binds human VEGF and human DLL4, and comprises: (a) a first heavy chain variable region having at least 90% sequence identity to SEQ ID NO:11; (b) a second heavy chain variable region having at least 90% sequence identity to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:58, or SEQ ID NO:64; and (c) a first and a second light chain variable region having at least 90% sequence identity to SEQ ID NO:12. In some disclosure herein, the VEGF/DLL4 bispecific antibody comprises (a) a first heavy chain variable region having at least 95% sequence identity to SEQ ID NO:11; (b) a second heavy chain variable region having at least 95% sequence identity to SEQ ID NO:9; and (c) a first and a

second light chain variable region having at least 95% sequence identity to SEQ ID NO:12. In some disclosure herein, the VEGF/DLL4 bispecific antibody comprises (a) a first heavy chain variable region having at least 95% sequence identity to SEQ ID NO:11; (b) a second heavy chain variable region having at least 95% sequence identity to SEQ ID NO:10; and (c) a first and a second light chain variable region having at least 95% sequence identity to SEQ ID NO:12. In somedisclosure herein, the VEGF/DLL4 bispecific antibody comprises (a) a first heavy chain variable region having at least 95% sequence identity to SEQ ID NO:11; (b) a second heavy chain variable region having at least 95% sequence identity to SEQ ID NO:58; and (c) a first and a second light chain variable region having at least 95% sequence identity to SEQ ID NO:12. In somedisclosure herein, the VEGF/DLL4 bispecific antibody comprises (a) a first heavy chain variable region having at least 95% sequence identity to SEQ ID NO:11; (b) a second heavy chain variable region having at least 95% sequence identity to SEQ ID NO:64; and (c) a first and a second light chain variable region having at least 95% sequence identity to SEQ ID NO:12.

**[0035]** In somedisclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising (a) a first antigen-binding site that binds human VEGF with a  $K_D$  between about 0.1nM and about 1.0 nM and (b) a second antigen-binding site that specifically binds human DLL4 with a  $K_D$  between about 0.1nM and about 20 nM. In certain disclosure herein, the bispecific antibody comprises two identical light chains.

**[0036]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody selected from the group consisting of 219R45-MB-21M18, 219R45-MB-21R79, 219R45-MB-21R75, and 219R45-MB-21R83.

**[0037]** In certain disclosure herein of each of the aforementioned aspects, as well as other aspects and/or disclosure herein described elsewhere herein, the binding agent or antibody is isolated.

**[0038]** The disclosure also provides a polypeptide selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64. In some disclosure herein, the polypeptide is isolated. In certain disclosure herein, the polypeptide is substantially pure. In certain disclosure herein, the polypeptide is an antibody or part of an antibody, such as an antibody fragment.

**[0039]** The disclosure also provides isolated polynucleotide molecules comprising a polynucleotide that encodes the binding agents and/or polypeptides described herein. In some disclosure herein, the polynucleotide comprises a sequence selected from the group consisting of: SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, and SEQ ID NO:74. The disclosure further provides expression vectors that comprise the polynucleotides, as well as cells that

comprise the expression vectors and/or the polynucleotides. In some disclosure herein, the cell is a prokaryotic cell or a eukaryotic cell.

**[0040]** The disclosure also provides methods of inhibiting growth of a tumor, comprising contacting the tumor with an effective amount of an antibody (or other binding agent) that binds VEGF, DLL4, or both VEGF and DLL4, including each of those antibodies (or other binding agents) described herein.

**[0041]** The disclosure also provides a method of inhibiting the growth of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of an antibody (or other binding agent) that binds VEGF, DLL4, or both VEGF and DLL4, including each of those antibodies (or other binding agents) described herein.

**[0042]** The disclosure also provides a method of modulating angiogenesis in a subject, comprising administering to the subject a therapeutically effective amount of an antibody (or other binding agent) that binds VEGF, DLL4, or both VEGF and DLL4, including each of those antibodies (or other binding agents) described herein.

**[0043]** The disclosure also provides a method of reducing the tumorigenicity of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of an antibody (or other binding agent) that binds VEGF, DLL4, or both VEGF and DLL4, including each of those antibodies (or other binding agents) described herein.

**[0044]** The disclosure also provides a method of reducing the tumorigenicity of a tumor in a subject by reducing the frequency of cancer stem cells in the tumor, comprising administering to the subject a therapeutically effective amount of an antibody (or other binding agent) that binds VEGF, DLL4, or both VEGF and DLL4, including each of those antibodies (or other binding agents) described herein.

**[0045]** The disclosure also provides methods of treating cancer in a subject, comprising administering to the subject a therapeutically effective amount of an antibody (or other binding agent) that binds VEGF, DLL4, or both VEGF and DLL4, including each of those antibodies (or other binding agents) described herein.

**[0046]** Pharmaceutical compositions comprising a binding agent (e.g., antibody) described herein and a pharmaceutically acceptable carrier are further provided, as are cell lines that express and/or produce the binding agents. Methods of treating cancer and/or inhibiting tumor growth in a subject (e.g., a human) comprising administering to the subject an effective amount of a composition comprising the binding agents are also provided.

**[0047]** Where disclosure herein is described in terms of a Markush group or other grouping of alternatives, the present disclosure encompasses not only the entire group listed as a whole, but also each member of the group individually and all possible subgroups of the main group, and also the main group absent one or more of the group members. The present disclosure also envisages the explicit exclusion of one or more of any of the group members in the claimed disclosure.

## BRIEF DESCRIPTIONS OF THE DRAWINGS

## [0048]

Figure 1. 1A) Heavy chain and light chain CDRs of anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18, 219R45-MB-21M79, 219R45-MB-21M75, and 219R45-MB-21M83; 1B) Heavy chain and light chain variable region SEQ ID NOs; 1C) Heavy chain and light chain SEQ ID NOs.

Figure 2. HTRF assay for simultaneous binding of bispecific antibodies to human VEGF and human DLL4. Results are reported in Relative Fluorescence Units (RFU), which represent the ratio of the relative fluorescence intensity at 665nm to the relative fluorescence intensity at 620nm. 219R45-MB-21M18 (---); 219R45-MB-21R79 (-■-); 219R45 plus 21M18 (-▲-); 219R45 plus 21R79 (-□-); 219R45 (-▼-); 21M18 (-◊-); 21R79 (-○-); control antibody LZ-1 (-Δ-).

Figure 3. Inhibition of VEGF-induced HUVEC proliferation by anti-VEGF/anti-DLL4 bispecific antibodies. Fluorescence intensity is read using an excitation wavelength of 530nm and an emission wavelength of 590. 219R45-MB-21M18 (---); 219R45-MB-21R79 (-▲-); 219R45 (-■-); Medium with no VEGF (-◊-).

Figure 4. Inhibition of DLL4-induced Notch signaling by anti-VEGF/anti-DLL4 bispecific antibodies. Luciferase activity was measured using a dual luciferase assay kit with firefly luciferase activity normalized to Renilla luciferase activity. 219R45-MB-21M18 (---); 219R45-MB-21R79 (-■-); 21M18 (-○-); 21R79 (-□-).

Figure 5. Inhibition of colon tumor growth *in vivo* by an anti-VEGF/anti-DLL4 bispecific antibody. OMP-C8 colon tumor cells were injected subcutaneously into a human skin graft in NOD/SCID mice. Mice were treated with control antibody (-■-), anti-hDLL4 antibody 21M18 (-▲-), anti-VEGF antibody bevacizumab (-○-), or anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21M18 (-▼-). Data is shown as tumor volume (photons/sec) over days post-treatment. Antibodies were administered intraperitoneally at a dose of 25mg/kg once a week.

Figure 6. Tumorigenicity of pancreatic tumor cells after treatment with anti-VEGF/anti-DLL4 bispecific antibodies. OMP-PN8 tumor cells from mice treated with control antibody, anti-hDLL4 antibody 21M18, anti-VEGF antibody bevacizumab, or anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 or 219R45-MB-21R79 with or without gemcitabine were processed to single cell suspensions, and serially transplanted into mice. 90 cells from each treatment group were injected subcutaneously into NOD/SCID mice. Tumors were allowed to grow with no treatment. Data is shown as tumor volume (mm<sup>3</sup>) on day 55. Tumor frequency is shown as number of tumors over total number of mice injected in each group.

Figure 7. Bispecific antibody ELISA. Bispecific antibodies 219R45-MB-21M18, 219R45-MB-21R79, 219R45-MB-21R75, and 219R45-MB-21R83 were diluted in blocking buffer (1x PBS, 0.1% gelatin, 0.1% Polysorbate-20, pH 7.4) containing 2µg/ml biotin-DLL4-hFc. The antibodies were serially diluted 3-fold from 500ng/ml to 0.008ng/ml. The antibody samples were incubated for 2 hours in

blocking buffer containing the biotin-DLL4-hFc. After incubation, the antibody samples were transferred to a VEGF-coated assay plate (100  $\mu$ l/well) and incubated for 2 hours. Streptavidin-HRP was added to each well and incubated for 1 hr. TMB substrate was added to the wells with a 10 minute color development and the reaction was stopped with 2M sulfuric acid. Absorbance was read at 450-650nm and the data analyzed using the 4-parameter fit within the Softmax Pro analysis program.

Figure 8. Imaged capillary isoelectric focusing of anti-VEGF/anti-DLL4 bispecific antibodies.

Figure 9. Inhibition of colon tumor growth by anti-VEGF/anti-DLL4 bispecific antibodies in tumor recurrence model. OMP-C8 colon tumor cells were injected subcutaneously in NOD/SCID mice. Mice were treated with control antibody (- ■ -), anti-hDLL4 antibody 21M18 (-●-), anti-VEGF antibody bevacizumab (- ▲ -), a combination of 21M18 and bevacizumab (- ▽ -), anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21M18 (-◊-), or anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21R79 (-○-), all in combination with irinotecan. Antibodies 21M18 and bevacizumab were administered intraperitoneally at a dose of 7.5mg/kg once a week, bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79 were administered intraperitoneally at a dose of 15mg/kg once a week, and irinotecan was administered for the first 4 weeks at a dose of 45mg/kg. Data are shown as tumor volume ( $\text{mm}^3$ ) over days post-treatment.

Figure 10. Tumorigenicity of OMP-C3 colon tumor cells after treatment with anti-VEGF/anti-DLL4 bispecific antibodies. Tumors from mice treated with control antibody, anti-hDLL4 antibody 21M18, anti-VEGF antibody bevacizumab, a combination of 21M18 and bevacizumab, or anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 or 219R45-MB-21R79 with or without irinotecan were processed to single cell suspensions, and serially transplanted into mice. 150 cells from each treatment group were injected subcutaneously into NOD/SCID mice. Tumors were allowed to grow with no treatment. Data are shown as tumor volume ( $\text{mm}^3$ ) on day 68.

Figure 11. Inhibition of colon tumor growth *in vivo* by anti-VEGF/anti-DLL4 bispecific antibodies. OMP-C8 colon tumor cells were injected subcutaneously into NOD/SCID mice. Mice were treated with control antibody (- ■ -), anti-VEGF antibody bevacizumab (- ▲ -), or anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 (-◊-), 219R45-MB-21R75 (-●-), 219R45-MB-21R79 (-○-), or 219R45-MB-21R83 (-▽-). Mice were treated with antibodies as single agents (Fig. 10A) or in combination with irinotecan (Fig. 10B). Antibodies were administered intraperitoneally at a dose of 15mg/kg once a week and irinotecan at a dose of 7.5mg/kg one a week. Data are shown as tumor volume ( $\text{mm}^3$ ) over days post-treatment.

#### DETAILED DESCRIPTION OF THE INVENTION

**[0049]** The present disclosure provides novel binding agents, including but not limited to polypeptides such as antibodies, that bind VEGF and/or DLL4 (e.g., a VEGF/DLL4 binding agent). Related polypeptides and polynucleotides, compositions comprising the VEGF/DLL4-binding agents, and methods of making the VEGF/DLL4-binding agents are also provided. Methods of

using the novel VEGF/DLL4-binding agents, such as methods of inhibiting tumor growth, methods of treating cancer, methods of reducing tumorigenicity of a tumor, methods of reducing the frequency of cancer stem cells in a tumor, and/or methods of modulating angiogenesis, are further provided.

**[0050]** A monoclonal antibody that specifically binds human VEGF has been identified, 219R45. This antibody has a binding affinity for human VEGF of about 0.67nM, and a binding affinity for mouse VEGF of about 23nM. Several monoclonal antibodies that specifically bind human DLL4 have been identified, 21R79, 21R75 and 21R83. Antibody 21R79 has a binding affinity for human DLL4 of less than 0.1nM. Bispecific antibodies that specifically bind human VEGF and human DLL4 have been produced, 219R45-MB-21M18, 219R45-MB-21R79, 219R45-MB-21R75, and 219R45-MB-21R83 (CDR sequences in Figure 1). As used herein, the "MB" within an antibody name refers to "monovalent/bispecific". Bispecific antibody 219R45-MB-21M18 has a binding affinity for human VEGF of less than 1.0nM and a binding affinity for human DLL4 of about 16nM. Bispecific antibody 219R45-MB-21R79 has a binding affinity for human VEGF of less than 1.0nM and a binding affinity for human DLL4 of less than 1.0nM. Bispecific antibody 219R45-MB-21R75 has a binding affinity for human DLL4 of about 5nM, while bispecific antibody 219R45-MB-21R83 has a binding affinity for human DLL4 of about 1nM. Bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79 bind mouse VEGF (Example 1, Table 3). Anti-VEGF/anti-DLL4 bispecific antibodies bind human VEGF and human DLL4 simultaneously (Example 2, Figure 2). Anti-VEGF/anti-DLL4 bispecific antibodies inhibit VEGF-induced proliferation of HUVEC cells (Example 3, Figure 3). Anti-VEGF/anti-DLL4 bispecific antibodies inhibit DLL4-induced Notch signaling (Example 4, Figure 4). Anti-VEGF/anti-DLL4 bispecific antibodies inhibit tumor growth (Examples 5, 9, 11 and Figures 5, 9, 11). Anti-VEGF/anti-DLL4 bispecific antibodies inhibit tumorigenicity (Examples 6 and 10 and Figures 6, 10). Anti-VEGF/anti-DLL4 bispecific antibodies bind both VEGF and DLL4 in a bispecific ELISA (Example 7, Figure 7). Anti-VEGF/anti-DLL4 bispecific antibodies are isolated and purified to a product comprising at least 90% heterodimeric antibody (Example 8, Table 7).

## I. Definitions

**[0051]** To facilitate an understanding of the present disclosure, a number of terms and phrases are defined below.

**[0052]** The term "antibody" as used herein refers to an immunoglobulin molecule that recognizes and specifically binds a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing, through at least one antigen recognition site within the variable region of the immunoglobulin molecule. As used herein, the term encompasses intact polyclonal antibodies, intact monoclonal antibodies, single chain antibodies, antibody fragments (such as Fab, Fab', F(ab')2, and Fv fragments), single chain Fv (scFv) antibodies, multispecific antibodies such as bispecific antibodies, monospecific antibodies, monovalent antibodies, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen-binding site of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site (i.e., antigen-binding site) as long

as the antibodies exhibit the desired biological activity. An antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), based on the identity of their heavy chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well-known subunit structures and three-dimensional configurations. Antibodies can be naked or conjugated to other molecules, including but not limited to, toxins and radioisotopes.

**[0053]** The term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments. "Antibody fragment" as used herein comprises an antigen-binding site or epitope-binding site.

**[0054]** The term "variable region" of an antibody refers to the variable region of an antibody light chain, or the variable region of an antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chains each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs), also known as "hypervariable regions". The CDRs in each chain are held together in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of the antibody. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Edition, National Institutes of Health, Bethesda, MD), and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-Lazikani et al., 1997, J. Mol. Biol., 273:927-948). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

**[0055]** The term "monoclonal antibody" as used herein refers to a homogeneous antibody population involved in the highly specific recognition and binding of a single antigenic determinant or epitope. This is in contrast to polyclonal antibodies that typically include a mixture of different antibodies directed against a variety of different antigenic determinants. The term "monoclonal antibody" encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (e.g., Fab, Fab', F(ab')2, Fv), single chain (scFv) antibodies, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site (antigen-binding site). Furthermore, "monoclonal antibody" refers to such antibodies made by any number of techniques, including but not limited to, hybridoma production, phage selection, recombinant expression, and transgenic animals.

**[0056]** The term "humanized antibody" as used herein refers to forms of non-human (e.g., murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Typically, humanized antibodies are human immunoglobulins in which residues of the CDRs are replaced by residues from the CDRs of a non-human species (e.g., mouse, rat, rabbit, or hamster) that have the desired specificity, affinity, and/or binding capability (Jones et al., 1986, Nature, 321:522-525; Riechmann et al., 1988, Nature, 332:323-327; Verhoeyen et al., 1988, Science, 239:1534-1536). In some instances, the Fv framework region residues of a human immunoglobulin are replaced with the

corresponding residues in an antibody from a non-human species that has the desired specificity, affinity, and/or binding capability. The humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or binding capability. In general, the humanized antibody will comprise substantially all of at least one, and typically two or three, variable domains containing all or substantially all of the CDRs that correspond to the non-human immunoglobulin whereas all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Examples of methods used to generate humanized antibodies are described in, for example, U.S. Patent 5,225,539.

**[0057]** The term "human antibody" as used herein refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human. A human antibody may be made using any of the techniques known in the art. This definition of a human antibody specifically excludes a humanized antibody comprising non-human CDRs.

**[0058]** The term "chimeric antibody" as used herein refers to an antibody wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. Typically, the variable region of both light and heavy chains corresponds to the variable region of antibodies derived from one species of mammals (e.g., mouse, rat, rabbit, etc.) with the desired specificity, affinity, and/or binding capability, while the constant regions correspond to sequences in antibodies derived from another species (usually human).

**[0059]** The phrase "affinity-matured antibody" as used herein refers to an antibody with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). The definition also includes alterations in non-CDR residues made in conjunction with alterations to CDR residues. Preferred affinity-matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al., 1992, *Bio/Technology* 10:779-783, describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al., 1994, *PNAS*, 91:3809-3813; Schier et al., 1995, *Gene*, 169:147-155; Yelton et al., 1995, *J. Immunol.* 155:1994-2004; Jackson et al., 1995, *J. Immunol.*, 154:3310-9; and Hawkins et al., 1992, *J. Mol. Biol.*, 226:889-896. Site-directed mutagenesis may also be used to obtain affinity-matured antibodies.

**[0060]** The terms "epitope" and "antigenic determinant" are used interchangeably herein and refer to that portion of an antigen capable of being recognized and specifically bound by a particular antibody. When the antigen is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids (also referred to as linear epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding (also referred to as conformational epitopes) are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

**[0061]** The terms "heteromultimeric molecule" or "heteromultimer" or "heteromultimeric complex" or "heteromultimeric polypeptide" are used interchangeably herein to refer to a molecule comprising at least a first polypeptide and a second polypeptide, wherein the second polypeptide differs in amino acid sequence from the first polypeptide by at least one amino acid residue. The heteromultimeric molecule can comprise a "heterodimer" formed by the first and second polypeptide or can form higher order tertiary structures where additional polypeptides are present.

**[0062]** The terms "antagonist" and "antagonistic" as used herein refer to any molecule that partially or fully blocks, inhibits, reduces, or neutralizes a biological activity of a target and/or signaling pathway (e.g., the Notch pathway). The term "antagonist" is used herein to include any molecule that partially or fully blocks, inhibits, reduces, or neutralizes the activity of a protein. Suitable antagonist molecules specifically include, but are not limited to, antagonist antibodies or antibody fragments.

**[0063]** The terms "modulation" and "modulate" as used herein refer to a change or an alteration in a biological activity. Modulation includes, but is not limited to, stimulating or inhibiting an activity. Modulation may be an increase or a decrease in activity (e.g., a decrease in angiogenesis or an increase in angiogenesis), a change in binding characteristics, or any other change in the biological, functional, or immunological properties associated with the activity of a protein, pathway, or other biological point of interest.

**[0064]** The terms "selectively binds" or "specifically binds" mean that a binding agent or an antibody reacts or associates more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the epitope, protein, or target molecule than with alternative substances, including unrelated proteins. In certain disclosure herein "specifically binds" means, for instance, that an antibody binds a protein with a  $K_D$  of about 0.1mM or less, but more usually less than about 1 $\mu$ M. In certain disclosure herein, "specifically binds" means that an antibody binds a target at times with a  $K_D$  of at least about 0.1  $\mu$ M or less, at other times at least about 0.01 $\mu$ M or less, and at other times at least about 1nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include an antibody that recognizes a protein in more than one species (e.g., human VEGF and mouse VEGF). Likewise, because of homology within certain regions of polypeptide sequences of different proteins, specific binding can include an antibody (or other polypeptide or binding agent) that recognizes more than one protein (e.g., human VEGF-A and human VEGF-B). It is understood that, in certain disclosure herein, an antibody or binding moiety that specifically binds a first target may or may not specifically bind a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding, i.e. binding to a single target. Thus, an antibody may, in certain disclosure herein, specifically bind more than one target. In certain disclosure herein, multiple targets may be bound by the same antigen-binding site on the antibody. For example, an antibody may, in certain instances, comprise two identical antigen-binding sites, each of which specifically binds the same epitope on two or more proteins. In certain alternative disclosure herein, an antibody may be multispecific and comprise at least two antigen-binding sites with differing specificities. By way of non-limiting example, a bispecific antibody may comprise one antigen-binding site that recognizes an epitope on one protein (e.g., human VEGF) and further comprise a second, different antigen-binding site that recognizes a different epitope

on a second protein (e.g., human DLL4). Generally, but not necessarily, reference to binding means specific binding.

**[0065]** The terms "polypeptide" and "peptide" and "protein" are used interchangeably herein and refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure may be based upon antibodies, in certain disclosure herein, the polypeptides can occur as single chains or associated chains.

**[0066]** The terms "polynucleotide" and "nucleic acid" are used interchangeably herein and refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase.

**[0067]** "Conditions of high stringency" may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 15mM sodium chloride/1.5mM sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 in 5x SSC (0.75M NaCl, 75mM sodium citrate) at 42°C; or (3) employ during hybridization 50% formamide in 5x SSC, 50mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2x SSC and 50% formamide, followed by a high-stringency wash consisting of 0.1x SSC containing EDTA at 55°C.

**[0068]** The terms "identical" or percent "identity" in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity may be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that may be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variations thereof. In some disclosure herein, two nucleic acids or polypeptides of the disclosure are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some disclosure herein at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some disclosure herein, identity exists over a region of the sequences that is at least about 10, at least about 20, at least about 40-60 residues, at least about 60-80 residues

in length or any integral value therebetween. In some disclosure herein, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some disclosure herein the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a nucleotide sequence.

**[0069]** A "conservative amino acid substitution" is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Preferably, conservative substitutions in the sequences of the polypeptides and antibodies of the disclosure do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen to which the polypeptide or antibody binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen binding are well-known in the art.

**[0070]** The term "vector" as used herein means a construct, which is capable of delivering, and usually expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid, or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, and DNA or RNA expression vectors encapsulated in liposomes.

**[0071]** A polypeptide, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, antibodies, polynucleotides, vectors, cells, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some disclosure herein, a polypeptide, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure.

**[0072]** The term "substantially pure" as used herein refers to material which is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

**[0073]** The terms "cancer" and "cancerous" as used herein refer to or describe the physiological condition in mammals in which a population of cells are characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, blastoma, sarcoma, and hematologic cancers such as lymphoma and leukemia.

**[0074]** The terms "tumor" and "neoplasm" as used herein refer to any mass of tissue that results from excessive cell growth or proliferation, either benign (noncancerous) or malignant (cancerous) including pre-cancerous lesions.

**[0075]** The term "metastasis" as used herein refers to the process by which a cancer spreads or transfers from the site of origin to other regions of the body with the development of a similar cancerous lesion at a new location. A "metastatic" or "metastasizing" cell is one that loses adhesive contacts with neighboring cells and migrates via the bloodstream or lymph from the primary site of disease to invade neighboring body structures.

**[0076]** The terms "cancer stem cell" and "CSC" and "tumor stem cell" and "tumor initiating cell" are used interchangeably herein and refer to cells from a cancer or tumor that: (1) have extensive proliferative capacity; 2) are capable of asymmetric cell division to generate one or more types of differentiated cell progeny wherein the differentiated cells have reduced proliferative or developmental potential; and (3) are capable of symmetric cell divisions for self-renewal or self-maintenance. These properties confer on the cancer stem cells the ability to form or establish a tumor or cancer upon serial transplantation into an immunocompromised host (e.g., a mouse) compared to the majority of tumor cells that fail to form tumors. Cancer stem cells undergo self-renewal versus differentiation in a chaotic manner to form tumors with abnormal cell types that can change over time as mutations occur.

**[0077]** The terms "cancer cell" and "tumor cell" refer to the total population of cells derived from a cancer or tumor or pre-cancerous lesion, including both non-tumorigenic cells, which comprise the bulk of the cancer cell population, and tumorigenic stem cells (cancer stem cells). As used herein, the terms "cancer cell" or "tumor cell" will be modified by the term "non-tumorigenic" when referring solely to those cells lacking the capacity to renew and differentiate to distinguish those tumor cells from cancer stem cells.

**[0078]** The term "tumorigenic" as used herein refers to the functional features of a cancer stem cell including the properties of self-renewal (giving rise to additional tumorigenic cancer stem cells) and proliferation to generate all other tumor cells (giving rise to differentiated and thus non-tumorigenic tumor cells).

**[0079]** The term "tumorigenicity" as used herein refers to the ability of a random sample of cells from the tumor to form palpable tumors upon serial transplantation into immunocompromised hosts (e.g., mice). This definition also includes enriched and/or isolated populations of cancer stem cells that form palpable tumors upon serial transplantation into immunocompromised hosts (e.g., mice).

**[0080]** The term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, canines, felines, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

**[0081]** The term "pharmaceutically acceptable" refers to a product or compound approved (or approvable) by a regulatory agency of the Federal government or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans.

**[0082]** The terms "pharmaceutically acceptable excipient, carrier or adjuvant" or "acceptable

"pharmaceutical carrier" refer to an excipient, carrier or adjuvant that can be administered to a subject, together with at least one binding agent (e.g., an antibody) of the present disclosure, and which does not destroy the activity of the binding agent. The excipient, carrier or adjuvant should be nontoxic when administered with a binding agent in doses sufficient to deliver a therapeutic effect.

**[0083]** The terms "effective amount" or "therapeutically effective amount" or "therapeutic effect" refer to an amount of a binding agent, an antibody, polypeptide, polynucleotide, small organic molecule, or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of cancer, the therapeutically effective amount of a drug (e.g., an antibody) has a therapeutic effect and as such can reduce the number of cancer cells; decrease tumorigenicity, tumorigenic frequency or tumorigenic capacity; reduce the number or frequency of cancer stem cells; reduce the tumor size; reduce the cancer cell population; inhibit and/or stop cancer cell infiltration into peripheral organs including, for example, the spread of cancer into soft tissue and bone; inhibit and/or stop tumor or cancer cell metastasis; inhibit and/or stop tumor or cancer cell growth; relieve to some extent one or more of the symptoms associated with the cancer; reduce morbidity and mortality; improve quality of life; or a combination of such effects. To the extent the agent, for example an antibody, prevents growth and/or kills existing cancer cells, it can be referred to as cytostatic and/or cytotoxic.

**[0084]** The terms "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both 1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. In some disclosure herein, a subject is successfully "treated" according to the methods of the disclosure if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer cells into soft tissue and bone; inhibition of or an absence of tumor or cancer cell metastasis; inhibition or an absence of cancer growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity; reduction in the number or frequency of cancer stem cells; or some combination of effects.

**[0085]** As used in the present disclosure and claims, the singular forms "a", "an" and "the" include plural forms unless the context clearly dictates otherwise.

**[0086]** It is understood that wherever disclosure herein is described herein with the language "comprising" otherwise analogous disclosure herein described in terms of "consisting of" and/or "consisting essentially of" are also provided. It is also understood that wherever disclosure herein is described herein with the language "consisting essentially of" otherwise analogous disclosure herein described in terms of "consisting of" are also provided.

**[0087]** The term "and/or" as used in a phrase such as "A and/or B" herein is intended to include

both A and B; A or B; A (alone); and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following disclosure herein: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

## II. Antibodies

**[0088]** The present disclosure provides agents that specifically bind human VEGF proteins and/or human DLL4 proteins. These agents are referred to herein as "VEGF/DLL4-binding agents". The phrase "VEGF/DLL4-binding agent" encompasses agents that bind only VEGF, agents that bind only DLL4, and bispecific agents that bind both VEGF and DLL4. In certain disclosure herein, in addition to specifically binding VEGF and/or DLL4, the VEGF/DLL4-binding agents further specifically bind at least one additional target or antigen. In some disclosure herein the VEGF/DLL4-binding agent is an antibody. In some disclosure herein the VEGF/DLL4-binding agent is a polypeptide. In certain disclosure herein, the VEGF/DLL4-binding agent specifically binds human VEGF. In certain disclosure herein, the VEGF/DLL4-binding agent specifically binds human DLL4. In certain disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody. In certain disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody that specifically binds human VEGF and human DLL4. The full-length amino acid (aa) sequences for human VEGF (VEGF-A) and human DLL4 are known in the art and are provided herein as SEQ ID NO:27 (VEGF) and SEQ ID NO:23 (DLL4).

**[0089]** In certain disclosure herein, the VEGF/DLL4-binding agent or antibody binds VEGF and/or DLL4 with a dissociation constant ( $K_D$ ) of about 1 $\mu$ M or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In some disclosure herein, a VEGF/DLL4-binding agent or antibody binds VEGF and/or DLL4 with a  $K_D$  of about 20nM or less. In some disclosure herein, a VEGF/DLL4-binding agent or antibody binds VEGF and/or DLL4 with a  $K_D$  of about 10nM or less. In some disclosure herein, a VEGF/DLL4-binding agent or antibody binds VEGF and/or DLL4 with a  $K_D$  of about 1nM or less. In some disclosure herein, a VEGF/DLL4-binding agent or antibody binds VEGF and/or DLL4 with a  $K_D$  of about 0.1nM or less. In some disclosure herein, the VEGF/DLL4-binding agent binds both human VEGF and mouse VEGF with a  $K_D$  of about 100nM or less. In some disclosure herein, the VEGF/DLL4-binding agent binds both human VEGF and mouse VEGF with a  $K_D$  of about 50nM or less. In some disclosure herein, a VEGF/DLL4-binding agent binds both human DLL4 and mouse DLL4 with a  $K_D$  of about 100nM or less. In some disclosure herein, a VEGF/DLL4-binding agent binds both human DLL4 and mouse DLL4 with a  $K_D$  of about 50nM or less. In some disclosure herein, the dissociation constant of the binding agent (e.g., an antibody) to VEGF is the dissociation constant determined using a VEGF fusion protein comprising at least a portion of VEGF immobilized on a Biacore chip. In some disclosure herein, the dissociation constant of the binding agent (e.g., an antibody) to DLL4 is the dissociation constant determined using a DLL4-fusion protein comprising at least a portion of DLL4 immobilized on a Biacore chip.

**[0090]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which

comprises a first antigen-binding site that specifically binds VEGF and a second antigen-binding site that specifically binds DLL4. In some disclosure herein, a VEGF/DLL4-binding agent or antibody binds both VEGF and DLL4 with a  $K_D$  of about 100nM or less. In somedisclosure herein, a VEGF/DLL4-binding agent or antibody binds both VEGF and DLL4 with a  $K_D$  of about 50nM or less. In some disclosure herein, a VEGF/DLL4-binding agent or antibody binds both VEGF and DLL4 with a  $K_D$  of about 20nM or less. In some disclosure herein, a VEGF/DLL4-binding agent or antibody binds both VEGF and DLL4 with a  $K_D$  of about 10nM or less. In some disclosure herein, a VEGF/DLL4-binding agent or antibody binds both VEGF and DLL4 with a  $K_D$  of about 1nM or less. In somedisclosure herein, the affinity of one of the antigen-binding sites may be weaker than the affinity of the other antigen-binding site. For example, the  $K_D$  of one antigen binding site may be about 1nM and the  $K_D$  of the second antigen-binding site may be about 10nM. In some disclosure herein, the difference in affinity between the two antigen-binding sites may be about 2-fold or more, about 3-fold or more, about 5-fold or more, about 8-fold or more, about 10-fold or more, about 15-fold or more, about 20-fold or more, about 30-fold or more, about 50-fold or more, or about 100-fold or more. Modulation of the affinities of the two antigen-binding sites may affect the biological activity of the bispecific antibody. For example, decreasing the affinity of the antigen-binding site for DLL4 or VEGF, may have a desirable effect, for example decreased toxicity of the binding agent or increased therapeutic index.

**[0091]** By way of non-limiting example, the bispecific antibody may comprise (a) a first antigen-binding site that binds human VEGF with a  $K_D$  between about 0.1 nM and about 1.0 nM, and (b) a second antigen-binding site that specifically binds human DLL4 with a  $K_D$  between about 0.1 nM and about 20 nM, between about 0.5nM and about 20nM, between about 1.0 nM and 10nM. In certain disclosure herein, the bispecific antibody comprises two identical light chains.

**[0092]** In certain disclosure herein, the VEGF/DLL4-binding agent (e.g., an antibody) binds VEGF and/or DLL4 with a half maximal effective concentration ( $EC_{50}$ ) of about 1 $\mu$ M or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In certain disclosure herein, a VEGF/DLL4-binding agent (e.g., an antibody) binds VEGF and/or DLL4 with a half maximal effective concentration ( $EC_{50}$ ) of about 1 $\mu$ M or less, about 100nM or less, about 40nM or less, about 20nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less.

**[0093]** In certain disclosure herein, the VEGF/DLL4-binding agent is an antibody. In somedisclosure herein, the antibody is a recombinant antibody. In somedisclosure herein, the antibody is a monoclonal antibody. In some disclosure herein, the antibody is a chimeric antibody. In some disclosure herein, the antibody is a humanized antibody. In somedisclosure herein, the antibody is a human antibody. In certain disclosure herein, the antibody is an IgA, IgD, IgE, IgG, or IgM antibody. In certain disclosure herein, the antibody is an IgG1 antibody. In certain disclosure herein, the antibody is an IgG2 antibody. In certain disclosure herein, the antibody is an antibody fragment comprising an antigen-binding site. In some disclosure herein, the antibody is a bispecific antibody. In some disclosure herein, the antibody is monovalent, monospecific, bivalent, or multispecific. In somedisclosure herein, the antibody is conjugated to a cytotoxic moiety. In some disclosure herein, the antibody is isolated. In some disclosure herein, the antibody is

substantially pure.

**[0094]** The VEGF/DLL4-binding agents (e.g., antibodies) of the present disclosure can be assayed for specific binding by any method known in the art. The immunoassays which can be used include, but are not limited to, competitive and non-competitive assay systems using techniques such as Biacore analysis, FACS analysis, immunofluorescence, immunocytochemistry, Western blot analysis, radioimmunoassay, ELISA, "sandwich" immunoassay, immunoprecipitation assay, precipitation reaction, gel diffusion precipitin reaction, immunodiffusion assay, agglutination assay, complement-fixation assay, immunoradiometric assay, fluorescent immunoassay, homogeneous time-resolved fluorescence assay (HTRF), and protein A immunoassay. Such assays are routine and well-known in the art (see, e.g., Ausubel et al., Editors, 1994-present, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York, NY).

**[0095]** For example, the specific binding of an antibody to human VEGF and/or human DLL4 may be determined using ELISA. An ELISA assay comprises preparing antigen, coating wells of a 96 well microtiter plate with antigen, adding the antibody or other binding agent conjugated to a detectable compound such as an enzymatic substrate (e.g. horseradish peroxidase or alkaline phosphatase) to the well, incubating for a period of time, and detecting the presence of the binding agent bound to the antigen. In some disclosure herein, the binding agent or antibody is not conjugated to a detectable compound, but instead a second antibody that recognizes the binding agent or antibody (e.g., an anti-Fc antibody) and is conjugated to a detectable compound is added to the well. In some disclosure herein, instead of coating the well with the antigen, the binding agent or antibody can be coated to the well and a second antibody conjugated to a detectable compound can be added following the addition of the antigen to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art.

**[0096]** In another example, the specific binding of an antibody to human VEGF and/or human DLL4 may be determined using FACS. A FACS screening assay may comprise generating a cDNA construct that expresses an antigen as a fusion protein, transfected the construct into cells, expressing the antigen on the surface of the cells, mixing the binding agent or antibody with the transfected cells, and incubating for a period of time. The cells bound by the binding agent or antibody may be identified by using a secondary antibody conjugated to a detectable compound (e.g., PE-conjugated anti-Fc antibody) and a flow cytometer. One of skill in the art would be knowledgeable as to the parameters that can be modified to optimize the signal detected as well as other variations of FACS that may enhance screening (e.g., screening for blocking antibodies).

**[0097]** The binding affinity of an antibody or other binding-agent to an antigen (e.g., VEGF or DLL4) and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., <sup>3</sup>H or <sup>125</sup>I), or fragment or variant thereof, with the antibody of interest in the presence of increasing amounts of unlabeled antigen followed by the detection of the antibody bound to the labeled antigen. The affinity of the antibody for the antigen and the binding off-rates can be determined from the data by Scatchard plot analysis. In some disclosure herein, Biacore kinetic analysis is used to determine the binding on and off rates of antibodies or

agents that bind an antigen (e.g., VEGF or DLL4). Biacore kinetic analysis comprises analyzing the binding and dissociation of antibodies from chips with immobilized antigen (e.g., VEGF or DLL4) on their surface.

**[0098]** The disclosure also provides a VEGF-binding agent (e.g., an antibody) that specifically binds human VEGF, wherein the VEGF-binding agent (e.g., an antibody) comprises one, two, three, four, five, and/or six of the CDRs of antibody 219R45 (see Table 1). In some disclosure herein, the VEGF-binding agent comprises one or more of the CDRs of 219R45, two or more of the CDRs of 219R45, three or more of the CDRs of 219R45, four or more of the CDRs of 219R45, five or more of the CDRs of 219R45, or all six of the CDRs of 219R45. In some disclosure herein, the VEGF-binding agent binds human VEGF and mouse VEGF.

Table 1

219R45	
HC CDR1	NYWMH (SEQ ID NO:17)
HC CDR2	DINPSNGRTSYKEKFKR (SEQ ID NO:18)
HC CDR3	HYDDKYYPLMDY (SEQ ID NO:19)
LC CDR1	RASESVDNYGISFMK (SEQ ID NO:20)
LC CDR2	AASNQGS (SEQ ID NO:21)
LC CDR3	QQSKEVPWTFGG (SEQ ID NO:22)

**[0099]** The disclosure also provides a VEGF-binding agent (e.g., an antibody) that specifically binds human VEGF, wherein the VEGF-binding agent comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19). In some disclosure herein, the VEGF-binding agent further comprises a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In certain disclosure herein, the VEGF-binding agent comprises: (a) a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and (b) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0100]** The disclosure also provides a VEGF-binding agent (e.g., an antibody) that specifically binds human VEGF, wherein the VEGF-binding agent comprises: (a) a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (c) a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (d) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (e) a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions;

and (f) a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain disclosure herein, the amino acid substitutions are conservative substitutions.

**[0101]** The disclosure also provides a VEGF-binding agent (e.g., an antibody) that specifically binds VEGF, wherein the VEGF-binding agent comprises a heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:11, and a light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In certain disclosure herein, the VEGF-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:11. In certain disclosure herein, the VEGF-binding agent comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:12. In certain disclosure herein, the VEGF-binding agent comprises a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:11, and a light chain variable region having at least about 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the VEGF-binding agent comprises a heavy chain variable region comprising SEQ ID NO:11, and a light chain variable region comprising SEQ ID NO:12. In certain disclosure herein, the VEGF-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:11, and a light chain variable region consisting essentially of SEQ ID NO:12. In some disclosure herein, the VEGF-binding agent comprises a heavy chain comprising SEQ ID NO:49, and a light chain comprising SEQ ID NO:8. In some disclosure herein, the VEGF-binding antibody or other agent comprises a heavy chain comprising SEQ ID NO:7, and a light chain comprising SEQ ID NO:8.

**[0102]** In some disclosure herein, the VEGF-binding agent binds VEGF with a  $K_D$  of about 10nM or less. In some disclosure herein, the VEGF-binding agent binds VEGF with a  $K_D$  of about 1nM or less. In some disclosure herein, the VEGF-binding agent binds VEGF with a  $K_D$  of about 0.1 nM or less. In some disclosure herein, the VEGF-binding agent binds VEGF with a  $K_D$  of about 0.01nM or less. In some disclosure herein, at least one amino acid residue in at least one CDR of the VEGF-binding agent is substituted with a different amino acid so that the affinity of the VEGF-binding agent for VEGF is altered. In some disclosure herein, the affinity of the VEGF-binding agent is increased. In some disclosure herein, the affinity of the VEGF-binding agent is decreased. In some disclosure herein, the VEGF-binding agent binds human VEGF. In some disclosure herein, the VEGF-binding agent binds human VEGF and mouse VEGF.

**[0103]** In certain disclosure herein, the VEGF-binding agent comprises the heavy chain variable region and light chain variable region of the 219R45 antibody. In certain disclosure herein, the VEGF-binding agent comprises the heavy chain and light chain of the 219R45 antibody (with or without the leader sequence). In certain disclosure herein, a VEGF-binding agent is the 219R45 antibody.

**[0104]** In certain disclosure herein, a VEGF-binding agent comprises, consists essentially of, or consists of, the antibody 219R45.

**[0105]** In certain disclosure herein, a VEGF-binding agent (e.g., an antibody) binds the same

epitope, or essentially the same epitope, on VEGF as an antibody of the disclosure. In other disclosure herein, a VEGF-binding agent is an antibody that binds an epitope on VEGF that overlaps with the epitope on VEGF bound by an antibody of the disclosure. In certain disclosure herein, a VEGF-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on VEGF as antibody 219R45. In other disclosure herein, the VEGF-binding agent is an antibody that binds an epitope on VEGF that overlaps with the epitope on VEGF bound by antibody 219R45.

**[0106]** In some disclosure herein, the VEGF-binding agent inhibits binding of VEGF to at least one VEGF receptor. In certain disclosure herein, the VEGF-binding agent inhibits binding of human VEGF to VEGFR-1 or VEGFR-2. In some disclosure herein, the VEGF-binding agent specifically binds VEGF and modulates angiogenesis. In some disclosure herein, the VEGF-binding agent specifically binds VEGF and inhibits angiogenesis. In some disclosure herein, the VEGF-binding agent specifically binds VEGF and inhibits tumor growth.

**[0107]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent (e.g., an antibody) comprises one, two, three, four, five, and/or six of the CDRs of antibody 21R79 (see Table 2). In some disclosure herein, the DLL4-binding agent comprises one or more of the CDRs of 21R79, two or more of the CDRs of 21R79, three or more of the CDRs of 21R79, four or more of the CDRs of 21R79, five or more of the CDRs of 21R79, or all six of the CDRs of 21R79. The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent (e.g., an antibody) comprises one, two, three, four, five, and/or six of the CDRs of antibody 21R75 (see Table 2). In some disclosure herein, the DLL4-binding agent comprises one or more of the CDRs of 21R75, two or more of the CDRs of 21R75, three or more of the CDRs of 21R75, four or more of the CDRs of 21R75, five or more of the CDRs of 21R75, or all six of the CDRs of 21R75. The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent (e.g., an antibody) comprises one, two, three, four, five, and/or six of the CDRs of antibody 21R83 (see Table 2). In some disclosure herein, the DLL4-binding agent comprises one or more of the CDRs of 21R83, two or more of the CDRs of 21R83, three or more of the CDRs of 21R83, four or more of the CDRs of 21R83, five or more of the CDRs of 21R83, or all six of the CDRs of 21R83. In some disclosure herein, the DLL4-binding agent binds human DLL4 and mouse DLL4.

Table 2

	21R79	21R75	21R83
HC CDR1	TAYYIH (SEQ ID NO: 13)	TAYYIH (SEQ ID NO: 13)	TAYYIH (SEQ ID NO:13)
HC CDR2	YIANYNRATNYNQKFKG (SEQ ID NO:14)	YIAGYKDATNYNQKFKG (SEQ ID NO:59)	YISNYNRATNYNQKFKG (SEQ ID NO:65)
HC CDR3	RDYDYDVGMDY (SEQ ID NO:16)	RDYDYDVGMDY (SEQ ID NO:16)	RDYDYDVGMDY (SEQ ID NO:16)
LC CDR1	RASESVDNYGISFMK (SEQ ID NO:20)	RASESVDNYGISFMK (SEQ ID NO:20)	RASESVDNYGISFMK (SEQ ID NO:20)
LC CDR2	AASNQGS (SEQIDNO:21)	AASNQGS (SEQ ID NO:21)	AASNQGS (SEQ ID NO:21)

	21R79	21R75	21R83
LC CDR3	QQSKEVPWTFGG (SEQ ID NO:22)	QQSKEVPWTFGG (SEQ ID NO:22)	QQSKEVPWTFGG (SEQ ID NO:22)

**[0108]** In certain disclosure herein, the heavy chain CDR1 of the DLL4-binding antibody is a minimal HC CDR1 comprising AYYIH (SEQ ID NO:79).

**[0109]** In somedisclosure herein, the binding agent is an antibody that binds human DLL4 and comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising YIX<sub>1</sub>X<sub>2</sub>YX<sub>3</sub>X<sub>4</sub>ATNYNQKFKG (SEQ ID NO:80), wherein X<sub>1</sub> is serine or alanine, X<sub>2</sub> is serine, asparagine, or glycine, X<sub>3</sub> is asparagine or lysine, and X<sub>4</sub> is glycine, arginine, or aspartic acid, and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0110]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein, the DLL4-binding agent further comprises a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In certain disclosure herein, the DLL4-binding agent comprises: (a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO: 13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO: 16), and (b) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0111]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent comprises: (a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO: 13), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO: 14), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (c) a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO: 16), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (d) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (e) a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and (f) a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain disclosure herein, the amino acid substitutions are conservative substitutions.

**[0112]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds DLL4, wherein the DLL4-binding agent comprises a heavy chain variable region having at

least about 80% sequence identity to SEQ ID NO:10, and a light chain variable region having at least 80% sequence identity to SEQ ID NO: 12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO: 10. In certain disclosure herein, the DLL4-binding agent comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO: 12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO: 10, and a light chain variable region having at least about 95% sequence identity to SEQ ID NO: 12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region comprising SEQ ID NO:10, and a light chain variable region comprising SEQ ID NO:12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:10, and a light chain variable region consisting essentially of SEQ ID NO:12. In some disclosure herein, the DLL4-binding agent comprises a heavy chain comprising SEQ ID NO:48, and a light chain comprising SEQ ID NO:8. In some disclosure herein, the DLL4-binding antibody or other agent comprises a heavy chain comprising SEQ ID NO:6, and a light chain comprising SEQ ID NO:8. In some disclosure herein, the antibody is a bispecific antibody.

**[0113]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIAGYKDATNYNQKFKG (SEQ ID NO:59), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein, the DLL4-binding agent further comprises a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In certain disclosure herein, the DLL4-binding agent comprises: (a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIAGYKDATNYNQKFKG (SEQ ID NO:59), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16), and (b) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0114]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent comprises: (a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO: 13), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising YIAGYKDATNYNQKFKG (SEQ ID NO:59), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (c) a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (d) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (e) a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and (f) a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain disclosure herein, the amino acid substitutions are conservative substitutions.

**[0115]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically

binds DLL4, wherein the DLL4-binding agent comprises a heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:58, and a light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:58. In certain disclosure herein, the DLL4-binding agent comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:58, and a light chain variable region having at least about 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region comprising SEQ ID NO:58, and a light chain variable region comprising SEQ ID NO:12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:58, and a light chain variable region consisting essentially of SEQ ID NO:12. In some disclosure herein, the DLL4-binding agent comprises a heavy chain comprising SEQ ID NO:56, and a light chain comprising SEQ ID NO:8.

**[0116]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein, the DLL4-binding agent further comprises a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In certain disclosure herein, the DLL4-binding agent comprises: (a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16), and (b) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0117]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds human DLL4, wherein the DLL4-binding agent comprises: (a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (b) a heavy chain CDR2 comprising YISYNRATNYNQKFKG (SEQ ID NO:65), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (c) a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (d) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; (e) a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and (f) a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22), or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In certain disclosure herein, the amino acid substitutions are conservative substitutions.

**[0118]** The disclosure also provides a DLL4-binding agent (e.g., an antibody) that specifically binds DLL4, wherein the DLL4-binding agent comprises a heavy chain variable region having at

least about 80% sequence identity to SEQ ID NO:64, and a light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:64. In certain disclosure herein, the DLL4-binding agent comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:12. In certain disclosure herein the DLL4-binding agent comprises a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:64, and a light chain variable region having at least about 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region comprising SEQ ID NO:64, and a light chain variable region comprising SEQ ID NO:12. In certain disclosure herein, the DLL4-binding agent comprises a heavy chain variable region consisting essentially of SEQ ID NO:64, and a light chain variable region consisting essentially of SEQ ID NO:12. In some disclosure herein, the DLL4-binding agent comprises a heavy chain comprising SEQ ID NO:62, and a light chain comprising SEQ ID NO:8. In some disclosure herein, the agent is a bispecific antibody.

**[0119]** In some disclosure herein, the DLL4-binding agent is an antibody that comprises a heavy chain comprising SEQ ID NO:5, and a light chain comprising SEQ ID NO:8. In some disclosure herein, the antibody is a bispecific antibody.

**[0120]** In some disclosure herein, the DLL4-binding agent binds DLL4 with a  $K_D$  of 25nM or less. In some disclosure herein, the DLL4-binding agent binds DLL4 with a  $K_D$  of 10nM or less. In some disclosure herein, the DLL4-binding agent binds DLL4 with a  $K_D$  of about 1nM or less. In some disclosure herein, the DLL4-binding agent binds DLL4 with a  $K_D$  of about 0.1nM or less. In some disclosure herein, the DLL4-binding agent binds DLL4 with a  $K_D$  of about 0.01nM or less. In some disclosure herein, at least one amino acid residue in at least one CDR of the DLL4-binding agent is substituted with a different amino acid so that the affinity of the DLL4-binding agent for DLL4 is altered. In some disclosure herein, the affinity of the DLL4-binding agent is increased. In some disclosure herein, the affinity of the DLL4-binding agent is decreased.

**[0121]** In certain disclosure herein the DLL4-binding agent comprises the heavy chain variable region and the light chain variable region of the 21R79 antibody. In certain disclosure herein, the DLL4-binding agent comprises the heavy chain and light chain of the 21R79 antibody (with or without the leader sequence). In certain disclosure herein, the DLL4-binding agent is the 21R79 antibody.

**[0122]** In certain disclosure herein, a DLL4-binding agent comprises, consists essentially of, or consists of, the antibody 21R79.

**[0123]** In certain disclosure herein, the DLL4-binding agent comprises the heavy chain variable region and the light chain variable region of the 21R75 antibody. In certain disclosure herein, the DLL4-binding agent comprises the heavy chain and light chain of the 21R75 antibody (with or without the leader sequence). In certain disclosure herein, the DLL4-binding agent is the 21R75 antibody.

**[0124]** In certain disclosure herein a DLL4-binding agent comprises, consists essentially of, or consists of, the antibody 21R75.

**[0125]** In certain disclosure herein the DLL4-binding agent comprises the heavy chain variable region and the light chain variable region of the 21R83 antibody. In certain disclosure herein the DLL4-binding agent comprises the heavy chain and light chain of the 21R83 antibody (with or without the leader sequence). In certain disclosure herein, the DLL4-binding agent is the 21R83 antibody.

**[0126]** In certain disclosure herein, a DLL4-binding agent comprises, consists essentially of, or consists of, the antibody 21R83.

**[0127]** In some disclosure herein, a DLL4-binding agent binds an N-terminal fragment of human DLL4 (amino acids 1-191 of SEQ ID NO:24). In some disclosure herein, the DLL4-binding agent binds an epitope comprising amino acids 40-47 of SEQ ID NO:25. In some disclosure herein, the DLL4-binding agent binds an epitope comprising amino acids 113-120 of SEQ ID NO:25. In some disclosure herein, the DLL4-binding agent binds an epitope comprising amino acids 40-47 of SEQ ID NO:25 and amino acids 113-120 of SEQ ID NO:25.

**[0128]** In certain disclosure herein, a DLL4-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on DLL4 as an antibody of the disclosure . In other, disclosure herein , a DLL4-binding agent is an antibody that binds an epitope on DLL4 that overlaps with the epitope on DLL4 bound by an antibody of the disclosure . In certain disclosure herein, a DLL4-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on DLL4 as antibody 21R79. In other disclosure , the DLL4-binding agent is an antibody that binds an epitope on DLL4 that overlaps with the epitope on DLL4 bound by antibody 21R79. In certain disclosure herein, a DLL4-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on DLL4 as antibody 21R75. In other disclosure , the DLL4-binding agent is an antibody that binds an epitope on DLL4 that overlaps with the epitope on DLL4 bound by antibody 21R75. In certain disclosure herein, a DLL4-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on DLL4 as antibody 21R83. In other disclosure , the DLL4-binding agent is an antibody that binds an epitope on DLL4 that overlaps with the epitope on DLL4 bound by antibody 21R83.

**[0129]** In some disclosure herein, the DLL4-binding agent inhibits binding of DLL4 to at least one Notch receptor. In certain disclosure herein, the Notch receptor is Notch1, Notch2, Notch3, or Notch4. In some disclosure herein, the DLL4-binding agent specifically binds DLL4 and inhibits DLL4 activity. In some disclosure herein, the DLL4-binding agent specifically binds DLL4 and inhibits Notch signaling. In some disclosure herein, the DLL4-binding agent specifically binds DLL4 and modulates angiogenesis. In some disclosure herein, the DLL4-binding agent specifically binds DLL4 and inhibits tumor growth. In some disclosure herein, the DLL4-binding agent specifically binds DLL4 and inhibits tumorigenicity. In some disclosure herein, the DLL4-binding agent specifically binds DLL4 and reduces the number or frequency of CSCs in a tumor.

**[0130]** The disclosure also provides a VEGF/DLL4-binding agent that is a bispecific antibody. In

some disclosure herein, the VEGF/DLL4 binding agent is a bispecific antibody comprising a first antigen-binding site that specifically binds human VEGF. In some disclosure herein, the VEGF/DLL4 binding agent is a bispecific antibody comprising a first antigen-binding site that specifically binds human VEGF and a second antigen-binding site that binds a tumor-associated target. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human VEGF, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19). In some disclosure herein, the bispecific antibody further comprises: a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human VEGF, wherein the first antigen-binding site comprises (a) a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and (b) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0131]** In some disclosure herein, the VEGF/DLL4 binding agent is a bispecific antibody comprising a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:11. In some disclosure herein, the bispecific antibody further comprises a light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:11, and a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:12.

**[0132]** The disclosure also provides a VEGF/DLL4-binding agent that is a bispecific antibody. In some disclosure herein, the VEGF/DLL4 binding agent is a bispecific antibody comprising a first antigen-binding site that specifically binds human DLL4. In some disclosure herein, the VEGF/DLL4 binding agent is a bispecific antibody comprising a first antigen-binding site that specifically binds human DLL4 and a second antigen-binding site that binds a tumor-associated target. In some disclosure herein the VEGF/DLL4-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising YIX<sub>1</sub>X<sub>2</sub>YX<sub>3</sub>X<sub>4</sub>ATNYNQKFKG (SEQ ID NO:80), wherein X<sub>1</sub> is serine or alanine, X<sub>2</sub> is serine, asparagine, or glycine, X<sub>3</sub> is asparagine or lysine, and X<sub>4</sub> is glycine, arginine, or aspartic acid, and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein the VEGF/DLL4-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID

NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), YISSYNGATNYNQKFKG (SEQ ID NO:15), YIAGYKDATNYNQKFKG (SEQ ID NO:59), or YISYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein, the bispecific antibody comprises a first antigen-binding site comprising a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein, the bispecific antibody comprises a first antigen-binding site comprising a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISSYNGATNYNQKFKG (SEQ ID NO:15), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein the bispecific antibody comprises a first antigen-binding site comprising a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIAGYKDATNYNQKFKG (SEQ ID NO:59), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein, the bispecific antibody comprises a first antigen-binding site comprising a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16). In some disclosure herein, the bispecific antibody further comprises: a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising: a first antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises (a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), YISSYNGATNYNQKFKG (SEQ ID NO:15), YIAGYKDATNYNQKFKG (SEQ ID NO:59), or YISYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16), and (b) a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0133]** In some disclosure herein, the VEGF/DLL4 binding agent is a bispecific antibody comprising a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:58, or SEQ ID NO:64. In some disclosure herein, the bispecific antibody further comprises a light chain variable region having at least 80% sequence identity to SEQ ID NO: 12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:58, or SEQ ID NO:64; and/or a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO: 12.

**[0134]** The disclosure also provides a VEGF/DLL4-binding agent (e.g., a bispecific antibody) that specifically binds human VEGF and human DLL4. In some disclosure herein, the bispecific antibody comprises: a) a first antigen-binding site that specifically binds human VEGF, and b) a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO: 17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising

HYDDKYYPLMDY (SEQ ID NO: 19); wherein the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO: 13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising YIX<sub>1</sub>X<sub>2</sub>YX<sub>3</sub>X<sub>4</sub>ATNYNQKFKG (SEQ ID NO:80), wherein X<sub>1</sub> is serine or alanine, X<sub>2</sub> is serine, asparagine, or glycine, X<sub>3</sub> is asparagine or lysine, and X<sub>4</sub> is glycine, arginine, or aspartic acid, and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO: 16); and a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, a bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF, and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO: 19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), YISSYNGATNYNQKFKG (SEQ ID NO:15), YIAGYKDATNYNQKFKG (SEQ ID NO:59), or YISYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0135]** In some disclosure herein the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF, and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO: 17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO: 18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody is 219R45-MB-21R79.

**[0136]** In some disclosure herein, the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF, and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO: 17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO: 18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISSYNGATNYNQKFKG (SEQ ID NO: 15), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody is 219R45-M B-21 M18.

**[0137]** In some disclosure herein, the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF, and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site which comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIAGYKDATNYNQKFKG (SEQ ID NO:59), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody is 219R45-MB-21R75.

**[0138]** In some disclosure herein, the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF, and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISNYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody is 219R45-MB-21R83.

**[0139]** In some disclosure herein, the VEGF/DLL4 binding agent (e.g., a bispecific antibody) comprises a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:58, or SEQ ID NO:64, and a first and a second light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:11; a second heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:58, or SEQ ID NO:64; and a first and a second light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:9, and a first and a second light chain variable region having at least about 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region having at least about 95% sequence identity to SEQ ID NO: 11, a second heavy chain variable region having at least about 95% sequence identity to SEQ ID

NO:10, and a first and a second light chain variable region having at least about 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:58, and a first and a second light chain variable region having at least about 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:64, and a first and a second light chain variable region having at least about 95% sequence identity to SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region comprising SEQ ID NO:11, a second heavy chain variable region comprising SEQ ID NO:9, and a first and a second light chain variable region comprising SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region comprising SEQ ID NO:11, a second heavy chain variable region comprising SEQ ID NO:10, and a first and a second light chain variable region comprising SEQ ID NO:12. In certain disclosure herein the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region comprising SEQ ID NO:11, a second heavy chain variable region comprising SEQ ID NO:58, and a first and a second light chain variable region comprising SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region comprising SEQ ID NO:11, a second heavy chain variable region comprising SEQ ID NO:64, and a first and a second light chain variable region comprising SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region consisting essentially of SEQ ID NO:11, a second heavy chain variable region consisting essentially of SEQ ID NO:9, and a first and a second light chain variable region consisting essentially of SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region consisting essentially of SEQ ID NO:11, a second heavy chain variable region consisting essentially of SEQ ID NO:10, and a first and a second light chain variable region consisting essentially of SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region consisting essentially of SEQ ID NO:11, a second heavy chain variable region consisting essentially of SEQ ID NO:58, and a first and a second light chain variable region consisting essentially of SEQ ID NO:12. In certain disclosure herein, the bispecific VEGF/DLL4-binding agent comprises a first heavy chain variable region consisting essentially of SEQ ID NO:11, a second heavy chain variable region consisting essentially of SEQ ID NO:64, and a first and a second light chain variable region consisting essentially of SEQ ID NO:12.

**[0140]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-VEGF antibody 219R45. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-DLL4 antibody 21M18. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-DLL4 antibody 21R79. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-DLL4 antibody 21R75. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-DLL4 antibody 21R83. In some disclosure herein, the

VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-VEGF antibody 219R45, a heavy chain variable region from the anti-DLL4 antibody 21R79 and two identical light chain variable regions. In some disclosure herein the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-VEGF antibody 219R45, a heavy chain variable region from the anti-DLL4 antibody 21M18 and two identical light chain variable regions. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-VEGF antibody 219R45, a heavy chain variable region from the anti-DLL4 antibody 21R75 and two identical light chain variable regions. In some disclosure herein the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain variable region from the anti-VEGF antibody 219R45, a heavy chain variable region from the anti-DLL4 antibody 21R83 and two identical light chain variable regions.

**[0141]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a first CH3 domain and a second CH3 domain, each of which is modified to promote formation of heteromultimers. In some disclosure herein, the first and second CH3 domains are modified using a knobs-into-holes technique. In some disclosure herein, the first and second CH3 domains comprise changes in amino acids that result in altered electrostatic interactions. In some disclosure herein, the first and second CH3 domains comprise changes in amino acids that result in altered hydrophobic/hydrophilic interactions.

**[0142]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises heavy chain constant regions selected from the group consisting of: (a) a first human IgG1 constant region, wherein the amino acids at positions 253 and 292 are substituted with glutamate or aspartate, and a second human IgG1 constant region, wherein the amino acids at positions 240 and 282 are substituted with lysine; (b) a first human IgG2 constant region, wherein the amino acids at positions 249 and 288 are substituted with glutamate or aspartate, and a second human IgG2 constant region wherein the amino acids at positions 236 and 278 are substituted with lysine; (c) a first human IgG3 constant region, wherein the amino acids at positions 300 and 339 are substituted with glutamate or aspartate, and a second human IgG3 constant region wherein the amino acids at positions 287 and 329 are substituted with lysine; and (d) a first human IgG4 constant region, wherein the amino acids at positions 250 and 289 are substituted with glutamate or aspartate, and a second IgG4 constant region wherein the amino acids at positions 237 and 279 are substituted with lysine.

**[0143]** In some disclosure herein the VEGF/DLL4-binding agent is a bispecific antibody which comprises a first human IgG1 constant region with amino acid substitutions at positions 253 and 292, wherein the amino acids are glutamate or aspartate, and a second human IgG1 constant region with amino acid substitutions at positions 240 and 282, wherein the amino acids are lysine. In some disclosure herein the VEGF/DLL4-binding agent is a bispecific antibody which comprises a first human IgG2 constant region with amino acid substitutions at positions 249 and 288, wherein the amino acids are glutamate or aspartate, and a second human IgG2 constant region with amino acid substitutions at positions 236 and 278, wherein the amino acids are lysine. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a first human IgG3 constant region with amino acid substitutions at positions 300 and 339, wherein

the amino acids are glutamate or aspartate, and a second human IgG2 constant region with amino acid substitutions at positions 287 and 329, wherein the amino acids are lysine. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a first human IgG4 constant region with amino acid substitutions at positions 250 and 289, wherein the amino acids are glutamate or aspartate, and a second human IgG4 constant region with amino acid substitutions at positions 237 and 279, wherein the amino acids are lysine.

**[0144]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a first human IgG2 constant region with amino acid substitutions at positions 249 and 288, wherein the amino acids are glutamate, and a second human IgG2 constant region with amino acid substitutions at positions 236 and 278, wherein the amino acids are lysine. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a first human IgG2 constant region with amino acid substitutions at positions 249 and 288, wherein the amino acids are asparate, and a second human IgG2 constant region with amino acid substitutions at positions 236 and 278, wherein the amino acids are lysine.

**[0145]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain of SEQ ID NO:7. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain of SEQ ID NO:5. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain of SEQ ID NO:56. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain of SEQ ID NO:62. In some disclosure herein, the bispecific antibody further comprises a light chain of SEQ ID NO:12. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain of SEQ ID NO:7, a heavy chain of SEQ ID NO:5, and two light chains of SEQ ID NO:8. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain of SEQ ID NO:7, a heavy chain of SEQ ID NO:6, and two light chains of SEQ ID NO:8. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain of SEQ ID NO:7, a heavy chain of SEQ ID NO:56, and two light chains of SEQ ID NO:8. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises a heavy chain of SEQ ID NO:7, a heavy chain of SEQ ID NO:62, and two light chains of SEQ ID NO:8.

**[0146]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which binds VEGF with a  $K_D$  of about 50nM or less, about 25nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which binds DLL4 with a  $K_D$  of about 50nM or less, about 25nM or less, about 10nM or less, about 1nM or less, or about 0.1nM or less. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which binds VEGF with a  $K_D$  of about 50nM or less and binds DLL4 with a  $K_D$  of about 50nM or less. In some disclosure herein the bispecific antibody binds VEGF with a  $K_D$  of about 25nM or less and binds DLL4 with a  $K_D$  of about 25nM or less. In some disclosure herein, the bispecific antibody binds VEGF with a  $K_D$  of about 10nM or less and binds DLL4 with a  $K_D$  of about 10nM or less. In some disclosure herein, the bispecific antibody binds VEGF with a  $K_D$  of about 1nM or less and binds DLL4 with a  $K_D$  of about 1nM or less.

less.

**[0147]** In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody which comprises one antigen-binding site with a binding affinity that is weaker than the binding affinity of the second antigen-binding site. For example, in some disclosure herein the bispecific antibody may bind VEGF with a  $K_D$  ranging from about 0.1nM to 1nM and may bind DLL4 with a  $K_D$  ranging from about 1nM to 10nM. Or the bispecific antibody may bind VEGF with a  $K_D$  ranging from about 1nM to 10nM and may bind DLL4 with a  $K_D$  ranging from about 0.1nM to 1nM. In some disclosure herein the bispecific antibody may bind DLL4 with a  $K_D$  ranging from about 0.1nM to 1nM and may bind VEGF with a  $K_D$  ranging from about 1nM to 10nM. Or the bispecific antibody may bind DLL4 with a  $K_D$  ranging from about 1nM to 10nM and may bind VEGF with a  $K_D$  ranging from about 0.1nM to 1nM. In some disclosure herein, the difference in affinity between the two antigen-binding sites may be about 2-fold or more, about 3-fold or more, about 5-fold or more, about 8-fold or more, about 10-fold or more, about 15-fold or more, about 30-fold or more, about 50-fold or more, or about 100-fold or more. In some disclosure herein at least one amino acid residue in at least one CDR of the antigen-binding site for VEGF is substituted with a different amino acid so that the affinity of the VEGF-binding site is altered. In some disclosure herein the affinity of the VEGF-binding site is increased. In some disclosure herein, the affinity of the VEGF-binding site is decreased. In some disclosure herein at least one amino acid residue in at least one CDR of the antigen-binding site for DLL4 is substituted with a different amino acid so that the affinity of the DLL4-binding site is altered. In some disclosure herein the affinity of the DLL4-binding site is increased. In some disclosure herein, the affinity of the DLL4-binding site is decreased. In some disclosure herein the affinities of both the VEGF and DLL4 antigen-binding sites are altered.

**[0148]** The disclosure provides polypeptides, including but not limited to antibodies, that specifically bind VEGF and/or DLL4. In some disclosure herein, a polypeptide binds human VEGF. In some disclosure herein, a polypeptide binds human DLL4. In some disclosure herein, a polypeptide binds human VEGF and mouse VEGF. In some disclosure herein a polypeptide binds human DLL4 and mouse DLL4.

**[0149]** In some disclosure herein, a VEGF-binding agent comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:47, and SEQ ID NO:49.

**[0150]** In some disclosure herein a DLL4-binding agent comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ NO ID:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64.

**[0151]** In some disclosure herein, a VEGF/DLL4-binding agent comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:62, SEQ ID NO:63,

and SEQ ID NO:64.

**[0152]** In some disclosure herein, a VEGF/DLL4-binding agent comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64. In some disclosure herein the VEGF/DLL4 binding agent further comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:47, and SEQ ID NO:49. In some disclosure herein, the VEGF/DLL4 binding agent further comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:8, and SEQ ID NO:12.

**[0153]** In some disclosure herein, a VEGF/DLL4-binding agent comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:47, and SEQ ID NO:49. In some disclosure herein the VEGF/DLL4 binding agent further comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64. In some disclosure herein, the VEGF/DLL4 binding agent further comprises a polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:8, and SEQ ID NO:12.

**[0154]** In certain disclosure herein, a VEGF/DLL4-binding agent (e.g., antibody) competes for specific binding to VEGF with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:11 and a light chain variable region comprising SEQ ID NO:12. In certain disclosure herein, a VEGF/DLL4-binding agent competes with antibody 219R45 for specific binding to human VEGF. In some disclosure herein, a VEGF/DLL4-binding agent or antibody competes for specific binding to VEGF in an *in vitro* competitive binding assay. In some disclosure herein, the VEGF is human VEGF. In some disclosure herein, the VEGF is mouse VEGF.

**[0155]** In certain disclosure herein a VEGF-DLL4-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on VEGF as an antibody of the disclosure . In other disclosure , a VEGF/DLL4-binding agent is an antibody that binds an epitope on VEGF that overlaps with the epitope on VEGF bound by an antibody of the disclosure . In certain disclosure herein a VEGF/DLL4-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on VEGF as antibody 219R45. In other disclosure , the VEGF/DLL4-binding agent is an antibody that binds an epitope on VEGF that overlaps with the epitope on VEGF bound by antibody 219R45.

**[0156]** In certain disclosure herein, the VEGF/DLL4-binding agent is an agent that competes for specific binding to VEGF with the antibody 219R45 (e.g., in a competitive binding assay).

**[0157]** In certain disclosure herein, a VEGF/DLL4-binding agent (e.g., antibody) competes for specific binding to DLL4 with an antibody that comprises a heavy chain variable region comprising SEQ ID NO:9 SEQ ID NO:10, SEQ ID NO:58, or SEQ ID NO:64 and a light chain variable region

comprising SEQ ID NO:12. In certain disclosure herein, a VEGF/DLL4-binding agent competes with antibody 21R79 for specific binding to human DLL4. In certain disclosure herein, a VEGF/DLL4-binding agent competes with antibody 21R75 for specific binding to human DLL4. In certain disclosure herein, a VEGF/DLL4-binding agent competes with antibody 21R83 for specific binding to human DLL4. In some disclosure herein, a VEGF/DLL4-binding agent or antibody competes for specific binding to DLL4 in an *in vitro* competitive binding assay. In some disclosure herein, the DLL4 is human DLL4. In some disclosure herein, the DLL4 is mouse DLL4.

**[0158]** In certain disclosure herein a VEGF/DLL4-binding agent (e.g., an antibody) binds the same epitope, or essentially the same epitope, on DLL4 as an antibody of the disclosure . In other disclosure , a VEGF/DLL4-binding agent is an antibody that binds an epitope on DLL4 that overlaps with the epitope on DLL4 bound by an antibody of the disclosure . In certain disclosure herein, a VEGF/DLL4-binding agent binds the same epitope, or essentially the same epitope, on DLL4 as antibody 21R79. In certain disclosure herein, a VEGF/DLL4-binding agent binds the same epitope, or essentially the same epitope, on DLL4 as antibody 21R75. In certain disclosure herein, a VEGF/DLL4-binding agent binds the same epitope, or essentially the same epitope, on DLL4 as antibody 21R83. In other disclosure , the VEGF/DLL4-binding agent is an antibody that binds an epitope on DLL4 that overlaps with the epitope on DLL4 bound by antibody 21R79. In other disclosure , the VEGF/DLL4-binding agent is an antibody that binds an epitope on DLL4 that overlaps with the epitope on DLL4 bound by antibody 21R75. In other disclosure , the VEGF/DLL4-binding agent is an antibody that binds an epitope on DLL4 that overlaps with the epitope on DLL4 bound by antibody 21R83.

**[0159]** In certain disclosure herein, the VEGF/DLL4-binding agent is an agent that competes for specific binding to DLL4 with the antibody 21R79 (e.g., in a competitive binding assay). In certain disclosure herein, the VEGF/DLL4-binding agent is an agent that competes for specific binding to DLL4 with the antibody 21R75 (e.g., in a competitive binding assay). In certain disclosure herein the VEGF/DLL4-binding agent is an agent that competes for specific binding to DLL4 with the antibody 21R83 (e.g., in a competitive binding assay). In certain disclosure herein the VEGF/DLL4-binding agent is an agent that competes for specific binding to DLL4 with the antibody 21M18 (e.g., in a competitive binding assay).

**[0160]** In certain disclosure herein the VEGF/DLL4-binding agent is an agent that competes for specific binding to VEGF and/or DLL4 with the bispecific antibody 219R45-MB-21M18 (e.g., in a competitive binding assay). In certain disclosure herein the VEGF/DLL4-binding agent is an agent that competes for specific binding to VEGF and/or DLL4 with the bispecific antibody 219R45-MB-21M79 (e.g., in a competitive binding assay). In certain disclosure herein the VEGF/DLL4-binding agent is an agent that competes for specific binding to VEGF and/or DLL4 with the bispecific antibody 219R45-MB-21M75 (e.g., in a competitive binding assay). In certain disclosure herein, the VEGF/DLL4-binding agent is an agent that competes for specific binding to VEGF and/or DLL4 with the bispecific antibody 219R45-MB-21M83 (e.g., in a competitive binding assay).

**[0161]** In certain disclosure herein, the VEGF/DLL4-binding agent (e.g., an antibody) described herein binds VEGF and modulates VEGF activity. In some disclosure herein the VEGF/DLL4-binding agent is a VEGF antagonist and inhibits VEGF activity. In some disclosure herein, the VEGF/DLL4-binding agent is a VEGF antagonist and modulates angiogenesis. In some disclosure

herein, the VEGF/DLL4-binding agent is a VEGF antagonist and inhibits angiogenesis. In some disclosure herein the VEGF/DLL4-binding agent is a VEGF antagonist and inhibits tumor growth.

**[0162]** In certain disclosure herein, a VEGF/DLL4-binding agent (e.g., an antibody) described herein binds human DLL4 and modulates DLL4 activity. In some disclosure herein a VEGF/DLL4-binding agent is a DLL4 antagonist and inhibits DLL4 activity. In some disclosure herein, a VEGF/DLL4-binding agent is a DLL4 antagonist and inhibits Notch activity. In some disclosure herein, a VEGF/DLL4-binding agent is a DLL4 antagonist and inhibits Notch signaling. In some disclosure herein a VEGF/DLL4-binding agent is a DLL4 antagonist and modulates angiogenesis. In some disclosure herein a VEGF/DLL4-binding agent is a DLL4 antagonist and promotes aberrant angiogenesis. In some disclosure herein, a VEGF/DLL4-binding agent is a DLL4 antagonist and inhibits tumor growth.

**[0163]** In certain disclosure herein , a VEGF/DLL4-binding agent (e.g., an antibody) described herein is a bispecific antibody that binds human VEGF and modulates VEGF activity. In certain disclosure herein a VEGF/DLL4-binding agent (e.g., an antibody) described herein is a bispecific antibody that binds human DLL4 and modulates DLL4 activity. In certain disclosure herein, a VEGF/DLL4-binding agent (e.g., an antibody) described herein is a bispecific antibody that binds human VEGF and human DLL4 and modulates both VEGF and DLL4 activity. In some disclosure herein the bispecific antibody is a VEGF antagonist and a DLL4 antagonist and inhibits both VEGF activity and DLL4 activity. In some disclosure herein, the bispecific antibody is a VEGF antagonist and a DLL4 antagonist and inhibits VEGF activity and Notch activity. In some disclosure herein the bispecific antibody is a VEGF antagonist and a DLL4 antagonist and inhibits VEGF activity and Notch signaling. In some disclosure herein the bispecific antibody is a VEGF antagonist and a DLL4 antagonist and modulates angiogenesis. In some disclosure herein, the bispecific antibody is a VEGF antagonist and a DLL4 antagonist and promotes aberrant angiogenesis. In some disclosure herein, the bispecific antibody is a VEGF antagonist and a DLL4 antagonist and inhibits angiogenesis. In some disclosure herein, the bispecific antibody is a VEGF antagonist and a DLL4 antagonist and inhibits tumor growth.

**[0164]** In certain disclosure herein the VEGF/DLL4-binding agent (e.g., an antibody or a bispecific antibody) is an antagonist of VEGF. In some disclosure herein the VEGF/DLL4-binding agent is an antagonist of VEGF and inhibits VEGF activity. In certain disclosure herein, the VEGF/DLL4-binding agent inhibits VEGF activity by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human VEGF activity is antibody 219R45. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human VEGF activity is a bispecific antibody comprising the antigen-binding site of 219R45. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human VEGF activity is the bispecific antibody 219R45-MB-21M18. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human VEGF activity is the bispecific antibody 219R45-MB-21R79. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human VEGF activity is the bispecific antibody 219R45-MB-21R75. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits human VEGF activity is the bispecific antibody 219R45-MB-21R83.

**[0165]** In certain disclosure herein, the VEGF/DLL4-binding agent (e.g., an antibody) is an antagonist of DLL4. In some disclosure herein, the VEGF/DLL4-binding agent is an antagonist of DLL4 and inhibits DLL4 activity. In certain disclosure herein the VEGF/DLL4-binding agent inhibits DLL4 activity by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human DLL4 activity is antibody 21R79. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits human DLL4 activity is antibody 21R75. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits human DLL4 activity is antibody 21R83. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human DLL4 activity is a bispecific antibody comprising the antigen-binding site of 21R79. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits human DLL4 activity is a bispecific antibody comprising the antigen-binding site of 21R75. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human DLL4 activity is a bispecific antibody comprising the antigen-binding site of 21R83. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human DLL4 activity is the bispecific antibody 219R45-MB-21M18. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits human DLL4 activity is the bispecific antibody 219R45-MB-21R79. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits human DLL4 activity is the bispecific antibody 219R45-MB-21R75. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits human DLL4 activity is the bispecific antibody 219R45-MB-21R83.

**[0166]** In certain disclosure herein the VEGF/DLL4-binding agent (e.g., antibody) is an antagonist of Notch signaling. In certain disclosure herein the VEGF/DLL4-binding agent inhibits Notch signaling by at least about 10%, at least about 20%, at least about 30%, at least about 50%, at least about 75%, at least about 90%, or about 100%. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits Notch signaling is antibody 21R79. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits Notch signaling is antibody 21R75. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits Notch signaling is antibody 21R83. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits Notch signaling is a bispecific antibody comprising the antigen-binding site of 21R79. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits Notch signaling is a bispecific antibody comprising the antigen-binding site of 21R75. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits Notch signaling is a bispecific antibody comprising the antigen-binding site of 21R83. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits Notch signaling is the bispecific antibody 219R45-MB-21M18. In certain disclosure herein a VEGF/DLL4-binding agent that inhibits Notch signaling is the bispecific antibody 219R45-MB-21R79. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits Notch signaling is the bispecific antibody 219R45-MB-21R75. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits Notch signaling is the bispecific antibody 219R45-MB-21R83.

**[0167]** In certain disclosure herein the VEGF/DLL4-binding agent (e.g., antibody) inhibits binding of VEGF to at least one receptor. In some disclosure herein the VEGF/DLL4-binding agent inhibits binding of VEGF to VEGFR-1 or VEGFR-2. In certain disclosure herein the VEGF/DLL4-binding agent inhibits binding of VEGF to at least one VEGF receptor by at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human VEGF to at least one VEGF receptor is antibody 219R45. In certain disclosure herein, a VEGF/DLL4-binding agent that

inhibits binding of human VEGF to at least one VEGF receptor is a bispecific antibody comprising the antigen-binding site of 219R45. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human VEGF to at least one VEGF receptor is the bispecific antibody 219R45-MB-21M18. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human VEGF to at least one VEGF receptor is the bispecific antibody 219R45-MB-21R79. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human VEGF to at least one VEGF receptor is the bispecific antibody 219R45-MB-21R75. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human VEGF to at least one VEGF receptor is the bispecific antibody 219R45-MB-21R83.

**[0168]** In certain disclosure herein, the VEGF/DLL4-binding agent (e.g., antibody) inhibits binding of DLL4 protein to at least one Notch receptor. In some disclosure herein, the VEGF/DLL4-binding agent inhibits binding of DLL4 to Notch 1, Notch2, Notch3, and/or Notch4. In certain disclosure herein, the VEGF/DLL4-binding agent inhibits binding of DLL4 to at least one Notch receptor by at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, or at least about 95%. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is antibody 21R79. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is antibody 21R75. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is antibody 21R83. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is a bispecific antibody comprising the antigen-binding site of 21R79. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is a bispecific antibody comprising the antigen-binding site of 21R75. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is a bispecific antibody comprising the antigen-binding site of 21R83. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is the bispecific antibody 219R45-MB-21M18. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is the bispecific antibody 219R45-MB-21R79. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is the bispecific antibody 219R45-MB-21R75. In certain disclosure herein, a VEGF/DLL4-binding agent that inhibits binding of human DLL4 to at least one Notch receptor is the bispecific antibody 219R45-MB-21R83.

**[0169]** *In vivo* and *in vitro* assays for determining whether a VEGF/DLL4-binding agent (or candidate VEGF/DLL4-binding agent) inhibits VEGF or affects angiogenesis are known in the art. *In vitro* assays of angiogenesis include but are not limited to, HUVEC proliferation assays, endothelial cell tube formation assays, sprouting (or sprout formation) assays, HUVEC cell migration assays, and invasion assays. In some disclosure herein, cells in the presence of VEGF and the presence of a VEGF/DLL4-binding agent are compared to cells in the presence of VEGF without the VEGF/DLL4-binding agent present, and evaluated for effects on angiogenesis (or biological effects associated with angiogenesis). *In vivo* assays of angiogenesis include, but are not limited to, matrigel plug assays, corneal micropocket assays, and chicken chorioallantoic membrane (CAM) assays.

**[0170]** *In vivo* and *in vitro* assays for determining whether a VEGF/DLL4-binding agent (or candidate VEGF/DLL4-binding agent) inhibits Notch activation or signaling are known in the art. For example, cell-based, luciferase reporter assays utilizing a TCF/Luc reporter vector containing multiple copies of the TCF-binding domain upstream of a firefly luciferase reporter gene may be used to measure Notch signaling levels *in vitro* (Gazit et al., 1999, Oncogene, 18; 5959-66; TOPflash, Millipore, Billerica MA). In some disclosure herein, a cell-based, luciferase reporter assay utilizing a CBF/Luc reporter vector containing multiple copies of the CBF-binding domain upstream of a firefly luciferase report genes may be used. The level of Notch signaling in the presence of one or more Notch ligands (e.g., DLL4 expressed on the surface of transfected cells or soluble DLL4-Fc fusion protein) and in the presence of a VEGF/DLL4-binding agent is compared to the level of Notch signaling without the VEGF/DLL4-binding agent present.

**[0171]** In certain disclosure herein, the VEGF/DLL4-binding agents have one or more of the following effects: inhibit proliferation of tumor cells, inhibit tumor growth, reduce the tumorigenicity of a tumor, reduce the frequency of cancer stem cells in a tumor, trigger cell death of tumor cells, prevent metastasis of tumor cells, decrease survival of tumor cells, modulate angiogenesis, inhibit angiogenesis, inhibit productive angiogenesis, or promote aberrant angiogenesis.

**[0172]** In certain disclosure herein the VEGF/DLL4-binding agents are capable of inhibiting tumor growth. In certain disclosure herein, the VEGF/DLL4-binding agents are capable of inhibiting tumor growth *in vivo* (e.g., in a xenograft mouse model, and/or in a human having cancer). In certain disclosure herein, tumor growth is inhibited at least about two-fold, about three-fold, about five-fold, about ten-fold, about 50-fold, about 100-fold, or about 1000-fold as compared to an untreated tumor.

**[0173]** In certain disclosure herein, the VEGF/DLL4-binding agents are capable of reducing the tumorigenicity of a tumor. In certain disclosure herein, the VEGF/DLL4-binding agent or antibody is capable of reducing the tumorigenicity of a tumor comprising cancer stem cells in an animal model, such as a mouse xenograft model. In certain disclosure herein the VEGF/DLL4-binding agent or antibody is capable of reducing the tumorigenicity of a tumor by decreasing the number or frequency of cancer stem cells in the tumor. In certain disclosure herein, the number or frequency of cancer stem cells in a tumor is reduced by at least about two-fold, about three-fold, about five-fold, about ten-fold, about 50-fold, about 100-fold, or about 1000-fold. In certain disclosure herein the reduction in the number or frequency of cancer stem cells is determined by limiting dilution assay using an animal model. Additional examples and guidance regarding the use of limiting dilution assays to determine a reduction in the number or frequency of cancer stem cells in a tumor can be found, e.g., in International Publication Number WO 2008/042236; U.S. Patent Publication No. 2008/0064049; and U.S. Patent Publication No. 2008/0178305.

**[0174]** In certain disclosure herein, the VEGF/DLL4-binding agents are capable of modulating angiogenesis. In certain disclosure herein, the VEGF/DLL4-binding agents are capable of modulating angiogenesis *in vivo* (e.g., in a xenograft mouse model, and/or in a human having cancer). In certain disclosure herein, VEGF/DLL4-binding agents are capable of inhibiting angiogenesis. In certain disclosure herein, VEGF/DLL4-binding agents are capable of promoting aberrant angiogenesis. In certain disclosure herein, VEGF/DLL4-binding agents are capable of inhibiting angiogenesis and/or promoting aberrant angiogenesis, leading to unproductive

vascularization.

**[0175]** In certain disclosure herein, the VEGF/DLL4-binding agents described herein have a circulating half-life in mice, cynomolgus monkeys, or humans of at least about 2 hours, at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. In certain disclosure herein, the VEGF/DLL4-binding agent is an IgG (e.g., IgG1 or IgG2) antibody that has a circulating half-life in mice, cynomolgus monkeys, or humans of at least about 2 hours, at least about 5 hours, at least about 10 hours, at least about 24 hours, at least about 3 days, at least about 1 week, or at least about 2 weeks. Methods of increasing (or decreasing) the half-life of agents such as polypeptides and antibodies are known in the art. For example, known methods of increasing the circulating half-life of IgG antibodies include the introduction of mutations in the Fc region which increase the pH-dependent binding of the antibody to the neonatal Fc receptor (FcRn) at pH 6.0 (see, e.g., U.S. Patent Publication Nos. 2005/0276799, 2007/0148164, and 2007/0122403). Known methods of increasing the circulating half-life of antibody fragments lacking the Fc region include such techniques as PEGylation.

**[0176]** In some disclosure herein the VEGF/DLL4-binding agents are antibodies. Polyclonal antibodies can be prepared by any known method. In some disclosure herein, polyclonal antibodies are produced by immunizing an animal (e.g., a rabbit, rat, mouse, goat, donkey) with an antigen of interest (e.g., a purified peptide fragment, full-length recombinant protein, or fusion protein) by multiple subcutaneous or intraperitoneal injections. The antigen can be optionally conjugated to a carrier such as keyhole limpet hemocyanin (KLH) or serum albumin. The antigen (with or without a carrier protein) is diluted in sterile saline and usually combined with an adjuvant (e.g., Complete or Incomplete Freund's Adjuvant) to form a stable emulsion. After a sufficient period of time, polyclonal antibodies are recovered from the immunized animal, usually from blood or ascites. The polyclonal antibodies can be purified from serum or ascites according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

**[0177]** In some disclosure herein, the VEGF/DLL4-binding agents are monoclonal antibodies. Monoclonal antibodies can be prepared using hybridoma methods known to one of skill in the art (see e.g., Kohler and Milstein, 1975, *Nature*, 256:495-497). In some disclosure herein, using the hybridoma method, a mouse, hamster, or other appropriate host animal, is immunized as described above to elicit from lymphocytes the production of antibodies that specifically bind the immunizing antigen. In some disclosure herein, lymphocytes can be immunized *in vitro*. In some disclosure herein, the immunizing antigen can be a human protein or a portion thereof. In some disclosure herein, the immunizing antigen can be a mouse protein or a portion thereof.

**[0178]** Following immunization, lymphocytes are isolated and fused with a suitable myeloma cell line using, for example, polyethylene glycol. The hybridoma cells are selected using specialized media as known in the art and unfused lymphocytes and myeloma cells do not survive the selection process. Hybridomas that produce monoclonal antibodies directed specifically against a chosen antigen may be identified by a variety of methods including, but not limited to, immunoprecipitation, immunoblotting, and *in vitro* binding assays (e.g., flow cytometry, FACS,

ELISA, and radioimmunoassay). The hybridomas can be propagated either in *in vitro* culture using standard methods (J.W. Goding, 1996, *Monoclonal Antibodies: Principles and Practice*, 3rd Edition, Academic Press, San Diego, CA) or *in vivo* as ascites tumors in an animal. The monoclonal antibodies can be purified from the culture medium or ascites fluid according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

**[0179]** In certain disclosure herein monoclonal antibodies can be made using recombinant DNA techniques as known to one skilled in the art. The polynucleotides encoding a monoclonal antibody are isolated from mature B-cells or hybridoma cells, such as by RT-PCR using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using standard techniques. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors which produce the monoclonal antibodies when transfected into host cells such as *E. coli*, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin proteins.

**[0180]** In certain other disclosure herein recombinant monoclonal antibodies, or fragments thereof, can be isolated from phage display libraries expressing variable domains or CDRs of a desired species (see e.g., McCafferty et al., 1990, *Nature*, 348:552-554; Clackson et al., 1991, *Nature*, 352:624-628; and Marks et al., 1991, *J. Mol. Biol.*, 222:581-597).

**[0181]** The polynucleotide(s) encoding a monoclonal antibody can be modified, for example, by using recombinant DNA technology to generate alternative antibodies. In some disclosure herein the constant domains of the light and heavy chains of, for example, a mouse monoclonal antibody can be substituted for those regions of, for example, a human antibody to generate a chimeric antibody, or for a non-immunoglobulin polypeptide to generate a fusion antibody. In some disclosure herein, the constant regions are truncated or removed to generate the desired antibody fragment of a monoclonal antibody. Site-directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of a monoclonal antibody.

**[0182]** In some disclosure herein a monoclonal antibody against VEGF and/or DLL4 is a humanized antibody. Typically, humanized antibodies are human immunoglobulins in which residues from the CDRs are replaced by residues from a CDR of a non-human species (e.g., mouse, rat, rabbit, hamster, etc.) that have the desired specificity, affinity, and/or binding capability using methods known to one skilled in the art. In some disclosure herein the Fv framework region residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species that has the desired specificity, affinity, and/or binding capability. In some disclosure herein, a humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or capability. In general, a humanized antibody will comprise substantially all of at least one, and typically two or three, variable domain regions containing all, or substantially all, of the CDRs that correspond to the non-human immunoglobulin whereas all, or substantially all, of the framework regions are those of a human immunoglobulin consensus sequence. In some disclosure herein, a humanized antibody can also comprise at least a portion of an immunoglobulin constant region or domain (Fc),

typically that of a human immunoglobulin. In certain disclosure herein such humanized antibodies are used therapeutically because they may reduce antigenicity and HAMA (human anti-mouse antibody) responses when administered to a human subject. One skilled in the art would be able to obtain a functional humanized antibody with reduced immunogenicity following known techniques (see e.g., U.S. Patent Nos. 5,225,539; 5,585,089; 5,693,761; and 5,693,762).

**[0183]** In certain disclosure herein, the VEGF/DLL4-binding agent is a human antibody. Human antibodies can be directly prepared using various techniques known in the art. In some disclosure herein human antibodies may be generated from immortalized human B lymphocytes immunized *in vitro* or from lymphocytes isolated from an immunized individual. In either case, cells that produce an antibody directed against a target antigen can be generated and isolated (see, e.g., Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77; Boemer et al., 1991, *J. Immunol.*, 147:86-95; and U.S. Patent Nos. 5,750,373; 5,567,610; and 5,229,275). In some disclosure herein, the human antibody can be selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., 1996, *Nature Biotechnology*, 14:309-314; Sheets et al., 1998, *PNAS*, 95:6157-6162; Hoogenboom and Winter, 1991, *J. Mol. Biol.*, 227:381; Marks et al., 1991, *J. Mol. Biol.*, 222:581). Alternatively, phage display technology can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable domain gene repertoires from unimmunized donors. Techniques for the generation and use of antibody phage libraries are also described in U.S. Patent Nos. 5,969,108; 6,172,197; 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915; 6,593,081; 6,300,064; 6,653,068; 6,706,484; and 7,264,963; and Rothe et al., 2008, *J. Mol. Bio.*, 376:1182-1200. Once antibodies are identified, affinity maturation strategies known in the art, including but not limited to, chain shuffling (Marks et al., 1992, *Bio/Technology*, 10:779-783) and site-directed mutagenesis, may be employed to generate high affinity human antibodies.

**[0184]** In some disclosure herein, human antibodies can be made in transgenic mice that contain human immunoglobulin loci. Upon immunization these mice are capable of producing the full repertoire of human antibodies in the absence of endogenous immunoglobulin production. This approach is described in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016.

**[0185]** This disclosure also encompasses bispecific antibodies. Bispecific antibodies are capable of specifically recognizing and binding at least two different antigens or epitopes. The different epitopes can either be within the same molecule (e.g., two epitopes on a single protein) or on different molecules (e.g., one epitope on a protein and one epitope on a second protein). In some disclosure herein, a bispecific antibody has enhanced potency as compared to an individual antibody or to a combination of more than one antibody. In some disclosure herein, a bispecific antibody has reduced toxicity as compared to an individual antibody or to a combination of more than one antibody. It is known to those of skill in the art that any binding agent (e.g., antibody) may have unique pharmacokinetics (PK) (e.g., circulating half-life). In some disclosure herein, a bispecific antibody has the ability to synchronize the PK of two active binding agents wherein the two individual binding agents have different PK profiles. In some disclosure herein, a bispecific antibody has the ability to concentrate the actions of two binding agents (e.g., antibodies) in a common area (e.g., a tumor and/or tumor environment). In some disclosure herein a bispecific

antibody has the ability to concentrate the actions of two binding agents (e.g., antibodies) to a common target (e.g., a tumor or a tumor cell). In some disclosure herein, a bispecific antibody has the ability to target the actions of two binding agents (e.g., antibodies) to more than one biological pathway or function.

**[0186]** In certain disclosure herein the bispecific antibody specifically binds VEGF and a second target. In certain disclosure herein, the bispecific antibody specifically binds DLL4 and a second target. In certain disclosure herein, the bispecific antibody specifically binds VEGF and DLL4. In some disclosure herein, the bispecific antibody specifically binds human VEGF and human DLL4. In some disclosure herein the bispecific antibody is a monoclonal human or a humanized antibody. In some disclosure herein the bispecific antibody inhibits angiogenesis and reduces cancer stem cell number or frequency. In some disclosure herein, the bispecific antibody inhibits blood vessel growth and inhibits blood vessel maturation. In some disclosure herein the bispecific antibody prevents endothelial hyperproliferation. In some disclosure herein, the bispecific antibody has decreased toxicity and/or side effects. In some disclosure herein the bispecific antibody has decreased toxicity and/or side effects as compared to a mixture of the two individual antibodies or the antibodies as single agents. In some disclosure herein, the bispecific antibody has an increased therapeutic index. In some disclosure herein, the bispecific antibody has an increased therapeutic index as compared to a mixture of the two individual antibodies or the antibodies as single agents.

**[0187]** In some disclosure herein, the bispecific antibody can specifically recognize and bind a first antigen target, (e.g., DLL4) as well as a second antigen target, such as an effector molecule on a leukocyte (e.g., CD2, CD3, CD28, or B7) or a Fc receptor (e.g., CD64, CD32, or CD16) so as to focus cellular defense mechanisms to the cell expressing the first antigen target. In some disclosure herein the bispecific antibodies can be used to direct cytotoxic agents to cells which express a particular target antigen. These antibodies possess an antigen-binding site (e.g., to human DLL4) and a second site which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA.

**[0188]** Techniques for making bispecific antibodies are known by those skilled in the art, see for example, Millstein et al., 1983, *Nature*, 305:537-539; Brennan et al., 1985, *Science*, 229:81; Suresh et al., 1986, *Methods in Enzymol.*, 121:120; Traunecker et al., 1991, *EMBO J.*, 10:3655-3659; Shalaby et al., 1992, *J. Exp. Med.*, 175:217-225; Kostelny et al., 1992, *J. Immunol.*, 148:1547-1553; Gruber et al., 1994, *J. Immunol.*, 152:5368; U.S. Patent No. 5,731,168; International Publication No. WO 2009/089004; and U.S. Patent Publication No. 2011/0123532. In some disclosure herein, the bispecific antibodies comprise heavy chain constant regions with modifications in the amino acids which are part of the interface between the two heavy chains. In some disclosure herein the bispecific antibodies can be generated using a "knobs-into-holes" strategy (see, e.g., U.S. Patent No. 5,731,168; Ridgway et. al., 1996, *Prot. Engin.*, 9:617-621). At times the "knobs" and "holes" terminology is replaced with the terms "protuberances" and "cavities". In some disclosure herein, the bispecific antibodies may comprise variant hinge regions incapable of forming disulfide linkages between the heavy chains (see, e.g., WO 2006/028936). In some disclosure herein, the modifications may comprise changes in amino acids that result in altered electrostatic interactions. In some disclosure herein the modifications may comprise changes in amino acids that result in altered hydrophobic/hydrophilic interactions.

**[0189]** Bispecific antibodies can be intact antibodies or antibody fragments comprising antigen-binding sites. Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared (Tutt et al., 1991, *J. Immunol.*, 147:60). Thus, in certain disclosure herein the antibodies to VEGF and/or DLL4 are multispecific.

**[0190]** In certain disclosure herein, the antibodies (or other polypeptides) described herein may be monospecific. In certain disclosure herein each of the one or more antigen-binding sites that an antibody contains is capable of binding (or binds) a homologous epitope on different proteins.

**[0191]** In certain disclosure herein the VEGF/DLL4-binding agent is an antibody fragment. Antibody fragments may have different functions or capabilities than intact antibodies; for example, antibody fragments can have increased tumor penetration. Various techniques are known for the production of antibody fragments including, but not limited to, proteolytic digestion of intact antibodies. In some disclosure herein, antibody fragments include a F(ab')2 fragment produced by pepsin digestion of an antibody molecule. In some disclosure herein antibody fragments include a Fab fragment generated by reducing the disulfide bridges of an F(ab')2 fragment. In other disclosure herein, antibody fragments include a Fab fragment generated by the treatment of the antibody molecule with papain and a reducing agent. In certain disclosure herein, antibody fragments are produced recombinantly. In some disclosure herein, antibody fragments include Fv or single chain Fv (scFv) fragments. Fab, Fv, and scFv antibody fragments can be expressed in and secreted from *E. coli* or other host cells, allowing for the production of large amounts of these fragments. In some disclosure herein antibody fragments are isolated from antibody phage libraries as discussed herein. For example, methods can be used for the construction of Fab expression libraries (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for VEGF and/or DLL4 or derivatives, fragments, analogs or homologs thereof. In some disclosure herein, antibody fragments are linear antibody fragments. In certain disclosure herein, antibody fragments are monospecific or bispecific. In certain disclosure herein, the VEGF/DLL4-binding agent is a scFv. Various techniques can be used for the production of single-chain antibodies specific to VEGF or DLL4 (see, e.g., U.S. Patent No. 4,946,778).

**[0192]** It can further be desirable, especially in the case of antibody fragments, to modify an antibody in order to alter (e.g., increase or decrease) its serum half-life. This can be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment by mutation of the appropriate region in the antibody fragment or by incorporating the epitope into a peptide tag that is then fused to the antibody fragment at either end or in the middle (e.g., by DNA or peptide synthesis).

**[0193]** Heteroconjugate antibodies are also within the scope of the present disclosure. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune cells to unwanted cells (see, e.g., U.S. Patent No. 4,676,980). It is also contemplated that the heteroconjugate antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by

forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptoputyrimidate.

**[0194]** For the purposes of the present disclosure, it should be appreciated that modified antibodies can comprise any type of variable region that provides for the association of the antibody with the target (i.e., human VEGF or human DLL4). In this regard, the variable region may comprise or be derived from any type of mammal that can be induced to mount a humoral response and generate immunoglobulins against the desired antigen. As such, the variable region of the modified antibodies can be, for example, of human, murine, non-human primate (e.g. cynomolgus monkeys, macaques, etc.) or rabbit origin. In some disclosure herein, both the variable and constant regions of the modified immunoglobulins are human. In other disclosure herein, the variable regions of compatible antibodies (usually derived from a non-human source) can be engineered or specifically tailored to improve the binding properties or reduce the immunogenicity of the molecule. In this respect, variable regions useful in the present disclosure can be humanized or otherwise altered through the inclusion of imported amino acid sequences.

**[0195]** In certain disclosure herein, the variable domains in both the heavy and light chains are altered by at least partial replacement of one or more CDRs and, if necessary, by partial framework region replacement and sequence modification and/or alteration. Although the CDRs may be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs may be derived from an antibody of different class and often from an antibody from a different species. It may not be necessary to replace all of the CDRs with all of the CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are required to maintain the activity of the antigen-binding site.

**[0196]** Alterations to the variable region notwithstanding, those skilled in the art will appreciate that the modified antibodies of this disclosure will comprise antibodies (e.g., full-length antibodies or immunoreactive fragments thereof) in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical characteristics such as increased tumor localization or increased serum half-life when compared with an antibody of approximately the same immunogenicity comprising a native or unaltered constant region. In some disclosure herein, the constant region of the modified antibodies will comprise a human constant region. Modifications to the constant region compatible with this disclosure comprise additions, deletions or substitutions of one or more amino acids in one or more domains. The modified antibodies disclosed herein may comprise alterations or modifications to one or more of the three heavy chain constant domains (CH1, CH2 or CH3) and/or to the light chain constant domain (CL). In some disclosure herein, one or more domains are partially or entirely deleted from the constant regions of the modified antibodies. In some disclosure herein, the modified antibodies will comprise domain deleted constructs or variants wherein the entire CH2 domain has been removed ( $\Delta$ CH2 constructs). In some disclosure herein, the omitted constant region domain is replaced by a short amino acid spacer (e.g., 10 amino acid residues) that provides some of the molecular flexibility typically imparted by the absent constant region.

**[0197]** In some disclosure herein, the modified antibodies are engineered to fuse the CH3 domain directly to the hinge region of the antibody. In other disclosure herein, a peptide spacer is inserted between the hinge region and the modified CH2 and/or CH3 domains. For example, constructs may be expressed wherein the CH2 domain has been deleted and the remaining CH3 domain (modified or unmodified) is joined to the hinge region with a 5-20 amino acid spacer. Such a spacer may be added to ensure that the regulatory elements of the constant domain remain free and accessible or that the hinge region remains flexible. However, it should be noted that amino acid spacers may, in some cases, prove to be immunogenic and elicit an unwanted immune response against the construct. Accordingly, in certain disclosure herein, any spacer added to the construct will be relatively non-immunogenic so as to maintain the desired biological qualities of the modified antibodies.

**[0198]** In some disclosure herein, the modified antibodies may have only a partial deletion of a constant domain or substitution of a few or even a single amino acid. For example, the mutation of a single amino acid in selected areas of the CH2 domain may be enough to substantially reduce Fc binding and thereby increase cancer cell localization and/or tumor penetration. Similarly, it may be desirable to simply delete the part of one or more constant region domains that control a specific effector function (e.g. complement Clq binding) to be modulated. Such partial deletions of the constant regions may improve selected characteristics of the antibody (scrum half-life) while leaving other desirable functions associated with the subject constant region domain intact. Moreover, as alluded to above, the constant regions of the disclosed antibodies may be modified through the mutation or substitution of one or more amino acids that enhances the profile of the resulting construct. In this respect it may be possible to disrupt the activity provided by a conserved binding site (e.g., Fc binding) while substantially maintaining the configuration and immunogenic profile of the modified antibody. In certain disclosure herein, the modified antibodies comprise the addition of one or more amino acids to the constant region to enhance desirable characteristics such as decreasing or increasing effector function or provide for more cytotoxin or carbohydrate attachment sites.

**[0199]** It is known in the art that the constant region mediates several effector functions. For example, binding of the C1 component of complement to the Fc region of IgG or IgM antibodies (bound to antigen) activates the complement system. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can also be involved in autoimmune hypersensitivity. In addition, the Fc region of an antibody can bind a cell expressing a Fc receptor (FcR). There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell cytotoxicity or ADCC), release of inflammatory mediators, placental transfer, and control of immunoglobulin production.

**[0200]** In certain disclosure herein, the modified antibodies provide for altered effector functions that, in turn, affect the biological profile of the administered antibody. For example, in some

disclosure herein, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating modified antibody thereby increasing cancer cell localization and/or tumor penetration. In other disclosure herein, the constant region modifications increase the serum half-life of the antibody. In other disclosure herein, the constant region modifications reduce the serum half-life of the antibody. In some disclosure herein, the constant region is modified to eliminate disulfide linkages or oligosaccharide moieties. Modifications to the constant region in accordance with this disclosure may easily be made using well known biochemical or molecular engineering techniques known to those of skill in the art.

**[0201]** In certain disclosure herein, a VEGF/DLL4-binding agent that is an antibody does not have one or more effector functions. For instance, in some disclosure herein, the antibody has no ADCC activity, and/or no complement-dependent cytotoxicity (CDC) activity. In certain disclosure herein, the antibody does not bind an Fc receptor, and/or complement factors. In certain disclosure herein, the antibody has no effector function.

**[0202]** The present disclosure further embraces variants and equivalents which are substantially homologous to the chimeric, humanized, and human antibodies, or antibody fragments thereof, set forth herein. These can contain, for example, conservative substitution mutations, i.e. the substitution of one or more amino acids by similar amino acids. For example, conservative substitution refers to the substitution of an amino acid with another amino acid within the same general class such as, for example, one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid or one neutral amino acid by another neutral amino acid. What is intended by a conservative amino acid substitution is well known in the art and described herein.

**[0203]** Thus, the present disclosure provides methods for producing an antibody that binds VEGF and/or DLL4, including bispecific antibodies that specifically bind both VEGF and DLL4. In some disclosure herein, the method for producing an antibody that binds VEGF and/or DLL4 comprises using hybridoma techniques. In some disclosure herein, the method of generating an antibody that binds VEGF or DLL4 or a bispecific antibody that binds VEGF and DLL4 comprises screening a human phage library. The present disclosure further provides methods of identifying an antibody that binds VEGF and/or DLL4. In some disclosure herein, the antibody is identified by FACS screening for binding to VEGF or a portion thereof. In some disclosure herein, the antibody is identified by FACS screening for binding to DLL4 or a portion thereof. In some disclosure herein, the antibody is identified by FACS screening for binding to both VEGF and DLL4 or a portion thereof. In some disclosure herein, the antibody is identified by screening using ELISA for binding to VEGF. In some disclosure herein, the antibody is identified by screening using ELISA for binding to DLL4. In some disclosure herein, the antibody is identified by screening using ELISA for binding to VEGF and DLL4. In some disclosure herein, the antibody is identified by FACS screening for blocking of binding of human VEGF to a human VEGF receptor. In some disclosure herein, the antibody is identified by FACS screening for blocking of binding of human DLL4 to a human Notch receptor. In some disclosure herein, the antibody is identified by screening for inhibition or blocking of Notch signaling. In some disclosure herein, the antibody is identified by screening for inhibition or blocking of VEGF activity (e.g., induction of HUVEC proliferation). In some disclosure herein, the antibody is identified by screening for modulation of angiogenesis.

**[0204]** In some disclosure herein, a method of generating an antibody to human VEGF comprises immunizing a mammal with a polypeptide comprising amino acids 27-232 of human VEGF. In some disclosure herein, a method of generating an antibody to human VEGF comprises immunizing a mammal with a polypeptide comprising at least a portion of amino acids 27-232 of human VEGF. In some disclosure herein, the method further comprises isolating antibodies or antibody-producing cells from the mammal. In some disclosure herein, a method of generating a monoclonal antibody which binds VEGF comprises: immunizing a mammal with a polypeptide comprising at least a portion of amino acids 27-232 of human VEGF, and isolating antibody-producing cells from the immunized mammal. In some disclosure herein, the method further comprises fusing the antibody-producing cells with cells of a myeloma cell line to form hybridoma cells. In some disclosure herein, the method further comprises selecting a hybridoma cell expressing an antibody that binds VEGF. In certain disclosure herein, the mammal is a mouse. In some disclosure herein, the antibody is selected using a polypeptide comprising at least a portion of amino acids 27-232 of human VEGF.

**[0205]** In some disclosure herein, a method of generating an antibody to human DLL4 comprises immunizing a mammal with a polypeptide comprising amino acids 27-529 of human DLL4. In some disclosure herein, a method of generating an antibody to human DLL4 comprises immunizing a mammal with a polypeptide comprising at least a portion of amino acids 27-529 of human DLL4. In some disclosure herein, a method of generating a monoclonal antibody which binds DLL4 comprises: immunizing a mammal with a polypeptide comprising at least a portion of amino acids 27-529 of human DLL4, and isolating antibody producing cells from the immunized mammal. In some disclosure herein, the method further comprises fusing the antibody-producing cells with cells of a myeloma cell line to form hybridoma cells. In some disclosure herein, the method further comprises selecting a hybridoma cell expressing an antibody that binds DLL4. In certain disclosure herein, the mammal is a mouse. In some disclosure herein, the antibody is selected using a polypeptide comprising at least a portion of amino acids 27-529 of human DLL4.

**[0206]** In some disclosure herein, a method of generating an antibody to human VEGF comprises screening an antibody-expressing library for antibodies that bind human VEGF. In some disclosure herein, a method of generating an antibody human DLL4 comprises screening an antibody-expressing library for antibodies that bind human DLL4. In some disclosure herein, a method of generating an antibody to human VEGF and/or human DLL4 comprises screening an antibody-expressing library for bispecific antibodies that bind human VEGF and human DLL4. In some disclosure herein, the antibody-expressing library is a phage library. In some disclosure herein, the screening comprises panning. In some disclosure herein, the antibody-expressing library (e.g., a phage library) is screened using at least a portion of amino acids 27-232 of human VEGF. In some disclosure herein, antibodies identified in the first screening, are screened again using at least a portion of amino acids 27-529 of human DLL4 to identify a bispecific antibody that binds VEGF and DLL4. In some disclosure herein, the antibody-expressing library (e.g., a phage library) is screened using at least a portion of amino acids 27-529 of human DLL4. In some disclosure herein, antibodies identified in the first screening, are screened again using at least a portion of amino acids 27-232 of human VEGF to identify a bispecific antibody that binds VEGF and DLL4. In some disclosure herein, the antibody identified in the screening is a VEGF

antagonist. In some disclosure herein, the antibody identified in the screening inhibits biological activities induced by VEGF. In some disclosure herein, the antibody identified in the screening is a DLL4 antagonist. In some disclosure herein, the antibody identified in the screening inhibits Notch signaling induced by DLL4. In some disclosure herein, the antibody identified in the screening binds both human VEGF and mouse VEGF. In some disclosure herein, the antibody identified in the screening binds both human DLL4 and mouse DLL4.

**[0207]** In certain disclosure herein, the antibodies described herein are isolated. In certain disclosure herein, the antibodies described herein are substantially pure.

**[0208]** In some disclosure herein, the VEGF/DLL4-binding agents are polypeptides. The polypeptides can be recombinant polypeptides, natural polypeptides, or synthetic polypeptides comprising an antibody, or fragment thereof, that bind VEGF and/or DLL4. It will be recognized in the art that some amino acid sequences of the binding agents described herein can be varied without significant effect on the structure or function of the protein. Thus, the disclosure further includes variations of the polypeptides which show substantial activity or which include regions of an antibody, or fragment thereof, against human VEGF and/or DLL4. In some disclosure herein, amino acid sequence variations of VEGF/DLL4-binding polypeptides include deletions, insertions, inversions, repeats, and/or other types of substitutions.

**[0209]** In some disclosure herein, the polypeptides described herein are isolated. In some disclosure herein, the polypeptides described herein are substantially pure.

**[0210]** The polypeptides, analogs and variants thereof, can be further modified to contain additional chemical moieties not normally part of the polypeptide. The derivatized moieties can improve or otherwise modulate the solubility, the biological half-life, and/or absorption of the polypeptide. The moieties can also reduce or eliminate undesirable side effects of the polypeptides and variants. An overview for chemical moieties can be found in Remington: The Science and Practice of Pharmacy, 21st Edition, 2005, University of the Sciences, Philadelphia, PA.

**[0211]** The polypeptides described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods to constructing a DNA sequence encoding polypeptide sequences and expressing those sequences in a suitable host. In some disclosure herein, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional analogs thereof. See, e.g., Zoeller et al., 1984, PNAS, 81:5662-5066 and U.S. Patent No. 4,588,585.

**[0212]** In some disclosure herein, a DNA sequence encoding a polypeptide of interest may be constructed by chemical synthesis using an oligonucleotide synthesizer. Oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize a polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence

coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

**[0213]** Once assembled (by synthesis, site-directed mutagenesis, or another method), the polynucleotide sequences encoding a particular polypeptide of interest can be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction enzyme mapping, and/or expression of a biologically active polypeptide in a suitable host. As is well-known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

**[0214]** In certain disclosure herein, recombinant expression vectors are used to amplify and express DNA encoding antibodies, or fragments thereof, against human VEGF and/or DLL4. For example, recombinant expression vectors can be replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of a VEGF/DLL4-binding agent, such as an anti-VEGF antibody or an anti-DLL4 antibody, or fragment thereof, operatively linked to suitable transcriptional and/or translational regulatory elements derived from mammalian, microbial, viral, or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are "operatively linked" when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. In some disclosure herein, structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. In other disclosure herein, in situations where recombinant protein is expressed without a leader or transport sequence, it can include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

**[0215]** The choice of an expression control sequence and an expression vector depends upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including pCR1, pBR322, pMB9, and their derivatives, and wider host range plasmids, such

as M13 and other filamentous single-stranded DNA phages.

**[0216]** The VEGF/DLL4-binding agents (e.g., polypeptides) of the present disclosure can be expressed from one or more vectors. For example, in some disclosure herein, one heavy chain polypeptide is expressed by one vector, a second heavy chain polypeptide is expressed by a second vector and a light chain polypeptide is expressed by a third vector. In some disclosure herein, a first heavy chain polypeptide and a light chain polypeptide is expressed by one vector and a second heavy chain polypeptide is expressed by a second vector. In some disclosure herein, two heavy chain polypeptides are expressed by one vector and a light chain polypeptide is expressed by a second vector. In some disclosure herein, three polypeptides are expressed from one vector. Thus, in some disclosure herein, a first heavy chain polypeptide, a second heavy chain polypeptide, and a light chain polypeptide are expressed by a single vector.

**[0217]** Suitable host cells for expression of a VEGF/DLL4-binding polypeptide or antibody (or a VEGF or DLL4 protein to use as an antigen) include prokaryotes, yeast cells, insect cells, or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram-negative or gram-positive organisms, for example *E. coli* or *Bacillus*. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems may also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described in Pouwels et al., 1985, *Cloning Vectors: A Laboratory Manual*, Elsevier, New York, NY. Additional information regarding methods of protein production, including antibody production, can be found, e.g., in U.S. Patent Publication No. 2008/0187954; U.S. Patent Nos. 6,413,746; 6,660,501; and International Patent Publication No. WO 04/009823.

**[0218]** Various mammalian or insect cell culture systems may be used to express recombinant polypeptides. Expression of recombinant proteins in mammalian cells may be desirable because these proteins are generally correctly folded, appropriately modified, and biologically functional. Examples of suitable mammalian host cell lines include, but are not limited to, COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants of these cell lines. Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences. Expression of recombinant proteins in baculovirus also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art (see, e.g., Luckow and Summers, 1988, *Bio/Technology*, 6:47).

**[0219]** Thus, the present disclosure provides cells comprising the VEGF/DLL4-binding agents described herein. In some disclosure herein, the cells produce the VEGF/DLL4-binding agents described herein. In certain disclosure herein, the cells produce an antibody. In some disclosure herein, the cells produce a VEGF-binding agent, such as an anti-VEGF antibody. In some

disclosure herein, the cells produce a bispecific antibody that binds VEGF. In some disclosure herein, the cells produce a DLL4-binding agent, such as an anti-DLL4 antibody. In some disclosure herein, the cells produce a bispecific antibody that binds DLL4. In certain disclosure herein, the cells produce a bispecific VEGF/DLL4-binding agent, such as a bispecific antibody that binds VEGF and DLL4. In certain disclosure herein, the cells produce antibody 219R45. In certain disclosure herein, the cells produce antibody 21R79. In certain disclosure herein, the cells produce antibody 21R75. In certain disclosure herein, the cells produce antibody 21R83. In certain disclosure herein, the cells produce a bispecific antibody which comprises an antigen-binding site from antibody 219R45. In certain disclosure herein, the cells produce a bispecific antibody which comprises an antigen-binding site from antibody 21R79. In certain disclosure herein, the cells produce a bispecific antibody which comprises an antigen-binding site from antibody 21R75. In certain disclosure herein, the cells produce a bispecific antibody which comprises an antigen-binding site from antibody 21R83. In certain disclosure herein, the cells produce a bispecific antibody which comprises an antigen-binding site from antibody 219R45 and an antigen-binding site from antibody 21R79. In certain disclosure herein, the cells produce a bispecific antibody which comprises an antigen-binding site from antibody 219R45 and an antigen-binding site from antibody 21M18. In certain disclosure herein, the cells produce a bispecific antibody which comprises an antigen-binding site from antibody 219R45 and an antigen-binding site from antibody 21R75. In certain disclosure herein, the cells produce a bispecific antibody which comprises an antigen-binding site from antibody 219R45 and an antigen-binding site from antibody 21R83. In certain disclosure herein, the cells produce the bispecific antibody 219R45-MB-21M18. In certain disclosure herein, the cells produce the bispecific antibody 219R45-MB-21 R79. In certain disclosure herein, the cells produce the bispecific antibody 219R45-MB-21R75. In certain disclosure herein, the cells produce the bispecific antibody 219R45-MB-21R83.

**[0220]** The proteins produced by a transformed host can be purified according to any suitable method. Standard methods include chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexa-histidine, maltose binding domain, influenza coat sequence, and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Affinity chromatography used for purifying immunoglobulins can include Protein A, Protein G, and Protein L chromatography. Isolated proteins can be physically characterized using such techniques as proteolysis, size exclusion chromatography (SEC), mass spectrometry (MS), nuclear magnetic resonance (NMR), isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and x-ray crystallography. The purity of isolated proteins can be determined using techniques known to those of skill in the art, including but not limited to, SDS-PAGE, SEC, capillary gel electrophoresis, IEF, and capillary isoelectric focusing (cIEF).

**[0221]** In some disclosure herein, supernatants from expression systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. In some disclosure herein, an anion exchange resin can be employed, for example, a matrix or

substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. In some disclosure herein, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In some disclosure herein, a hydroxyapatite media can be employed, including but not limited to, ceramic hydroxyapatite (CHT). In certain disclosure herein, one or more reverse-phase HPLC steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a recombinant protein (e.g., a VEGF/DLL4-binding agent). Some or all of the foregoing purification steps, in various combinations, can be employed to provide a homogeneous recombinant protein.

**[0222]** In some disclosure herein, heterodimeric proteins such as bispecific antibodies are purified according to any of the methods described herein. In some disclosure herein, anti-VEGF/anti-DLL4 bispecific antibodies are isolated and/or purified using at least one chromatography step. In some disclosure herein, the at least one chromatography step comprises affinity chromatography. In some disclosure herein, the at least one chromatography step further comprises anion exchange chromatography. In some disclosure herein, the isolated and/or purified antibody product comprises at least 90% heterodimeric antibody. In some disclosure herein, the isolated and/or purified antibody product comprises at least 95%, 96%, 97%, 98% or 99% heterodimeric antibody. In some disclosure herein, the isolated and/or purified antibody product comprises about 100% heterodimeric antibody.

**[0223]** In some disclosure herein, recombinant protein produced in bacterial culture can be isolated, for example, by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange, or size exclusion chromatography steps. HPLC can be employed for final purification steps. Microbial cells employed in expression of a recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

**[0224]** Methods known in the art for purifying antibodies and other proteins also include, for example, those described in U.S. Patent Publication Nos. 2008/0312425; 2008/0177048; and 2009/0187005.

**[0225]** In certain disclosure herein, the VEGF/DLL4-binding agent is a polypeptide that is not an antibody. A variety of methods for identifying and producing non-antibody polypeptides that bind with high affinity to a protein target are known in the art. See, e.g., Skerra, 2007, *Curr. Opin. Biotechnol.*, 18:295-304; Hosse et al., 2006, *Protein Science*, 15:14-27; Gill et al., 2006, *Curr. Opin. Biotechnol.*, 17:653-658; Nygren, 2008, *FEBS J.*, 275:2668-76; and Skerra, 2008, *FEBS J.*, 275:2677-83. In certain disclosure herein, phage or mammalian cell display technology may be used to produce and/or identify a VEGF/DLL4-binding polypeptide that is not an antibody. In certain disclosure herein, the polypeptide comprises a protein scaffold of a type selected from the group consisting of protein A, protein G, a lipocalin, a fibronectin domain, an ankyrin consensus repeat domain, and thioredoxin.

**[0226]** In certain disclosure herein, the VEGF/DLL4-binding agents or antibodies can be used in any one of a number of conjugated (i.e. an immunoconjugate or radioconjugate) or non-

conjugated forms. In certain disclosure herein, the antibodies can be used in a non-conjugated form to harness the subject's natural defense mechanisms including complement-dependent cytotoxicity and antibody-dependent cellular toxicity to eliminate malignant or cancer cells.

**[0227]** In some disclosure herein, the VEGF/DLL4-binding agent (e.g., an antibody or polypeptide) is conjugated to a cytotoxic agent. In some disclosure herein, the cytotoxic agent is a chemotherapeutic agent including, but not limited to, methotrexate, adriamicin, doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents. In some disclosure herein, the cytotoxic agent is an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof, including, but not limited to, diphtheria A chain, non-binding active fragments of diphtheria toxin, exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, Sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the trichothecenes. In some disclosure herein, the cytotoxic agent is a radioisotope to produce a radioconjugate or a radioconjugated antibody. A variety of radionuclides are available for the production of radioconjugated antibodies including, but not limited to,  $^{90}\text{Y}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{123}\text{I}$ ,  $^{111}\text{In}$ ,  $^{131}\text{In}$ ,  $^{105}\text{Rh}$ ,  $^{153}\text{Sm}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{166}\text{Ho}$ ,  $^{177}\text{Lu}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$  and  $^{212}\text{Bi}$ . Conjugates of an antibody and one or more small molecule toxins, such as calicheamicins, maytansinoids, triethothecenes, and CC1065, and the derivatives of these toxins that have toxin activity, can also be used. Conjugates of an antibody and cytotoxic agent can be made using a variety of bifunctional protein-coupling agents including, but not limited to, N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

### III. Polynucleotides

**[0228]** This disclosure encompasses polynucleotides comprising polynucleotides that encode a polypeptide (or a fragment of a polypeptide) that specifically binds VEGF, DLL4, both VEGF and DLL4. The term "polynucleotides that encode a polypeptide" encompasses a polynucleotide which includes only coding sequences for the polypeptide, as well as a polynucleotide which includes additional coding and/or non-coding sequences. For example, the disclosure provides a polynucleotide comprising a polynucleotide sequence that encodes an antibody to human VEGF or encodes a fragment of such an antibody (e.g., a fragment comprising the antigen-binding site).

**[0229]** The disclosure provides a polynucleotide comprising a polynucleotide sequence that encodes an antibody to human DLL4 or encodes a fragment of such an antibody (e.g., a fragment comprising the antigen-binding site). The polynucleotides of the disclosure can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be doublestranded or single-stranded, and if single-stranded can be the coding strand or non-coding

(anti-sense) strand.

**[0230]** In certain disclosure herein, the polynucleotide comprises a polynucleotide encoding a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:64. In certain disclosure herein, the polynucleotide comprises a polynucleotide encoding a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:62, and SEQ ID NO:64. In some disclosure herein, the polynucleotide comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78.

**[0231]** In certain disclosure herein, the polynucleotide comprises a polynucleotide having a nucleotide sequence at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, and in some disclosure herein, at least about 96%, 97%, 98% or 99% identical to a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78. In certain disclosure herein, the polynucleotide comprises a polynucleotide having a nucleotide sequence at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, and in some disclosure herein, at least about 96%, 97%, 98% or 99% identical to a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78. Also provided is a polynucleotide that comprises a polynucleotide that hybridizes to SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78. In certain disclosure herein, the hybridization is under conditions of high stringency.

**[0232]** In certain disclosure herein, the polynucleotides comprise the coding sequence for the mature polypeptide fused in the same reading frame to a polynucleotide which aids, for example,

in expression and secretion of a polypeptide from a host cell (e.g., a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell). The polypeptide having a leader sequence is a preprotein and can have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides can also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

**[0233]** In certain disclosure herein, the polynucleotides comprise the coding sequence for the mature polypeptide fused in the same reading frame to a marker sequence that allows, for example, for purification of the encoded polypeptide. For example, the marker sequence can be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or the marker sequence can be a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (e.g., COS-7 cells) is used. In some disclosure herein, the marker sequence is a FLAG-tag, a peptide of sequence DYKDDDDK (SEQ ID NO:45) which can be used in conjunction with other affinity tags.

**[0234]** The present disclosure further relates to variants of the hereinabove described polynucleotides encoding, for example, fragments, analogs, and/or derivatives.

**[0235]** In certain disclosure herein, the present disclosure provides polynucleotides comprising polynucleotides having a nucleotide sequence at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, and in some disclosure herein, at least about 96%, 97%, 98% or 99% identical to a polynucleotide encoding a polypeptide comprising a VEGF/DLL4-binding agent (e.g., an antibody), or fragment thereof, described herein.

**[0236]** As used herein, the phrase a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence is intended to mean that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence can include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence can be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence can be inserted into the reference sequence. These mutations of the reference sequence can occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

**[0237]** The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some disclosure herein, a polynucleotide variant contains alterations which produce silent substitutions, additions, or deletions, but does not alter the properties or activities of the encoded polypeptide. In some disclosure herein, a polynucleotide variant comprises silent substitutions that results in no change to the amino acid sequence of the polypeptide (due to the

degeneracy of the genetic code). Polynucleotide variants can be produced for a variety of reasons, for example, to optimize codon expression for a particular host (i.e., change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*). In some disclosure herein, a polynucleotide variant comprises at least one silent mutation in a non-coding or a coding region of the sequence.

**[0238]** In some disclosure herein, a polynucleotide variant is produced to modulate or alter expression (or expression levels) of the encoded polypeptide. In some disclosure herein, a polynucleotide variant is produced to increase expression of the encoded polypeptide. In some disclosure herein, a polynucleotide variant is produced to decrease expression of the encoded polypeptide. In some disclosure herein, a polynucleotide variant has increased expression of the encoded polypeptide as compared to a parental polynucleotide sequence. In some disclosure herein, a polynucleotide variant has decreased expression of the encoded polypeptide as compared to a parental polynucleotide sequence.

**[0239]** In some disclosure herein, at least one polynucleotide variant is produced (without changing the amino acid sequence of the encoded polypeptide) to increase production of a heteromultimeric molecule. In some disclosure herein, at least one polynucleotide variant is produced (without changing the amino acid sequence of the encoded polypeptide) to increase production of a bispecific antibody.

**[0240]** In certain disclosure herein, the polynucleotides are isolated. In certain disclosure herein, the polynucleotides are substantially pure.

**[0241]** Vectors and cells comprising the polynucleotides described herein are also provided. In some disclosure herein, an expression vector comprises a polynucleotide molecule. In some disclosure herein, a host cell comprises an expression vector comprising the polynucleotide molecule. In some disclosure herein, a host cell comprises a polynucleotide molecule.

#### **IV. Methods of use and pharmaceutical compositions**

**[0242]** The -binding agents (including polypeptides and antibodies) of the disclosure that bind (e.g., specifically bind) VEGF and/or DLL4 are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as the treatment of cancer. In certain disclosure herein, the agents are useful for inhibiting VEGF activity, inhibiting DLL4-induced Notch signaling, inhibiting tumor growth, reducing tumor volume, reducing the frequency of cancer stem cells in a tumor, reducing the tumorigenicity of a tumor, modulating angiogenesis, and/or inhibiting angiogenesis. The methods of use may be *in vitro*, *ex vivo*, or *in vivo*. In certain disclosure herein, a VEGF/DLL4-binding agent is an antagonist of human VEGF. In certain disclosure herein, a VEGF/DLL4-binding agent is an antagonist of human DLL4. In certain disclosure herein, a VEGF/DLL4-binding agent is an antagonist of both VEGF and DLL4.

**[0243]** In certain disclosure herein, the VEGF/DLL4-binding agents are used in the treatment of a disease associated with angiogenesis, i.e. increased angiogenesis and/or aberrant angiogenesis.

In certain disclosure herein, the disease is a disease dependent upon angiogenesis. In certain disclosure herein, the VEGF/DLL4-binding agents are used in the treatment of disorders characterized by increased levels of stem cells and/or progenitor cells.

**[0244]** The present disclosure provides methods for inhibiting growth of a tumor using the VEGF/DLL4-binding agents or antibodies described herein. In certain disclosure herein, the method of inhibiting growth of a tumor comprises contacting a tumor cell with a VEGF/DLL4-binding agent (e.g., antibody) *in vitro*. For example, an immortalized cell line or a cancer cell line is cultured in medium to which is added an anti-VEGF antibody, an anti-DLL4 antibody, or an anti-VEGF/anti-DLL4 bispecific antibody to inhibit tumor cell growth. In some disclosure herein, tumor cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and cultured in medium to which is added a VEGF/DLL4-binding agent to inhibit tumor cell growth.

**[0245]** In some disclosure herein, the method of inhibiting growth of a tumor comprises contacting a tumor or tumor cells with a VEGF/DLL4-binding agent (e.g., antibody) *in vivo*. In certain disclosure herein, contacting a tumor or tumor cell with a VEGF/DLL4-binding agent is undertaken in an animal model. For example, an anti-VEGF antibody, an anti-DLL4 antibody, or an anti-VEGF/anti-DLL4 bispecific antibody may be administered to an immunocompromised host animal (e.g., NOD/SCID mice) which has a tumor xenograft. In some disclosure herein, tumor cells and/or cancer stem cells are isolated from a patient sample such as, for example, a tissue biopsy, pleural effusion, or blood sample and injected into an immunocompromised host animal (e.g., NOD/SCID mice) that is then administered a VEGF/DLL4-binding agent to inhibit tumor cell growth. In some disclosure herein, the VEGF/DLL4-binding agent is administered at the same time or shortly after introduction of tumorigenic cells into the animal to prevent tumor growth ("preventative model"). In some disclosure herein, the VEGF/DLL4-binding agent is administered as a therapeutic after tumors have grown to a specified size ("therapeutic model"). In certain disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody that specifically binds human VEGF and human DLL4.

**[0246]** In certain disclosure herein, the method of inhibiting growth of a tumor comprises administering to a subject a therapeutically effective amount of a VEGF/DLL4-binding agent. In certain disclosure herein, the subject is a human. In certain disclosure herein, the subject has a tumor or has had a tumor which was removed. In certain disclosure herein, the tumor comprises cancer stem cells. In certain disclosure herein, the frequency of cancer stem cells in the tumor is reduced by administration of the VEGF/DLL4-binding agent. The disclosure also provides a method of reducing the frequency of cancer stem cells in a tumor, comprising contacting the tumor with an effective amount of a VEGF/DLL4-binding agent (e.g., an anti-VEGF/anti-DLL4 bispecific antibody). In some disclosure herein, a method of reducing the frequency of cancer stem cells in a tumor in a subject, comprises administering to the subject a therapeutically effective amount of a VEGF/DLL4-binding agent.

**[0247]** In some disclosure herein, the tumor is a solid tumor. In certain disclosure herein, the tumor is a tumor selected from the group consisting of colorectal tumor, colon tumor, pancreatic tumor, lung tumor, ovarian tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and

neck tumor. In certain disclosure herein, the tumor is a colorectal tumor or a colon tumor. In certain disclosure herein, the tumor is an ovarian tumor. In some disclosure herein, the tumor is a lung tumor. In certain disclosure herein, the tumor is a pancreatic tumor. In certain disclosure herein, the tumor is a breast tumor.

**[0248]** The present disclosure further provides methods for treating cancer comprising administering a therapeutically effective amount of a VEGF/DLL4-binding agent to a subject. In some disclosure herein, the VEGF/DLL4-binding agent binds VEGF, and inhibits or reduces growth of the cancer. In some disclosure herein, the VEGF/DLL4-binding agent binds DLL4, and inhibits or reduces growth of the cancer. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody that binds VEGF and DLL4, and inhibits or reduces growth of the cancer. In some disclosure herein, the VEGF/DLL4-binding agent binds VEGF, interferes with VEGF/VEGF receptor interactions, and inhibits or reduces growth of the cancer. In some disclosure herein, the VEGF/DLL4-binding agent binds DLL4, interferes with DLL4/Notch interactions, and inhibits or reduces growth of the cancer. In some disclosure herein, the VEGF/DLL4-binding agent binds both VEGF and DLL4, interferes with VEGF/VEGF receptor interactions and with DLL4/Notch interactions, and inhibits or reduces growth of the cancer. In some disclosure herein, the VEGF/DLL4-binding agent binds DLL4, and reduces the frequency of cancer stem cells in the cancer.

**[0249]** The present disclosure provides methods of treating cancer comprising administering a therapeutically effective amount of a VEGF/DLL4-binding agent to a subject (e.g., a subject in need of treatment). In certain disclosure herein, the subject is a human. In certain disclosure herein, the subject has a cancerous tumor. In certain disclosure herein, the subject has had a tumor removed.

**[0250]** The subject's cancer/tumor, may, in some disclosure herein, be refractory to certain treatment(s). As a non-limiting example, the subject's cancer (or tumor) may be chemorefractory. In certain disclosure herein, the subject's cancer may be resistant to anti-VEGF therapy or anti-DLL4 therapy, or both.

**[0251]** In certain disclosure herein, the cancer is a cancer selected from the group consisting of colorectal cancer, pancreatic cancer, lung cancer, ovarian cancer, liver cancer, breast cancer, kidney cancer, prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, bladder cancer, glioblastoma, and head and neck cancer. In certain disclosure herein, the cancer is ovarian cancer. In certain disclosure herein, the cancer is colorectal cancer or colon cancer. In certain disclosure herein, the cancer is pancreatic cancer. In certain disclosure herein, the cancer is breast cancer. In certain disclosure herein, the cancer is prostate cancer. In certain disclosure herein, the cancer is lung cancer. In some disclosure herein, the cancer is a hematologic cancer such as leukemia or lymphoma. In some disclosure herein, the leukemia or lymphoma is a B-cell leukemia or lymphoma. In some disclosure herein, the leukemia or lymphoma is a T-cell leukemia or lymphoma. In some disclosure herein the hematologic cancer is acute myelogenous leukemia, Hodgkin lymphoma, non-Hodgkin's lymphoma, acute lymphocytic leukemia, hairy cell leukemia, chronic lymphocytic leukemia, multiple myeloma, cutaneous T-cell lymphoma, or T-cell acute lymphoblastic leukemia.

**[0252]** The disclosure also provides methods of treating a disease or disorder in a subject, wherein the disease or disorder is associated with angiogenesis. In some disclosure herein, the disease or disorder is associated with aberrant angiogenesis. In some disclosure herein, the disease or disorder is associated with increased angiogenesis. Thus, the present disclosure provides methods for modulating angiogenesis in a subject, comprising administering to the subject a therapeutically effective amount of any of the VEGF/DLL4-binding agents described herein. In some disclosure herein, the VEGF/DLL4-binding agent is an antibody that binds human VEGF. In some disclosure herein, the VEGF/DLL4-binding agent is an antibody that binds human DLL4. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody that binds human VEGF. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody that binds human DLL4. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody that binds human VEGF and human DLL4.

**[0253]** Methods of treating a disease or disorder in a subject, wherein the disease or disorder is characterized by an increased level of stem cells and/or progenitor cells are further provided. In some disclosure herein, the treatment methods comprise administering a therapeutically effective amount of a VEGF/DLL4-binding agent, polypeptide, or antibody to the subject.

**[0254]** In certain disclosure herein, of any of the methods described herein, the VEGF/DLL4-binding agent is a bispecific antibody that specifically binds human VEGF and human DLL4. In some disclosure herein, the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYTH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), YISSYNGATNYNQKFKG (SEQ ID NO:15), YIAGYKDATNYNQKFKG (SEQ ID NO:59), or YISNYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIANYNRATNYNQKFKG (SEQ ID NO:14), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising

NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISSYNGATNYNQKFKG (SEQ ID NO:15), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and second antigen-binding site which comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YIAGYKDATNYNQKFKG (SEQ ID NO:59), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22). In some disclosure herein, the bispecific antibody comprises a first antigen-binding site that specifically binds human VEGF and a second antigen-binding site that specifically binds human DLL4, wherein the first antigen-binding site comprises a heavy chain CDR1 comprising NYWMH (SEQ ID NO:17), a heavy chain CDR2 comprising DINPSNGRTSYKEKFKR (SEQ ID NO:18), and a heavy chain CDR3 comprising HYDDKYYPLMDY (SEQ ID NO:19), and the second antigen-binding site comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising YISYNRATNYNQKFKG (SEQ ID NO:65), and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16); and wherein both the first and second antigen-binding sites comprise a light chain CDR1 comprising RASESVDNYGISFMK (SEQ ID NO:20), a light chain CDR2 comprising AASNQGS (SEQ ID NO:21), and a light chain CDR3 comprising QQSKEVPWTFGG (SEQ ID NO:22).

**[0255]** In certain disclosure herein of any of the methods described herein, the VEGF/DLL4 bispecific antibody comprises a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:58, or SEQ ID NO:64, and a first and a light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In some disclosure herein, the VEGF/DLL4 bispecific antibody comprises a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:9, and a first and a second light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In some disclosure herein, the VEGF/DLL4 bispecific antibody comprises a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:10, and a first and a second light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In some disclosure herein, the VEGF/DLL4 bispecific antibody comprises a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:58, and a first and a

second light chain variable region having at least 80% sequence identity to SEQ ID NO:12. In some disclosure herein, the VEGF/DLL4 bispecific antibody comprises a first heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:11, a second heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:64, and a first and a second light chain variable region having at least 80% sequence identity to SEQ ID NO:12.

**[0256]** In some disclosure herein, of any of the methods described herein, the VEGF/DLL4-binding agent is an antibody. In some disclosure herein, , the VEGF/DLL4-binding agent is an anti-VEGF antibody. In some disclosure herein, the anti-VEGF antibody is antibody 219R45. In some disclosure herein, the VEGF/DLL4-binding agent is an anti-DLL4 antibody. In some disclosure herein, the anti-DLL4 antibody is antibody 21R79. In some disclosure herein, the anti-DLL4 antibody is antibody 21R75. In some disclosure herein, the anti-DLL4 antibody is antibody 21R83. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising an antigen-binding site from antibody 219R45. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising an antigen-binding site from antibody 21R79. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising an antigen-binding site from antibody 21R75. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising an antigen-binding site from antibody 21R83. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising a first antigen-binding site from antibody 219R45 and a second antigen-binding site from antibody 21R79. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising a first antigen-binding site from antibody 219R45 and a second antigen-binding site from antibody 21M18. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising a first antigen-binding site from antibody 219R45 and a second antigen-binding site from antibody 21R75. In some disclosure herein, the VEGF/DLL4-binding agent is a bispecific antibody comprising a first antigen-binding site from antibody 219R45 and a second antigen-binding site from antibody 21 R83. In some disclosure herein, the VEGF/DLL4-binding agent is the bispecific antibody 219R45-MB-21M18. In some disclosure herein, the VEGF/DLL4-binding agent is the bispecific antibody 219R45-MB-21R79. In some disclosure herein, the VEGF/DLL4-binding agent is the bispecific antibody 219R45-MB-21R75. In some disclosure herein, the VEGF/DLL4-binding agent is the bispecific antibody 219R45-MB-21R83.

**[0257]** The present disclosure further provides pharmaceutical compositions comprising the binding agents described herein. In certain disclosure herein, the pharmaceutical compositions further comprise a pharmaceutically acceptable vehicle. These pharmaceutical compositions find use in inhibiting tumor growth and/or treating cancer in a subject (e.g., a human patient).

**[0258]** In certain disclosure herein, the disclosure provides pharmaceutical compositions comprising bispecific antibodies, wherein at least about 90%, at least about 95%, at least about 98%, at least about 99% of the antibodies in the composition are bispecific antibodies or heterodimeric antibodies. In certain disclosure herein, the bispecific antibodies are IgG (e.g., IgG2 or IgG1) antibodies. In certain disclosure herein, less than about 10%, less than about 5%, less than about 2% or less than about 1% of the total antibodies in the compositions are monospecific antibodies or homodimeric antibodies. In certain disclosure herein, the antibodies in the composition are at least about 98% heterodimeric.

**[0259]** In certain disclosure herein, formulations are prepared for storage and use by combining a purified antibody or agent of the present disclosure with a pharmaceutically acceptable vehicle (e.g., a carrier or excipient). Suitable pharmaceutically acceptable vehicles include, but are not limited to, non-toxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (e.g., less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes; and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG). (Remington: The Science and Practice of Pharmacy, 21st Edition, 2005, University of the Sciences, Philadelphia, PA).

**[0260]** The pharmaceutical compositions of the present disclosure can be administered in any number of ways for either local or systemic treatment. Administration can be topical by epidermal or transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders; pulmonary by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, and intranasal; oral; or parenteral including intravenous, intraarterial, intratumoral, subcutaneous, intraperitoneal, intramuscular (e.g., injection or infusion), or intracranial (e.g., intrathecal or intraventricular).

**[0261]** The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and diluents (e.g., water). These can be used to form a solid preformulation composition containing a homogeneous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. The solid preformulation composition is then subdivided into unit dosage forms of a type described above. The tablets, pills, etc. of the formulation or composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

**[0262]** The VEGF/DLL4-binding agents or antibodies described herein can also be entrapped in microcapsules. Such microcapsules are prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-

(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions as described in Remington: The Science and Practice of Pharmacy, 21st Edition, 2005, University of the Sciences in Philadelphia, PA.

**[0263]** In certain disclosure herein, pharmaceutical formulations include a VEGF/DLL4-binding agent (e.g., an antibody) of the present disclosure complexed with liposomes. Methods to produce liposomes are known to those of skill in the art. For example, some liposomes can be generated by reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes can be extruded through filters of defined pore size to yield liposomes with the desired diameter.

**[0264]** In certain disclosure herein, sustained-release preparations can be produced. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing a VEGF/DLL4-binding agent (e.g., an antibody), where the matrices are in the form of shaped articles (e.g., films or microcapsules). Additional examples of sustained-release matrices include polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(vinyl alcohol), polylactides, copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

**[0265]** In certain disclosure herein, in addition to administering a VEGF/DLL4-binding agent (e.g., an antibody), the method or treatment further comprises administering at least one additional therapeutic agent. An additional therapeutic agent can be administered prior to, concurrently with, and/or subsequently to, administration of the VEGF/DLL4-binding agent. Pharmaceutical compositions comprising a VEGF/DLL4-binding agent and the additional therapeutic agent(s) are also provided. In some disclosure herein, the at least one additional therapeutic agent comprises 1, 2, 3, or more additional therapeutic agents.

**[0266]** Combination therapy with at least two therapeutic agents often uses agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action may result in additive or synergistic effects. Combination therapy may allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of at least one of the agents. Combination therapy may decrease the likelihood that resistant cancer cells will develop. In some disclosure herein, combination therapy comprises a therapeutic agent that primarily affects (e.g., inhibits or kills) non-tumorigenic cells and a therapeutic agent that primarily affects (e.g., inhibits or kills) tumorigenic CSCs.

**[0267]** Useful classes of therapeutic agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers,

duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain disclosure herein, the second therapeutic agent is an alkylating agent, an antimetabolite, an antimitotic, a topoisomerase inhibitor, or an angiogenesis inhibitor. In some disclosure herein, the second therapeutic agent is a platinum complex such as carboplatin or cisplatin. In some disclosure herein, the additional therapeutic agent is a platinum complex in combination with a taxane.

**[0268]** Therapeutic agents that may be administered in combination with the VEGF/DLL4-binding agents include chemotherapeutic agents. Thus, in some disclosure herein, the method or treatment involves the administration of an anti-VEGF-binding agent or antibody of the present disclosure in combination with a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents. In some disclosure herein, the method or treatment involves the administration of an anti-DLL4-binding agent or antibody of the present disclosure in combination with a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents. In some disclosure herein, the method or treatment involves the administration of a bispecific antibody of the present disclosure that binds VEGF and DLL4 in combination with a chemotherapeutic agent or cocktail of multiple different chemotherapeutic agents.

**[0269]** Chemotherapeutic agents useful in the instant disclosure include, but are not limited to, alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethyloloamelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytosine arabinoside, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mepidamol; nitracrine; pentostatin; phenacetin; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2'-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); taxoids, e.g. paclitaxel (TAXOL) and docetaxel

(TAXOTERE); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; ibandronate; CPT11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; cspcramicins; capecitabine (XELODA); and pharmaceutically acceptable salts, acids or derivatives of any of the above. Chemotherapeutic agents also include anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as antiestrogens including, for example, tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY1 17018, onapristone, and toremifene (FARESTON); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In certain disclosure herein, the second therapeutic agent is cisplatin. In certain disclosure herein, the second therapeutic agent is carboplatin. In certain disclosure herein, the second therapeutic agent is paclitaxel.

**[0270]** In certain disclosure herein, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapeutic agents that interfere with the action of a topoisomerase enzyme (e.g., topoisomerase I or II). Topoisomerase inhibitors include, but are not limited to, doxorubicin HCl, daunorubicin citrate, mitoxantrone HCl, actinomycin D, etoposide, topotecan HCl, teniposide (VM-26), and irinotecan, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In certain disclosure herein, the second therapeutic agent is irinotecan.

**[0271]** In certain disclosure herein, the chemotherapeutic agent is an anti-metabolite. An anti-metabolite is a chemical with a structure that is similar to a metabolite required for normal biochemical reactions, yet different enough to interfere with one or more normal functions of cells, such as cell division. Anti-metabolites include, but are not limited to, gemcitabine, fluorouracil, capecitabine, methotrexate sodium, ralitrexed, pemetrexed, tegafur, cytosine arabinoside, thioguanine, 5-azacytidine, 6-mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In certain disclosure herein, the second therapeutic agent is gemcitabine.

**[0272]** In certain disclosure herein, the chemotherapeutic agent is an antimitotic agent, including, but not limited to, agents that bind tubulin. In some disclosure herein, the agent is a taxane. In certain disclosure herein, the agent is paclitaxel or docetaxel, or a pharmaceutically acceptable salt, acid, or derivative of paclitaxel or docetaxel. In certain disclosure herein, the agent is paclitaxel (TAXOL), docetaxel (TAXOTERE), albumin-bound paclitaxel (ABRAXANE), DHA-paclitaxel, or PG-paclitaxel. In certain alternative disclosure herein, the antimitotic agent comprises a vinca alkaloid, such as vincristine, vinblastine, vinorelbine, or vindesine, or pharmaceutically acceptable salts, acids, or derivatives thereof. In some disclosure herein, the antimitotic agent is an inhibitor of kinesin Eg5 or an inhibitor of a mitotic kinase such as Aurora A or Plk1. In certain disclosure herein, where the chemotherapeutic agent administered in combination with a VEGF/DLL4-binding agent is an anti-mitotic agent, the cancer or tumor being treated is breast cancer or a breast tumor.

**[0273]** In some disclosure herein, a second therapeutic agent comprises an agent such as a small molecule. For example, treatment can involve the combined administration of a VEGF/DLL4-binding agent (e.g. an antibody) of the present disclosure with a small molecule that acts as an inhibitor against additional tumor-associated proteins including, but not limited to, EGFR, ErbB2, HER2, and/or VEGF. In certain disclosure herein, the second therapeutic agent is a small molecule that inhibits a cancer stem cell pathway. In some disclosure herein, the second therapeutic agent is a small molecule inhibitor of the Notch pathway. In some disclosure herein, the second therapeutic agent is a small molecule inhibitor of the Wnt pathway. In some disclosure herein, the second therapeutic agent is a small molecule inhibitor of the BMP pathway. In some disclosure herein, the second therapeutic agent is a small molecule that inhibits  $\beta$ -catenin signaling.

**[0274]** In some disclosure herein, a second therapeutic agent comprises a biological molecule, such as an antibody. For example, treatment can involve the combined administration of a VEGF/DLL4-binding agent (e.g. an antibody) of the present disclosure with other antibodies against additional tumor-associated proteins including, but not limited to, antibodies that bind EGFR, ErbB2, HER2, and/or VEGF. In certain disclosure herein, the second therapeutic agent is an antibody that is an anti-cancer stem cell marker antibody. In some disclosure herein, the second therapeutic agent is an antibody that binds a component of the Notch pathway. In some disclosure herein, the second therapeutic agent is an antibody that binds a component of the Wnt pathway. In certain disclosure herein, the second therapeutic agent is an antibody that inhibits a cancer stem cell pathway. In some disclosure herein, the second therapeutic agent is an antibody inhibitor of the Notch pathway. In some disclosure herein, the second therapeutic agent is an antibody inhibitor of the Wnt pathway. In some disclosure herein, the second therapeutic agent is an antibody inhibitor of the BMP pathway. In some disclosure herein, the second therapeutic agent is an antibody that inhibits  $\beta$ -catenin signaling. In certain disclosure herein, the second therapeutic agent is an antibody that is an angiogenesis inhibitor or modulator (e.g., an anti-VEGF or VEGF receptor antibody). In certain disclosure herein, the second therapeutic agent is bevacizumab (AVASTIN), trastuzumab (HERCEPTIN), panitumumab (VECTIBIX), or cetuximab (ERBITUX). Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously.

**[0275]** Furthermore, treatment with a VEGF/DLL4-binding agent described herein can include combination treatment with other biologic molecules, such as one or more cytokines (e.g., lymphokines, interleukins, tumor necrosis factors, and/or growth factors) or can be accompanied by surgical removal of tumors, cancer cells, or any other therapy deemed necessary by a treating physician.

**[0276]** In certain disclosure herein, the treatment involves the administration of a VEGF/DLL4-binding agent (e.g. an antibody) of the present disclosure in combination with radiation therapy. Treatment with a VEGF/DLL4-binding agent can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Dosing schedules for such radiation therapy can be determined by the skilled medical practitioner.

**[0277]** It will be appreciated that the combination of a VEGF/DLL4-binding agent and an additional therapeutic agent may be administered in any order or concurrently. Treatment with a VEGF/DLL4-binding agent (e.g., an antibody) can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *The Chemotherapy Source Book*, 4th Edition, 2008, M. C. Perry, Editor, Lippincott, Williams & Wilkins, Philadelphia, PA.

**[0278]** In some disclosure herein, the VEGF/DLL4-binding agent will be administered to patients that have previously undergone treatment with a second therapeutic agent. In certain other disclosure herein, the VEGF/DLL4-binding agent and a second therapeutic agent will be administered substantially simultaneously or concurrently. For example, a subject may be given a VEGF/DLL4-binding agent (e.g., an antibody) while undergoing a course of treatment with a second therapeutic agent (e.g., chemotherapy). In certain disclosure herein, a VEGF/DLL4-binding agent will be administered within 1 year of the treatment with a second therapeutic agent. In certain alternative disclosure herein, a VEGF/DLL4-binding agent will be administered within 10, 8, 6, 4, or 2 months of any treatment with a second therapeutic agent. In certain other disclosure herein, a VEGF/DLL4-binding agent will be administered within 4, 3, 2, or 1 weeks of any treatment with a second therapeutic agent. In some disclosure herein, a VEGF/DLL4-binding agent will be administered within 5, 4, 3, 2, or 1 days of any treatment with a second therapeutic agent. It will further be appreciated that the two (or more) agents or treatments may be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

**[0279]** For the treatment of a disease, the appropriate dosage of an VEGF/DLL4-binding agent (e.g., an antibody) of the present disclosure depends on the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, whether the VEGF/DLL4-binding agent or antibody is administered for therapeutic or preventative purposes, previous therapy, the patient's clinical history, and so on, all at the discretion of the treating physician. The VEGF/DLL4-binding agent or antibody can be administered one time or as a series of treatments spread over several days to several months, or until a cure is effected or a diminution of the disease state is achieved (e.g., reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual antibody or agent. The administering physician can determine optimum dosages, dosing methodologies, and repetition rates. In certain disclosure herein, dosage of a VEGF/DLL4-binding agent or antibody is from about 0.01 $\mu$ g to about 1 00mg/kg of body weight, from about 0.1 $\mu$ g, to about 100mg/kg of body weight, from about 1  $\mu$ g to about 100mg/kg of body weight, from about 1 mg to about 100mg/kg of body weight, about 1mg to about 80mg/kg of body weight from about 10mg to about 100mg/kg of body weight, from about 10mg to about 75mg/kg of body weight, or from about 10mg to about 50mg/kg of body weight. In certain disclosure herein, the dosage of the antibody or other VEGF/DLL4-binding agent is from about 0.1mg to about 20mg/kg of body weight. In certain disclosure herein, dosage can be given

once or more daily, weekly, monthly, or yearly. In certain disclosure herein, the antibody or other VEGF/DLL4-binding agent is given once every week, once every two weeks, once every three weeks, or once every month.

**[0280]** In some disclosure herein, a VEGF/DLL4-binding agent (e.g., an antibody) may be administered at an initial higher "loading" dose, followed by one or more lower doses. In some disclosure herein, the frequency of administration may also change. In some disclosure herein, a dosing regimen may comprise administering an initial dose, followed by additional doses (or "maintenance" doses) once a week, once every two weeks, once every three weeks, or once every month. For example, a dosing regimen may comprise administering an initial loading dose, followed by a weekly maintenance dose of, for example, one-half of the initial dose. Or a dosing regimen may comprise administering an initial loading dose, followed by maintenance doses of, for example one-half of the initial dose every other week. Or a dosing regimen may comprise administering three initial doses for 3 weeks, followed by maintenance doses of, for example, the same amount every other week. Or a dosing regimen may comprise administering an initial dose followed by additional doses every 3 weeks or once a month. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. The progress of therapy can be monitored by conventional techniques and assays.

**[0281]** As is known to those of skill in the art, administration of any therapeutic agent may lead to side effects and/or toxicities. In some cases, the side effects and/or toxicities are so severe as to preclude administration of the particular agent at a therapeutically effective dose. In some cases, drug therapy must be discontinued, and other agents may be tried. However, many agents in the same therapeutic class often display similar side effects and/or toxicities, meaning that the patient either has to stop therapy, or if possible, suffer from the unpleasant side effects associated with the therapeutic agent.

**[0282]** Side effects from therapeutic agents may include, but are not limited to, hives, skin rashes, itching, nausea, vomiting, decreased appetite, diarrhea, chills, fever, fatigue, muscle aches and pain, headaches, low blood pressure, high blood pressure, hypokalemia, low blood counts, bleeding, and cardiac problems.

**[0283]** Thus, one aspect of the present disclosure is directed to methods of treating cancer in a patient comprising administering an anti-VEGF/anti-DLL4 bispecific antibody using an intermittent dosing regimen, which may reduce side effects and/or toxicities associated with administration of the anti-VEGF/anti-DLL4 bispecific antibody. As used herein, "intermittent dosing" refers to a dosing regimen using a dosing interval of more than once a week, e.g., dosing once every 2 weeks, once every 3 weeks, once every 4 weeks, etc. In some disclosure herein, a method for treating cancer in a human patient comprises administering to the patient an effective dose of an anti-VEGF/anti-DLL4 bispecific antibody according to an intermittent dosing regimen. In some disclosure herein, a method for treating cancer in a human patient comprises administering to the patient an effective dose of an anti-VEGF/anti-DLL4 bispecific antibody according to an intermittent dosing regimen, and increasing the therapeutic index of the anti-VEGF/anti-DLL4 bispecific antibody. In some disclosure herein, the intermittent dosing regimen comprises administering an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody to the patient, and

administering subsequent doses of the anti-VEGF/anti-DLL4 bispecific antibody about once every 2 weeks. In some disclosure herein, the intermittent dosing regimen comprises administering an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody to the patient, and administering subsequent doses of the anti-VEGF/anti-DLL4 bispecific antibody about once every 3 weeks. In some disclosure herein, the intermittent dosing regimen comprises administering an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody to the patient, and administering subsequent doses of the anti-VEGF/anti-DLL4 bispecific antibody about once every 4 weeks.

**[0284]** In some disclosure herein, the subsequent doses in an intermittent dosing regimen are about the same amount or less than the initial dose. In other disclosure herein, the subsequent doses are a greater amount than the initial dose. As is known by those of skill in the art, doses used will vary depending on the clinical goals to be achieved. In some disclosure herein, the initial dose is about 0.25mg/kg to about 20mg/kg. In some disclosure herein, the initial dose is about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20mg/kg. In certain disclosure herein, the initial dose is about 0.5mg/kg. In certain disclosure herein, the initial dose is about 1mg/kg. In certain disclosure herein, the initial dose is about 2.5mg/kg. In certain disclosure herein, the initial dose is about 5mg/kg. In certain disclosure herein, the initial dose is about 7.5mg/kg. In certain disclosure herein, the initial dose is about 10mg/kg. In certain disclosure herein, the initial dose is about 12.5mg/kg. In certain disclosure herein, the initial dose is about 15mg/kg. In certain disclosure herein, the initial dose is about 20mg/kg. In some disclosure herein, the subsequent doses are about 0.25mg/kg to about 15mg/kg. In certain disclosure herein, the subsequent doses are about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15mg/kg. In certain disclosure herein, the subsequent doses are about 0.5mg/kg. In certain disclosure herein, the subsequent doses are about 1mg/kg. In certain disclosure herein, the subsequent doses are about 2.5mg/kg. In certain disclosure herein, the subsequent doses are about 5mg/kg. In some disclosure herein, the subsequent doses are about 7.5mg/kg. In some disclosure herein, the subsequent doses are about 10mg/kg. In some disclosure herein, the subsequent doses are about 12.5mg/kg.

**[0285]** In some disclosure herein, the intermittent dosing regimen comprises: (a) administering to the patient an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody of about 2.5mg/kg and (b) administering subsequent doses of about 2.5 mg/kg once every 2 weeks. In some disclosure herein, the intermittent dosing regimen comprises: (a) administering to the patient an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody of about 5mg/kg and (b) administering subsequent doses of about 5 mg/kg once every 2 weeks. In some disclosure herein, the intermittent dosing regimen comprises: (a) administering to the patient an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody of about 2.5mg/kg and (b) administering subsequent doses of about 2.5 mg/kg once every 3 weeks. In some disclosure herein, the intermittent dosing regimen comprises: (a) administering to the patient an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody of about 5mg/kg and (b) administering subsequent doses of about 5 mg/kg once every 3 weeks. In some disclosure herein, the intermittent dosing regimen comprises: (a) administering to the patient an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody of about 2.5mg/kg and (b) administering subsequent doses of about 2.5 mg/kg once every 4 weeks. In some disclosure herein, the intermittent dosing regimen comprises: (a) administering to the patient an initial dose of an anti-VEGF/anti-DLL4 bispecific antibody of about 5mg/kg and (b) administering subsequent doses of

about 5 mg/kg once every 4 weeks. In certain disclosure herein, the initial dose and the maintenance doses are different, for example, the initial dose is about 5mg/kg and the subsequent doses are about 2.5mg/kg. In certain disclosure herein, an intermittent dosing regimen may comprise a loading dose, for example, the initial dose is about 20mg/kg and the subsequent doses are about 2.5mg/kg or about 5mg/kg administered once every 2 weeks, once every 3 weeks, or once every 4 weeks.

**[0286]** The disclosure is also directed to methods for reducing toxicity of an anti-VEGF/anti-DLL4 bispecific antibody in a human patient comprises administering to the patient the anti-VEGH/anti-DLL4 bispecific antibody using an intermittent dosing regimen. The disclosure is also directed to methods for reducing side effects of an anti-VEGF/anti-DLL4 bispecific antibody in a human patient comprises administering to the patient the anti-VEGF/anti-DLL4 bispecific antibody using an intermittent dosing regimen. The disclosure is also directed to methods for increasing the therapeutic index of an anti-VEGF/anti-DLL4 bispecific antibody in a human patient comprises administering to the patient the anti-VEGF/anti-DLL4 bispecific antibody using an intermittent dosing regimen.

**[0287]** The choice of delivery method for the initial and subsequent doses is made according to the ability of the animal or human patient to tolerate introduction of the anti-VEGF/anti-DLL4 bispecific antibody into the body. Thus, in any of the aspects and/or disclosure herein described herein, the administration of the anti-VEGF/anti-DLL4 bispecific antibody may be by intravenous injection or intravenously. In some disclosure herein, the administration is by intravenous infusion. In any of the aspects and/or disclosure herein described herein, the administration of the anti-VEGF/anti-DLL4 bispecific antibody may be by a non-intravenous route.

#### **V. Kits comprising VEGF/DLL4-binding agents**

**[0288]** The present disclosure provides kits that comprise the VEGF/DLL4-binding agents (e.g., antibodies) described herein and that can be used to perform the methods described herein. In certain disclosure herein, a kit comprises at least one purified antibody against VEGF and/or DLL4 in one or more containers. In some disclosure herein, the kits contain all of the components necessary and/or sufficient to perform a detection assay, including all controls, directions for performing assays, and any necessary software for analysis and presentation of results. One skilled in the art will readily recognize that the disclosed VEGF/DLL4-binding agents of the present disclosure can be readily incorporated into one of the established kit formats which are well known in the art.

**[0289]** Further provided are kits comprising a VEGF/DLL4-binding agent (e.g., an anti-VEGF/anti-DLL4 bispecific antibody), as well as at least one additional therapeutic agent. In certain disclosure herein, the second (or more) therapeutic agent is a chemotherapeutic agent. In certain disclosure herein, the second (or more) therapeutic agent is an angiogenesis inhibitor.

**[0290]** The present disclosure can be further defined by reference to the following non-limiting examples, which describe in detail preparation of certain antibodies of the present disclosure and

methods for using antibodies of the present disclosure. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the present disclosure.

## EXAMPLES

### Example 1

#### Binding affinities of anti-VEGF/anti-DLL4 antibodies

**[0291]** The  $K_D$ s of parental antibodies anti-VEGF 219R45 (IgG format), anti-DLL4 21R79 (IgG format), anti-DLL4 21M18 (IgG format) and bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79 were determined using a Biacore 2000 system from Biacore LifeSciences (GE Healthcare). Recombinant human DLL4-Fc or mouse DLL4-Fc proteins were immobilized on CM5 carboxyl chips using standard amine-based chemistry (NHS/EDC) and blocked with ethanolamine. Recombinant human VEGF<sub>165</sub> or mouse VEGF<sub>165</sub> were biotinylated and immobilized on streptavidin chips. The antibodies were serially diluted 2-fold from 100nM to 0.78nM in HBS-P (0.01M HEPES pH7.4, 0.15M NaCl, 0.005% v/v Polysorbate 20). For each antibody, all 8 dilutions were sequentially injected over a specific chip. Kinetic data were collected over time and were fit using the simultaneous global fit equation to yield affinity constants ( $K_D$  values) for each bispecific antibody.

Table 3

Antibody	hVEGF (nM)	mVEGF(nM)	hDLL4 (nM)	mDLL4 (nM)
219R45	0.67	22.9	NB	NB
21M18	NB	NB	<0.1	NB
21R79	NB	NB	<0.1	NB
219R45-MB-21M18	0.36	25.5	16	NB
219R45-MB-21R79	0.68	12.5	0.53	NB

**[0292]** As shown in Table 3, bispecific antibody 219R45-MB-21M18 had an affinity constant ( $K_D$ ) for human VEGF of 0.36nM and a  $K_D$  for human DLL4 of 16nM. Bispecific antibody 219R45-MB-21R79 had a  $K_D$  for human VEGF of 0.68nM and a  $K_D$  for human DLL4 of 0.53nM. Both bispecific antibodies demonstrated weaker binding to mouse VEGF as compared to human VEGF and neither antibody bound mouse DLL4. Thus, both bispecific antibodies demonstrated similar binding affinity to human VEGF and 219R45-MB-21R79 demonstrated approximately 30-fold stronger binding to human DLL4 than 219R45-MB-21M18. Furthermore, bispecific antibody 219R45-MB-21R79 had a similar binding affinity to human VEGF despite the fact the bispecific antibody is monovalent for VEGF as compared to the bivalent parental antibody.

**[0293]** Several additional anti-DLL4 antibodies were identified that had binding affinities intermediate to the  $K_D$ s of 21M18 and 21R79. Two of these anti-DLL4 antibodies were used to produce anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21R75 and 219R45-MB-21R83. Using the Biacore 2000 system as described above, the  $K_D$ s of the bispecific antibodies 219R45-MB-21R75 and 219R45-MB-21R83 to human DLL4 were determined. A comparison of the binding affinity to human DLL4 of these four anti-VEGF/anti-DLL4 bispecific antibodies is shown in Table 4.

**[0294]** The CDRs for anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18, 219R45-MB-21R79, 219R45-MB-21R75, and 219R45-MB-21R83 are shown in Figure 1A. The heavy chain and light chain variable region SEQ ID NOs are shown in Figure 1B and the heavy chain and light chain SEQ ID NOs (with and without signal sequence) are shown in Figure 1C.

**[0295]** Anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21M18 comprises a (a) heavy chain encoded by the DNA comprising SEQ ID NO:75 deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA, USA, under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_, (b) a heavy chain encoded by the DNA comprising SEQ ID NO:33 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_, and (c) a light chain encoded by the DNA comprising SEQ ID NO:34 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_.

**[0296]** Anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21R79 comprises a (a) heavy chain encoded by the DNA comprising SEQ ID NO:31 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_, (b) a heavy chain encoded by the DNA comprising SEQ ID NO:33 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_, and (c) a light chain encoded by the DNA comprising SEQ ID NO:34 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_.

**[0297]** Anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21R83 comprises (a) a heavy chain encoded by the DNA comprising SEQ ID NO:72 deposited with ATCC under the conditions of the Budapest Treaty on \_\_\_\_\_ and assigned designation number PTA-\_\_\_\_, (b) a heavy chain encoded by the DNA comprising SEQ ID NO:33 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_, and (c) a light chain encoded by the DNA comprising SEQ ID NO:34 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_.

**[0298]** Anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21R75 comprises (a) a heavy chain encoded by the DNA comprising SEQ ID NO:74 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_, (b) a heavy chain encoded by the DNA comprising SEQ ID NO:33 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-\_\_\_\_, and

(c) a light chain encoded by the DNA comprising SEQ ID NO:34 deposited with ATCC under the conditions of the Budapest Treaty on September 21, 2012 and assigned designation number PTA-

Table 4

Antibody	Heavy chain CDR2	hDLL4 (nM)
219R45-MB-21M18	YISSYNGATNYNQKFKG (SEQ ID NO:15)	16.00
219R45-MB-21R79	YIANYNRATNYNQKFKG (SEQ ID NO:14)	0.53
219R45-MB-21R75	YIAGYKDATNYNQKFKG (SEQ ID NO:59)	5.10
219R45-MB-21R83	YISYNYNRATNYNQKFKG (SEQ ID NO:65)	1.30

**Example 2**

**HTRF Assay for simultaneous binding of bispecific antibodies to human VEGF and human DLL4**

**[0299]** To characterize the binding capabilities of certain antibodies and/or antibody mixtures to both VEGF and DLL4, homogeneous time resolved fluorescence (HTRF) assays were performed. Antibodies tested were anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79, parental antibodies 219R45 (anti-VEGF), 21M18 (anti-DLL4), 21R79 (anti-DLL4), a combination of 219R45 and 21M18, or a combination of 219R45 and 21R79. The antibodies or antibody mixtures were serially diluted 2-fold from 3000nM to 2.9nM in binding buffer (IX PBS, 0.1% gelatin, 0.1% Polysorbate 20, 400mM potassium fluoride) and placed in a white 96-well plate. An equal volume of solution containing 4 $\mu$ g/ml of d2-labeled hDLL4-Fc and 21.4ng/ml Europium cryptate-labeled hVEGF<sub>165</sub> was added to each well for a final volume of 100 $\mu$ l (final concentrations of acceptor and donor fluorophores were 2 $\mu$ g/ml and 10.7ng/ml, respectively). The assay plates were incubated for 2 hours to overnight and read on a SpectraMax M5e Microplate reader (Molecular Devices, Sunnyvale CA) at an excitation wavelength of 314nm.

**[0300]** As shown in Figure 2, anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79, were able to bind both hVEGF and hDLL4 simultaneously. Importantly, neither of the combinations of the parental antibodies (i.e., 219R45 and 21M18 or 219R45 and 21R79) was able to bind VEGF and DLL4 simultaneously. These results clearly demonstrate that the anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79 are capable of functioning differently than just a mixture of the two individual antibodies.

**Example 3**

**Inhibition of HUVEC proliferation by anti-VEGF/anti-DLL4 bispecific antibodies**

**[0301]** HUVEC cells were obtained from Lonza (Walkersville MD) and cultured in growth media (M199, 10% heat-inactivated FBS (HI-FBS), 50µg/ml EGS, IX heparin, 1mM L-glutamine). For the HUVEC proliferation assay, a 96-well plate was pre-coated with 50µl of 10µg/ml rat tail collagen type I solution (collagen I in 0.02N acetic acid) and incubated at 4°C overnight. After incubation, the plate was thoroughly aspirated to remove unbound collagen I solution and washed once with 200µl DPBS. The HUVEC cells were removed from the surface of the growth flasks using an endothelial cell subclone reagent and centrifuged at 1200 rpm for 5 minutes at 4°C. The cells were resuspended in starvation/assay medium (M199 and 2% HI-FBS, IX heparin, 5U/ml heparin-glutamine) at a density of 10<sup>5</sup> cells/ml. The cells were seeded into the collagen-coated assay plate at 5000 cells/well, 50ul/well. The cells were incubated for 3 hours at 37°C, washed one time, refed with 100ul assay media, and incubated overnight at 37°C. The next day, bispecific antibodies 219R45-MB-21M18, 219R45-MB-21R79, parental antibody 219R45, or control antibody LZ1 were prepared in a mixture with human VEGF (R&D Biosystems, Minneapolis MN). The antibodies were serially diluted 5-fold from 20µM to 0.25nM in assay buffer in combination with hVEGF (final concentration 5ng/ml). The mixture was pre-incubated at 37°C for 2 hours. The medium was removed from the assay plate, and 100µl of the antibody/hVEGF mixture was added to each well. After 3-4 days incubation, medium was removed and a fresh aliquot of the antibody/hVEGF mixture was added to each well and allowed to incubate for another 4 days. On day 7, 20µl of Alamar Blue reagent (Invitrogen, Carlsbad, CA) was added to each well and incubated at 37°C for 5-6 hours. The plate was read with a SpectraMax M5e Microplate reader (Molecular Devices, Sunnyvale CA) using a excitation wavelength of 539nm and an emission wavelength of 590nm.

**[0302]** As shown in Figure 3, anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79, as well as parental anti-VEGF antibody 219R45 inhibited HUVEC proliferation. These results demonstrated that the bispecific antibodies were capable of inhibiting VEGF-induced proliferation of HUVEC cells.

#### Example 4

#### Inhibition of DLL4-induced Notch signalling by bispecific antibodies

**[0303]** Human PC3 cells were transfected with an expression vector encoding a full-length human Notch2 receptor and a firefly luciferase reporter vector (8xCBF-luciferase reporter) that is responsive to Notch signaling. The cells were also transfected with a Renilla luciferase reporter (Promega, Madison WI) as an internal control for transfection efficiency. Purified human DLL4 protein was coated onto 96-well plates at 100ng/well and Notch2-expressing PC3-luc cells were added to the wells. Anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18, 219R45-MB-21R79, parental anti-DLL4 antibodies 21M18, 21R79 or a control antibody LZ1 were serially diluted 5-fold from 20ug/ml to 0.064ug/ml, added to the appropriate wells, and incubated overnight. Luciferase activity was determined using a dual luciferase assay kit (Promega,

Madison, WI) with firefly luciferase activity normalized to Renilla luciferase activity.

**[0304]** As shown in Figure 4, anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21R79 and parental anti-DLL4 antibodies 21M18 and 21R79 inhibited DLL4-induced Notch signaling. Bispecific antibody 219R45-MB-21M18 inhibited DLL4-induced Notch signaling only at high antibody concentrations. These results demonstrated that bispecific antibody 219R45-MB-21R79, and to a lesser extent bispecific antibody 219R45-MB-21M18, were capable of inhibiting DLL4-induced Notch signaling. Thus, in combination with the results presented in Example 3, the anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21R79 and 219R45-MB-21M18 have demonstrated the ability to inhibit both VEGF-induced and DLL4-induced signaling and/or proliferation functions.

#### **Example 5**

##### **Inhibition of tumor growth *in vivo* by a bispecific antibody in a human skin graft model**

**[0305]** A human skin graft model has been reported which comprises a human skin graft and human tumor cells. A human skin graft is established and then human tumor cells are implanted into the skin graft, allowing the tumor cells to grow in an environment with human stroma and vasculature (Tahtis et al., 2003, Mol. Cancer Ther. 2:229-737). Human skin samples were obtained from neonatal foreskin tissue and grafted onto the lateral flank of NOD-SCID mice. After establishment of the skin graft, luciferase-labeled OMP-C8 colon tumor cells (20,000 cells) were injected intradermally into the human skin. Tumor growth was monitored by bioluminescence imaging using an IVIS imaging system (Caliper Life Sciences, Mountain View, CA). Tumors were allowed to grow until they reached  $1.2 \times 10^6$  photons per second. Tumor-bearing mice ( $n = 6$  mice/group) were randomized and treated with control Ab, anti-hDLL4 antibody 21M18, anti-VEGF antibody bevacizumab, or anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21M18. Animals were treated once a week and antibodies were administered intraperitoneally at a dose of 25mg/kg. Tumor growth was monitored by bioluminescence imaging on the indicated days.

**[0306]** As shown in Figure 5, both anti-hDLL4 antibody 21M18 and anti-VEGF antibody bevacizumab inhibited tumor growth in this human skin graft/human tumor model. Furthermore, bispecific anti-VEGF/ anti-DLL4 bispecific antibody 219R45-MB-21M18 was more effective than either the anti-DLL4 antibody or the anti-VEGF antibody alone. These data demonstrate the utility of simultaneously targeting DLL4 and VEGF with a bispecific antibody.

#### **Example 6**

##### **Tumorigenicity of OMP-PN8 pancreatic tumor cells after treatment with anti-VEGF/anti-DLL4 bispecific antibodies**

**[0307]** Mice bearing OMP-PN8 pancreatic tumors were treated with control antibody (15 mg/kg), anti-hDLL4 antibody 21M18 (15 mg/kg), anti-VEGF antibody bevacizumab (15 mg/kg), or anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 or 219R45-MB-21R79 (30 mg/kg) with or without gemcitabine (70 mg/kg). Following four weeks of treatment, tumors were harvested, processed to single cell suspensions and the human tumor cells were purified by immunomagnetic depletion of murine cells. 90 human tumor cells from each treatment group were transferred to a new cohort of mice (n = 10 mice/group). Tumors were allowed to grow for 55 days without any treatment and tumor volumes were measured with electronic calipers.

**[0308]** Figure 6 shows the tumor volume from the individual mice in each group. Cells isolated from mice treated with anti-hDLL4 antibody 21M18 had greatly decreased tumorigenicity, 5 out of 10 mice had tumors, as compared to cells isolated from mice treated with control antibody where 9 out of 10 mice had tumors. The reduction in tumor growth frequency indicates a reduction in cancer stem cell frequency. In contrast, bevacizumab treatment resulted in no reduction of tumor growth frequency, 10 out of 10 mice had tumors. Similar to bevacizumab, treatment with gemcitabine as a single agent had no effect on tumor growth frequency as 10 out of 10 mice had tumors. The anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79 both reduced tumor growth frequency (5 out of 10 mice had tumors and 4 out of 10 mice had tumors, respectively). Combination treatment with gemcitabine appeared to have no effect on tumor growth frequency. These data indicate that targeting DLL4 reduces cancer stem cell frequency while targeting VEGF alone does not. Importantly, these data indicate that the anti-CSC activity of the anti-DLL4 antibody is retained in a bispecific antibody.

#### Example 7

##### Bispecific Antibody ELISA

**[0309]** VEGF (ATGEN, South Korea) was coated onto Nunc maxisorb plates at 2ug/ml (100 $\mu$ l/well) and incubated overnight at 2-8°C. Bispecific antibodies 219R45-MB-21M18, 219R45-MB-21R79, 219R45-MB-21R75, and 219R45-MB-21R83 were diluted in blocking buffer (1 $\times$  PBS, 0.1% gelatin, 0.1% Polysorbate-20, pH 7.4) containing 2 $\mu$ g/ml biotin-DLL4-hFc. The antibodies were serially diluted 3-fold from 500ng/ml to 0.008ng/ml. The antibody samples were incubated for 2 hours in blocking buffer containing the biotin-DLL4-hFc. After incubation, the antibody samples were transferred to the VEGF-coated assay plate (100 ul/well) and incubated for 2 hours. Streptavidin-HRP (Jackson ImmunoResearch, West Grove, PA) was added to each well and incubated for 1 hr. TMB substrate was added to the wells with a 10 minute color development and the reaction was stopped with 2M sulfuric acid. Absorbance was read at 450-650nm and the data analyzed using the 4-parameter fit within the Softmax Pro analysis program (Molecular Devices, Sunnyvale, CA).

**[0310]** Figure 7 shows the titration curves of bispecific antibodies 219R45-MB-21M18 (open circles), 219R45-MB-21R79 (open squares), 219R45-MB-21R75 (open triangles), and 219R45-

MB-21R83 (open diamonds) in comparison to a reference anti-VEGF/anti-DLL4 bispecific antibody (solid circles). Relative potencies for the bispecific antibodies as compared to the reference bispecific antibody are shown in Table 5.

Table 5

Antibody	Relative Potency (%)
219R45-MB-21M18	67
219R45-MB-21R79	501
219R45-MB-21R75	422
219R45-MB-21R83	222

**[0311]** Bispecific antibody 219R45-MB-21R79 was the most potent, about 7-fold more potent than 219R45-MB-21M18, which reflected the higher affinity of the 21R79 antigen-binding site.

#### Example 8

#### Bispecific Antibody Production

**[0312]** Bispecific antibodies were produced using a GS-CHO cell line. CHOK1SV cells (Lonza Biologics) were transfected via electroporation with the gene(s) of interest coupled with glutamine synthetase (GS) as the selectable marker. Transfectants and subclones were screened for antibody productivity and the high producers were selected for scaled-up production. Cells were grown using a fed-batch process and fed-batch bioreactors. Accumulated antibody in harvested cell culture fluid (HCCF) was isolated and purified using chromatography techniques.

**[0313]** Bispecific antibody cell lines 219R45-MB-21M18.010.017 and 219R45-MB-21R79.017.003 were cultured in 5L stirred tank bioreactors for 14 days. Cell line 219R45-MB-21M18.010.017 produced a final antibody titer of 3.0g/L and cell line 219R45-MB-21R79.017.003 produced a final antibody titer of 0.8g/L. Cell lines 219R45-MB-21R75.101 and 219R45-MB-21R83.113 were cultured in 25L WAVE bioreactor systems (GE Healthcare) using a fed-batch process that achieved final antibody titers of 0.4g/L. Bispecific antibody cell lines 219R45-MB-21M18AG.138.007, 219R45-MB-21M18AG.038.009, 219R45-MB-21M18AG.142.002, 219R45-MB-21R79AG.072.014 and 219R45-MB-21R83AG.129.003 were cultured in 5 L stirred tank bioreactors for 14 - 15 days. Cell line 219R45-MB-21M18AG.138.007 produced a final antibody titer of 1.0 g/L after 14 days. Cell line 219R45-MB-21M18AG.038.009 produced a final antibody titer of 1.6 g/L after 14 days. Cell line 219R45-MB-21M18AG.142.002 produced a final antibody titer of 2.6 g/L after 14 days. Cell line 219R45-MB-21R79AG.072.014 produced a final antibody titer of 2.1 g/L after 15 days. Cell line 219R45-MB-21M18AG.038.009 produced a final antibody titer of 2.4 g/L after 15 days. Culture fluid was harvested by filtration from each of these four cell lines and subjected to Protein A affinity chromatography. The Protein A column was washed with a series of buffers and the antibodies were eluted using a low pH elution buffer. Initial characterization of the purity of the bispecific antibodies was performed using size exclusion

chromatography (SEC-HPLC) and isoelectric focusing (IEF).

**[0314]** Size exclusion chromatography (SEC) was used to determine the purity of the antibody product. SEC is a well known chromatographic method in which molecules (e.g., antibodies) in solution are separated by their size. SEC may be used to distinguish an antibody product from aggregate and/or impurities, and to determine the percentage of the antibody product as compared to the total mixture. As used herein, SEC does not distinguish between a homomeric antibody and a heterodimeric bispecific antibody.

**[0315]** Imaged capillary isoelectric focusing (icIEF) was used to determine identity and purity of the bispecific antibody heterodimers. Using icIEF, the charge isoforms of an antibody are separated according to their pI and the result is a "fingerprint" of the antibody's charge distribution. The icIEF method can also serve as a determination of purity by separating the bispecific antibody heterodimers by their distinct pI from any homodimer products or impurities.

**[0316]** Bispecific antibody samples were analyzed by icIEF on a ProteinSimple ICE280 instrument (ProteinSimple, Santa Clara, CA). For this analysis, a protein mixture is introduced into a capillary, high voltage is applied across the capillary and ampholytes establish a linear pH gradient along the length of the capillary. Under the influence of the electric field, the pI markers and the protein mixture both migrate the length of the capillary until a pH value is reached where the net charge is zero. Once focused, the ICE280 instrument uses whole-column imaging detection with a 280-nm UV camera to monitor the pattern of protein isoforms within the capillary. The resulting electropherogram is calibrated using internal pI markers and integrated to establish the respective percentage areas of the different charged isoforms of the protein mixture. The charge profiles from several anti-VEGF/anti-DLL4 bispecific antibodies are shown in Figure 8. For this experiment, Protein A eluates were diluted with MilliQ water to a concentration of 6.6mg/ml. A total of 18 $\mu$ l of the sample was mixed with 100 $\mu$ L of 8M urea, 70 $\mu$ l of 0.5% methylcellulose, 8 $\mu$ L of 3-10 Pharmalyte, 2 $\mu$ l of high pI marker and 2 $\mu$ l of low pI marker to a final volume of 200 $\mu$ l. Table 6 shows the percentage of antibody product from cell lines 219R45-MB-21M18.010.017, 219R45-MB-21R79.017.002, 219R45-MB-21R75.101, 219R45-MB-21R83.113, 219R45-MB-21M18.138.007, 219R45-MB-21M18AG.038.009, 219R45-MB-21M18AG.142.002, 219R45-MB-21R79AG.072.014, and 219R45-MB-21R83AG.129.003 after Protein A affinity chromatography as determined by SEC-HPLC. Table 6 also shows the percentage of heterodimeric antibodies from cell lines 219R45-MB-21M18.010.017, 219R45-MB-21R79.017.002, 219R45-MB-21R75.101, 219R45-MB-21R83.113, 219R45-MB-21M18.138.007, 219R45-MB-21M18AG.038.009, 219R45-MB-21M18AG.142.002, 219R45-MB-21R79AG.072.014, and 219R45-MB-21R83AG.129.003 after Protein A affinity chromatography as analyzed by icIEF.

Table 6

Cell Line	Antibody Titer (g/L)	Purity by SEC (%)	Purity by IEF (%) heterodimer)
219R45-MB-21M18.010.017	3.0	73.9	47.2
219R45-MB-21R79.017.002	0.8	79.3	72.5
219R45-MB-21R75.101	0.4	91.2	84.9

Cell Line	Antibody Titer (g/L)	Purity by SEC (%)	Purity by IEF (% heterodimer)
219R45-MB-21R83.113	0.4	91.8	91.4
219R45-MB-21M18.138.007	1.0	92.6	95.8
219R45-MB-21M18AG.038.009	1.6	89.6	89.0
219R45-MB-21M18AG.142.002	2.6	91.2	84.6
219R45-MB-21R79AG.072.014	2.1	87.8	84.9
219R45-MB-21R83AG.129.003	2.4	89.4	90.5

**[0317]** The purity of the bispecific antibody product can be increased further by additional chromatography steps. After Protein A affinity chromatography, the eluate fraction was held at a low pH for no less than 60 minutes at room temperature for viral inactivation. The antibody solution (Protein A column eluate, pH adjusted) was loaded onto a strong anion-exchange column. Product- and process-related impurities bound to the anion exchange chromatography resin and the flow-through fraction (antibody product) was collected. In some cases, purity was further improved by use of a multi-modal chromatography resin such as ceramic hydroxyapatite. In some cases, buffer exchange of the antibody product was undertaken using ultrafiltration and diafiltration techniques, after which excipients were added. The formulated antibody was sterile filtered into sterile containers and stored refrigerated or frozen. Purity of the bispecific antibodies was re-assessed using SEC-HPLC and IEF.

Table 7

Cell Line	Purity by SEC (%)	Purity by IEF (% heterodimer)
219R45-MB-21M18.010.017	98.9	98.5
219R45-MB-21R79.017.002	95.1	99.3
219R45-MB-21R75.101	97.2	98.2
219R45-MB-21R83.113	95.3	91.4
219R45-MB-21M18.138.007	98.1	100
219R45-MB-21M18AG.142.002	99.6	100
219R45-MB-21R79AG.072.014	98.2	100
219R45-MB-21R83AG.129.003	998.6	100

**[0318]** As shown in Table 7, the purification of the anti-VEGF/anti-DLL4 bispecific antibodies with additional chromatography steps after Protein A resulted in isolation of antibody products that were 95% to about 99% pure as analyzed by SEC. Analysis by IEF determined that purified anti-VEGF/anti-DLL4 bispecific antibody from cell line 219R45-MB-21M18.010.017 was 98.5%

heterodimeric, anti-VEGF/anti-DLL4 bispecific antibody from cell line 219R45-MB-21R79.017.002 was 99.3% heterodimeric, anti-VEGF/anti-DLL4 bispecific antibody from cell line 219R45-MB-21R75.101 was 98.2% heterodimeric, anti-VEGF/anti-DLL4 bispecific antibody from cell line 219R45-MB-21R83.113 was 91.4% heterodimeric, anti-VEGF/anti-DLL4 bispecific antibody from cell line 219R45-MB-21M18.138.007 was 100% heterodimeric, anti-VEGF/anti-DLL4 bispecific antibody from cell line 219R45-MB-21M18AG.142.002 was 100% heterodimeric, anti-VEGF/anti-DLL4 bispecific antibody from cell line 219R45-MB-21R79AG.072.014 was 100% heterodimeric, and anti-VEGF/anti-DLL4 bispecific antibody from cell line 219R45-MB-21R83AG.129.003 was 100% heterodimeric. These results demonstrated that the anion-exchange chromatography step greatly increased the percentage of heterodimeric antibodies as compared to purification with Protein A chromatography alone. The addition of a multi-modal chromatography step such as ceramic hydroxyapatite can also improve monomeric purity (as determined by SEP-HPLC).

#### **Example 9**

##### **Inhibition of OMP-C8 colon tumor growth *in vivo* tumor recurrence model**

**[0319]** Single cell suspensions of OMP-C8 colon tumor xenografts (20,000 cells) were injected subcutaneously into the flanks of 6-8 week old NOD/SCID mice. Tumors were allowed to grow for 33 days until they reached an average volume of 240mm<sup>3</sup>. The mice were randomized (n = 10 per group) and treated with anti-hDLL4 antibody 21M18, anti-VEGF antibody bevacizumab, a combination of antibodies 21M18 and bevacizumab, anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21M18, anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21R79, or control antibody, all in combination with irinotecan. Antibodies and irinotecan were dosed weekly by injection into the intraperitoneal cavity. Antibodies 21M18 and bevacizumab were dosed at 7.5mg/kg, bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79 were dosed at 15mg/kg, and irinotecan was dosed at 45mg/kg. Irinotecan was dosed for four weeks, at which time, it was discontinued and the administration of the antibodies continued. Tumor growth was monitored and tumor volumes were measured with electronic calipers at the indicated time points. Data are expressed as mean ± S.E.M.

**[0320]** As shown in Figure 9, anti-hDLL4 antibody 21M18 continued to inhibit tumor growth after treatment with irinotecan was stopped. In contrast, anti-VEGF antibody bevacizumab was not able to inhibit regrowth of the tumor after irinotecan had been stopped. The combination of anti-DLL4 antibody 21M18 and anti-VEGF antibody bevacizumab resulted in greater inhibition of tumor regrowth than either agent alone. Furthermore, the anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21M18 was more effective at inhibiting tumor regrowth than the mixture of the two antibodies.

#### **Example 10**

##### **Reduction in tumorigenicity of OMP-C8 colon tumors**

**[0321]** Single cell suspensions of OMP-C8 colon tumor xenografts (20,000 cells) were injected subcutaneously into the flanks of 6-8 week old NOD/SCID mice. Tumors were allowed to grow for 33 days until they reached an average volume of 300mm<sup>3</sup>. The mice were randomized (n = 5 per group) and treated with anti-DLL4 antibody 21M18, anti-VEGF antibody bevacizumab, a combination of antibodies 21M18 and bevacizumab, anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21M18, anti-VEGF/anti-DLL4 bispecific antibody 219R45-MB-21R79, or control antibody, either in combination with irinotecan or without irinotecan. Antibodies and irinotecan were dosed weekly by injection into the intraperitoneal cavity. Antibodies 21M18 and bevacizumab were dosed at 7.5mg/kg, bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79 were dosed at 15mg/kg, and irinotecan was dosed at 45mg/kg. Tumors were harvested after 4 weeks, processed into single cell suspensions, and the human tumor cells were isolated. 150 tumor cells from each experimental group were injected subcutaneously into a new cohort of mice (n = 10 per group) and tumors were allowed to grow without treatment. Tumor growth was monitored and tumor volumes were measured with electronic calipers.

**[0322]** Individual tumor volumes at day 68 are shown in Figure 10. Anti-DLL4 antibody 21M18, the combination of 21M18 with anti-VEGF antibody bevacizumab, bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R79, and irinotecan all reduced tumor growth frequency as single agents. In contrast, anti-VEGF bevacizumab as a single agent had no effect on tumor growth frequency as compared to the control antibody. In the groups treated with a combination of irinotecan and antibodies, the bispecific antibody 219R45-MB-21M18 had the greatest effect in reducing tumor growth frequency.

#### Example 11

##### **Inhibition of OMP-C8 colon tumor growth *in vivo***

**[0323]** Single cell suspensions of OMP-C8 colon tumor xenografts (50,000 cells) were injected subcutaneously into the flanks of 6-8 week old NOD/SCID mice. Tumors were allowed to grow for 21 days until they reached an average volume of 80mm<sup>3</sup>. The mice were randomized (n = 8 per group) and treated with anti-DLL4 antibody 21M18, anti-VEGF antibody bevacizumab, anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18, 219R45-MB-21R75, 219R45-MB-21R79, 219R45-MB-21R83, or control antibody, either alone or in combination with irinotecan. Antibodies and irinotecan were dosed weekly by injection into the intraperitoneal cavity. Bevacizumab and bispecific antibodies 219R45-MB-21M18, 219R45-MB-21R75, 219R45-MB-21R79, and 219R45-MB-21R83 were dosed at 15mg/kg, and irinotecan was dosed at 7.5mg/kg. Tumor growth was monitored and tumor volumes were measured with electronic calipers at the indicated time points. Data are expressed as mean  $\pm$  S.E.M.

**[0324]** As single agents, all four anti-VEGF/anti-DLL4 bispecific antibodies showed enhanced

antitumor activity relative to anti-VEGF antibody bevacizumab. In combination with irinotecan, treatment with anti-VEGF/anti-DLL4 bispecific antibodies 219R45-MB-21M18 and 219R45-MB-21R83 resulted in the greatest inhibition of tumor growth (Figure 11).

**[0325]** Following the treatment phase, tumor sections were prepared and analyzed by hematoxylin and eosin (H&E) staining. The tumors treated with 219R45-MB-21M18 and 219R45-MB-21R83 in combination with irinotecan showed dark pink staining regions providing evidence of extensive calcification. This is characteristic of highly necrotic tumor tissue.

### Example 12

#### Non-GLP toxicity study of bispecific antibodies in cynomolgus monkeys

**[0326]** A non-GLP toxicity study in cynomolgus monkeys was initiated to evaluate and compare the toxicity profile of some of the bispecific antibodies. The animals were dosed with 0 mg/kg (control), 5 mg/kg (low dose), or 30 mg/kg (high dose) of anti-DLL4/anti-VEGF bispecific antibody (219R45-MB-21M18, 219R45-MB-21R83, or 219R45-MB-21R79) every 2 weeks via IV infusion. 3 males and 3 females were dosed in each group. After 15 weeks, mean body weights were lower in animals receiving the high dose of 219R45-MB-21R79 than in animals that received the high dose of either 219R45-MB-21R18 or 219R45-MB-21R83. In addition, mean serum albumin levels were lower in animals that received 219R45-MB-21R79 than in those that received either 219R45-MB-21R18 or 219R45-MB-21R83. Although preliminary in nature, these early data suggest that 219R45-MB-21R18 and 219R45-MB-21R83 may have a superior toxicity profile compared to 219R45-MB-21R79.

### SEQUENCES

#### [0327]

##### 21M18 Heavy chain with signal sequence (underlined) (SEQ ID NO:1)

MKHLWFFLLVAAPRWVLSQVQLVQSGAEVKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYISSYNGATNYNQKFGRVTFTDTSTSTAYMELRSLRSDDTAVYYCARDYDYDVGMDYWGQGTIVTVSSASTKGPSVFPLAPCSRSTSESTAA~~LGCLV~~KDYFPEPVTVSWNSGALTSGVHTFP~~AVL~~QSSGLYSLSVVTPSSNFGTQTYTCNVDHKPSNTKVDKTVERKC~~CVEC~~PPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHDPEVQFNWYVGVEVHN~~AKT~~TKPREEQFNSTFRVVSVLT~~V~~HQDWLN~~G~~KEYKCKVSNKGLPAPIEKTIS~~K~~TKGQP~~REP~~QVYTLPPSREEMTKNQVSLTCLVEGFYPSDIAVEWESNGQPENNYKTTPPMLSDGSFFLYSELTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

##### 21R79 Heavy chain with signal sequence (underlined) (SEQ ID NO:2)

MKHLWFFLLVAAPRWVLSQVQLVQSGAEVKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYIANYNRATNYNQKFGRVTFTDTSTSTAYMELRSLRSDDTAVYYCARDYDYDVGMDYWGQGTIVTVSSASTKGPSVFPLAPCSRSTSESTAA~~LGCLV~~KDYFPEPVTVSWNSGALTSGVHTFP~~AVL~~QSSGLYSLSVVTPSSNFGTQTYTCNVDHKPSNTKVDKTVERKC~~CVEC~~PPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHDPEVQFNWYVGVEVHN~~AKT~~TKPREEQFNSTFRVVSVLT~~V~~HQDWLN~~G~~KEYKCKVSNKGLPAPIEKTIS~~K~~TKGQP~~REP~~QVYTLPPSREEMTKNQVSLTCLVEGFYPSDIAVEWESNGQPENNYKTTPPMLSDGS

FFLYSELTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

**219R45 Heavy chain with signal sequence (underlined) (SEQ ID NO:3)**

MKHLWFFLLVAAPRWVLSQVQLVQSGAEVKPGASVKSCKASGYTFTNYWMHWVRQAP  
GOGLEWMGDINPSNGRTSYKEKFKRRVTLSDKSSSTAYMELSSLRSEDTAVYFCTIHYD  
DKYPLMDYWGQGTLTVVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS  
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTPSSNFGTQTYCNUDHKPSNTKVDKTVER  
KCCVECPPPCPAPPVAGPSVFLFPPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDG  
VEVHNAKTKPREEQFNSTFRVVSVLTVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKG  
QPREPQVYTLPPSREKMTKNQVSLTCLVKGFPSDIAVEEWSNGQPENNYKTTPMLKSD  
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

**Light chain with signal sequence (underlined) (SEQ ID NO:4)**

MVQTQVFISLLLWISGAYGDIMQSPDSLAVSLGERATISCRASESVDNYGISFMKWF  
QQKPGQPPKLLIYAASNQGSGVPDRFSGSGSGTDFLTISSLQAEDVAVYCQQSKEVPW  
TFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFPRAEKVQWKVDNALQS  
GNSQESVTEQDSKDSTYSLSSTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

**21M18 Heavy chain without predicted signal sequence (SEQ ID NO:5)**

QVQLVQSGAEVKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYISSYNGATNY  
NQKFKGRVTTTDTSTSAYMELRSLRSDDTAVYYCARDYDDVGMDYWGQGTLTVS  
STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVWNSGALTSGVHTFPAVLQSSG  
LYSLSSVVTPSSNFGTQTYCNUDHKPSNTKVDKTVERKCCVECPPCAPPVAGPSVF  
FPPKPDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV  
VSVLTVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ  
VSLTCLVKGFPSDIAVEEWSNGQPENNYKTTPMLSDGSSFLYSLTVDKSRWQQGNV  
FSCSVMHEALHNHYTQKSLSLSPGK

**21R79 Heavy chain without predicted signal sequence (SEQ ID NO:6)**

QVQLVQSGAEVKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYIANYNRATNY  
NQKFKGRVTTTDTSTSAYMELRSLRSDDTAVYYCARDYDDVGMDYWGQGTLTVS  
STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVWNSGALTSGVHTFPAVLQSSG  
LYSLSSVVTPSSNFGTQTYCNUDHKPSNTKVDKTVERKCCVECPPCAPPVAGPSVF  
FPPKPDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV

VSVLTVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ  
VSLTCLVKGFPSDIAVEEWSNGQPENNYKTTPMLSDGSSFLYSLTVDKSRWQQGNV  
FSCSVMHEALHNHYTQKSLSLSPGK

**219R45 Heavy chain without predicted signal sequence (SEQ ID NO:7)**

QVQLVQSGAEVKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYISSYNGATNY  
KEKFKRRVTLSDKSSSTAYMELSSLRSEDTAVYFCTIHYDDKYPLMDYWGQGTLTVS  
SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVWNSGALTSGVHTFPAVLQS  
SGLYSLSSVVTPSSNFGTQTYCNUDHKPSNTKVDKTVERKCCVECPPCAPPVAGPSVF  
FLFPPKPDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV  
RVVSVLTVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQ  
NQVSLCLVKGFPSDIAVEEWSNGQPENNYKTTPMLSDGSSFLYSLTVDKSRWQQGNV  
FSCSVMHEALHNHYTQKSLSLSPGK

**Light chain without predicted signal sequence (SEQ ID NO:8)**

DIVMQSPDSLAVSLGERATISCRASESVDNYGISFMKWFQQKPGQPPKLLIYAASNQGS  
GVPDRFSGSGSGTDFLTISSLQAEDVAVYCQQSKEVPWTFGGGTKVEIKRTVAAPSV  
IFPPSDEQLKSGTASVVCLLNNFPRAEKVQWKVDNALQSGNQESVTEQDSKDSTYSL  
STLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

**21M18 Heavy chain variable region (SEQ ID NO:9)**

QVQLVQSGAEVKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYISSYNGATNY  
NQKFKGRVTTTDTSTSAYMELRSLRSDDTAVYYCARDYDDVGMDYWGQGTLTVS

## 21R79 Heavy chain variable region (SEQ ID NO:10)

QVQLVQSGAEVKKPGASVKISCKASGYSTAYYIHWVKQAFQGLEWIGYIANYNRATNY  
NQKFKGRVIFTTDSTSTAYMELRSLRSDDTAVYYCARDYDYDVGMDYWGQGTLVTVSS

## 219R45 Heavy chain variable region (SEQ ID NO:11)

QVQLVQSGAEVKKPGASVKVSKASGYTFTNYWMHWVRQAPGQGLEWMGDINPSNGRTSY  
KEKFKRRVTLSDKSSSTAYMELSSLRSEDATAVYFCTIHYDDKYYPLMDYWGQGTLVTVSS

## Light chain variable region (SEQ ID NO:12)

DIVMTQSPDSLAVSLGERATISCRASESVDNYGISFMKWFQQKPGQPPKLLIYAASNQGS  
GVPDRFSGSGSGTDFITLTISSLQAEDEVAVYYCQQSKEVPWTFGGGKVEIK

## 21R75, 21R79, 21R83, and 21M18 Heavy chain CDR1 (SEQ ID NO:13)

TAYYIH

## Alternative 21R75, 21R79, 21R83, and 21M18 Heavy chain CDR1 (SEQ ID NO:79)

AYYIH

## 21R79 Heavy chain CDR2 (SEQ ID NO:14)

YIANYNRATNYNQKFKG

## 21M18 Heavy chain CDR2 (SEQ ID NO:15)

YISSYNGATNYNQKFKG

## 21R75, 21R79, 21R83, and 21M18 Heavy chain CDR3 (SEQ ID NO:16)

RDYDYDVGMDY

## 219R45 Heavy chain CDR1 (SEQ ID NO:17)

NYWMH

## 219R45 Heavy chain CDR2 (SEQ ID NO:18)

DINPSNGRTSYKEKFKR

## 219R45 Heavy chain CDR3 (SEQ ID NO:19)

HYDDKYYPLMDY

## Light chain CDR1 (SEQ ID NO:20)

RASESVDNYGISFMK

## Light chain CDR2 (SEQ ID NO:21)

AASNQGS

## Light chain CDR3 (SEQ ID NO:22)

QQSKEVPWTFGG

## Human DLL4 with signal sequence (underlined) (SEQ ID NO:23)

MAAARSASGWLLLVALWQRAAGSGVFQLQLFFINERGVLASGRPCEPGCRTFFRV  
CLKHFQAVVSPGPCTFGTVSTPVLGTNSFAVRRDSSGGGRNPLQLPFNFTWPGTFSLIIE  
AWHAPGDDDLRPEALPPDALISKIAIQGLSLAVGONWLLDEQTSTLTRLYSYRVICSDNY  
GDNCSRLCKRNDHFGHYVCQPDGNLSCLPGWTGEYCQQPICLSGCHEQNGYCSKPAECL  
CRPGWQGRLCNECIPHNGCRHGTCSTPWQCTDEGWGGLFCDQDLNYCTHSPCKNGATC  
SNSGQRSYTCTRPGYTGVDCEELSECDSNPCRNGGSCKDQEDGYHCLCPPGYYGLHCE  
HSTLSCADSPCFNGGSCRERNQGANYACECPPNFTGSNCEKVDRCTSNPCANGGQCLNR  
GPSRMCRPGFTGTYCELHVSDCARNPCAHGGTCHDLENGLMCTPAGFSGRRCEVRTS

IDACASSPCFN RATCYTDLSTDTFVCNC PYGFVGSRCEFPVG

**Human DLL4 without predicted signal sequence (SEQ ID NO:24)**

SGFQLQLQEFINERGVLASGRPCEPGCRTFRVCLKHQAVVSPGCTFGTVSTPVLGT  
NSFAVRDDSSGGGRNPLQLPFNFTWPGTFSLIEAWHAPGDDLPEALPPDALISKIAIQ  
GSLAVGQNWLDEQTSTLTRLRYSYRVCSDNYYGDNCRLCKKRNDHF GHYVCQPDGNL  
SCLPGWTGEYCYQQPICLSCHEQNGYCSKPAECLCRPGWQGRLCNECIPHNGCRHGTCT  
PWQCTCDEGWGGLFCDQDLYNCTHSPCKNGATCSNSGQRSYTCRPGFTGVDELELS  
ECDSNP CRNGGSKDQEDGYHCLCPPGYYGLHCEHSTLSCADSPCFNGGSCRERNQGANY  
ACECPPNFTGSNCEKKVDRCTS NPCANGQCLNRGSPSRMCRPGFTGTYCELHVSDCAR  
NPCAHGGTCHDLENGLMCTCPAGFSRRCEVRTSIDACASSPCFN RATCYTDLSTDTFVC  
NCPYGFVGSRCEFPVG

**Human DLL4 N-Terminal Region (SEQ ID NO:25)**

SGFQLQLQEFINERGVLASGRPCEPGCRTFRVCLKHQAVVSPGCTFGTVSTPVLGT  
NSFAVRDDSSGGGRNPLQLPFNFTWPGTFSLIEAWHAPGDDLPEALPPDALISKIAIQ  
GSLAVGQN

**Human DLL4 DSL Domain (SEQ ID NO:26)**

WILDEQTSTLTRLRYSYRVCSDNYYGDNCRLCKKRNDHF GHYVCQPDGNLSCLPWTG  
EYC

**Human VEGF-A with signal sequence (underlined)(SEQ ID NO:27)**

MNFLLSWVHSL ALLLYLHHAKWSQAAPMAEGGGQNHHEVVKFMDVYQR SYCHPIETLVD  
I FQ EY PDEIEYI FKPSCVPLMRCGCCNDE GLECVPTeesN ITMQIMRIKPHQGHIGEM  
SFLQHNKCECRPKDRARQEKKSVRGKGKQKRKKSRYKSWSVYVGARCLMPWSLPG  
PHPCGCSERRKHLFVQDPQTCKCSCNTDSRCKARQLELNERTCRCDKPRR

**Human VEGF-A without predicted signal sequence (SEQ ID NO:28)**

APMAEGGGQNHHEVVKFMDVYQR SYCHPIETLVDIFQ EY PDEIEYI FKPSCVPLMRCGGC  
CNDEGLECVPTeesN ITMQIMRIKPHQGHIGEM SFLQHNKCECRPKDRARQEKKSVRG  
KGKGQKRKKSRYKSWSVYVGARCLMPWSLPGPHPCGCSERRKHLFVQDPQTCKCSC  
KNTDSRCKARQLELNERTCRCDKPRR

**21M18 Heavy chain nucleotide sequence (13B Version 1) (SEQ ID NO:29)**

ATGAAGCACCCTGTGGTTCTTCTGCTGGTGGCCGCTCCAGATGGGTGCTGCCCCAG  
GTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCTGGCGCTCCGTGAAGATCTCC  
TGCAAGGCCTCCGGCTACTCCTCACCGCTTACTACATCCACTGGTCAAGCAGGCCCT  
GGCAGGGCCTGGAATGGATGGCTACATCTCCTCACAGGGGCCACCAACTACAAC  
CAGAAATTCAGGGCCCGTGA CTTTACACCGCCGACACCTCCACCTCACCGCCTACATG  
GA ACTGCGGCTCTGCGGAGCGACGACACCGCCGTGACTACTGCGCCAGAGACTACGAC  
TACGACGTGGCATGGACTACTGGGCCAGGGCACCTGGTACCGTGTCCCTGCGCTCC  
ACCAAGGGCCCATTGGTGTCCCTGCCCCCTTGCTCCGGTCCACCTCTGAGTCTACC  
GCCGCTCTGGCTGCCCTGGTGAAGGACTACTTCCCTGACCCGTGACCGTGTCCCTGGAAC  
TCTGGCGCCCTGACCTCTGGCGTGACACCTTCCCTGCCGTGCTGCAGTCTCCGGCTG  
TACTCCCTGTCTAGCGTGGTGA CCGTGCCTCCCTCAACTTCCGACCCAGACCTACACC  
TGTAACTGGA GACCACAAGCCCTTCAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGC  
TGGTGGAGTGGCCCTCCTGTCTGGCTGCCCTGGTCAATTGGTACGTGGAGCGGTGGAG  
GTGCACAACGCCAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGTGGT  
TCTGTGCTGACCGTGGTGCACCGAGACTGGCTGAACGGCAAAGAAATACAAGTGC  
TCCAACAAGGGCCTGCCCTGCCCCATCGAAAAGACCATCAGCAAGACCAAGGGCCAGCCT  
CGCGAGCCTCAGGTGTACACCCCTGCCCTCCAGCGGGAAAGAAATGACCAAGAACCCAGGTG  
TCCCTGACCTGTCTGGTGGAGGGCTTCTACCCCTCCGATATGCCGTGGAGTGGAGTCT  
AACGGCCAGCCTGAGAACAACTACAAGACCAACCCCTCTATGCTGGACTCCGACGGCTCC  
TTCTTCCCTGTACTCCGA ACTGACCGTGGACAAGTCCCGTGGCAGCAGGGCAACGTGTT  
TCTGCTCCGTGATGCACGAGGCCCTGCACAACC ACTACACCCAGAAGTCCCTGTCCTG  
TCTCCTGGCAAGTAG

**21R79 Heavy chain nucleotide sequence (13B Version 1) (SEQ ID NO:30)**

ATGAAGCACCCTGTGGTTCTTCTGCTGGTGGCCGCTCCAGATGGGTGCTGCCCCAG  
GTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCTGGCGCTCCGTGAAGATCTCC  
TGCAAGGCCTCCGGCTACTCCTCACCGCTACTACATCCACTGGTGAACACGGCACCA  
GGCCAGCCACTGCAATCGCATGCCCTATTCGCAACTACAGCAAGGGCCAGCCT

GGCCAGGGAACTGGAAATGGAACTGGGCTTATGCGCAACTACGGACGGGACACCAACATACAC  
 CAGAAATTCAAGGGCCCGTGACCTTACCCACCGACACCGCCGTGTACTACTGCGCCAGAGACTACGAC  
 GAACTGCGGTCCCTGCGGAGCGACCCGCGTGTACTACTGCGCCAGAGACTACGAC  
 TACGACGTGGCATGGACTACTGGGCCAGGGCACCCCTGGTGTACAGTGTCTCCGGCTCC  
 ACCAAGGGCCCTCCGTGTTCCCTCTGGCCCTTGCTCCCGTCCACCTCTGAGTCTACC  
 GCCGCTCTGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGTCTGGAAC  
 TCTGGCGCCCTGACCTCTGGCTGCACACCTTCCCTGCCGTGCTGCAGTCCTCCGGCTG  
 TACTCCCTGTCTAGCGTGGTGACCGTGCCTCTCCAACTCGGCACCCAGACCTACACC  
 TGTAAACGTGGACCACAAGCCTCCAAACACCAAGGTGGACAAGACCGTGGAGCGGAAGTGC  
 TGCCTGGAGTGCCCTCCGTGCTCTGGCTGGCCCTTGCTGTTCTGTT  
 CCTCCAAAGGCTAAGGACACCCGTGATGATCTCCGGACCCCTGAAGTGAACCTGCGTGGTG  
 GTGGACGTGCCCACGAGGACCCGTGGTGAGGTGCAATTGGTACGGTGGACGGCGTGGAG  
 GTGCACAACGCCAAGACCAAGCCTCGGGAGGAACAGTCAACTCCACCTCCGGGTGGTG  
 TCTGTGCTGACCGTGGTGACCCAGGACTGGCTGAACGGCAAAGAAATACAAGTGCAGGTG  
 TCCAACAAGGGCTGCTGCCCTATGAAAAGACCATCAGCAAGACCAAGGGCCAGCCT  
 CGCGAGCCTCAGGTGTACACCCCTGCCCTCAGCGGGAAAGAAATGACCAAGAACAGGTG  
 TCCCTGACCTGTCTGGGGAGGGCTTACCCCTCCGATATGCCGTGGAGTGGAGTCT  
 AACGGCCAGCCTGAGAACAACTACAAGACCAACCCCTCTGCTGGACTCCGACGGCTCC  
 TTCTCCTGTAACCGAAGTGGACAAGTCCGGCAGCAGGGCAACGTGTT  
 TCTCTGGCAAGTAG

**21R79 Heavy chain nucleotide sequence (13B Version 2) (SEQ ID NO:31)**

ATGAAGCACCTATGGTTCTTCTATTATTAGTGGCCGCTCCCGTGGGTGTTATCGCAG  
 GTTCAGCTAGTTCACTGGAGCGGAAGTTAAGAAACCTGGAGCATCGTAAAATAAGT  
 TGCAAGGCATCCGGTTACTCGTTACCGCATACTATATCCACTGGTTAAACAGGCACCA  
 GGACAGGGACTTGAATGGATCGGATATATCGCTAATTATAAGAGCTACAAACTATAAC  
 CAAAATTCAAAAGGACCGCTGACTTACAACACTGACACCTCAACCTCGACAGCATACTG  
 GAATTACGGTCCCTACGGTCTGACCGACACTGCCGTTACTATTGGCTAGAGATTATGAT  
 TATGATGTTGAATGGACTATTGGGGCAGGGAAACACTGGTGTACAGTGTCTGACATCC  
 ACTAAGGGACATCCGGTCTGAAAGATTACTTCCCTGCTCGTGCACCTCTGAATCGACT  
 GCGCTCTGGGATGCCGTGAAAGATTACTTCCCTGCTGACCGTGTGACCGTTCTGGAAC  
 TCGGGCGCCCTAACCTCGGCGTGCACACATTCCCTGCCGTGCTACAGTCTTCTGGCTA  
 TACTTTTATCTCGGTGTTACCGTACCTTCTCTAACTTCCGAAAGCTTACCCAAACTTACACC  
 TGTAAACGTAGACCACAAGCCTCGAACACCAAGGTGGACAAGACTGTTGAGCGAAAGTGC  
 TCGGTTGAGTGGCCCTCATGCTCGCACCTCCGTGGCTGGCCCTCTGTGTTCTGTT  
 CCTCCAAACACTTAAGGACACTCTAAATGATCTCGGACTCCTGAGGTGACTTGCCTGGTT  
 GTGGACGTGCCCACGAGGACCCGTGGTGAGGTGCAATTGGTACGGTGGACGGAGTCGAG  
 GTGCACAATGCAAAGCCAAGCCTCGGGAGGAACAGTCAACTCCACCTCCGGTGGTT  
 TCTGTGTTGACCGTTGTCACCAAGACTGGCTGAACGGCAAAGAAATACAAGTGCAGGTG  
 TCCAACAAGGGCTGCCCTGCCCCATCGAAAAGACCATCAGCAAGACCAAGGGCCAGCCT  
 CGCGAGCCTCAGGTGTACACCCCTGCCCTCAGCGGGAAAGAAATGACCAAGAACAGGTG  
 TCCCTGACCTGTCTGGGGAGGGCTTACCCCTCCGACATGCCGTGAGTGGAGTCT  
 AACGGACAGCGGAGAACAACTACAAGACTACGCCCTCAATGCTGGACTCCGACGGCTCC  
 TTCTCCTGTAACCGAAGTGGACAAGTCCGGTGGCAGCAGGGCAACGTGTT  
 TCATGCTCCGTAATGCACGAAGCCTTGACAACTACACTACACTCAAAGTCCCTATCCTTA  
 TCTCCTGGCAAGTAG

**219R45 Heavy chain nucleotide sequence (13A Version 1) (SEQ ID NO:32)**

ATGAAGCATCTGGTTTCTGTCGTCGGCCACCCAGATGGGTGTTCTCCAA  
 GTGCAGCTGGTCCAGAGCGGGCTGAGGTGAAGAAACCCGGAGCAAGCGTAAAAGTATCG  
 TGTAAGGCTCGGGTACACGTTACAAACTACTGGATGCAATTGGTGCGGCAGGCTCCG  
 GGACAGGGGTTGGAATGGATGGGTGACATTAACCCCTCAAATGGCAGAACATCATATAAG  
 GAAAAGTTCAAACGCCGCTCACACTCTCGTGGACAAGTCAAGCTGACTCGTACATG  
 GAACTTCGTCGCTGAGGTGGAGGACACGGCAGTGTACTTTGACCATCCATTATGAT  
 GACAAGTATTACCCCTGATGGATTATTGGGTGAGGTACGTTGGTACCGTCTCCAGC  
 GCGTCGACGAAAGGCTCGGTATTCCCTGCCCTGCTCGAGGTGACATCCGAA  
 TCAACAGCTGCCCTCGGCTGCCTGGTCAAAGACTACTTCCAGAGCCGGTAACGGTGTG  
 TGGAACTCGGGAGCGTACGTCGGAGTCCACACATTCCGGCGTACTGCAATCCTCG  
 GGACTGTATTGTTGTCGTCAGTGGTACTGTCGGCTCCAAATTCCGGACTCAGACC  
 TATACGTGCAACGTCGACCAACAAACCTCAAACACCAAGGTGGATAAGACAGTGGAGCGC  
 AAGTGTGCGTGGAGTGTCCCCCGTGTCCGGCACCCCTGTCGCCGACCCCTCAGTCTT  
 TTGTTCCGGCGAAGCCAAAGATAACTCATGATCTCAAGAACGCCGAGGTAACATGC  
 GTGGTGGTCGATGTAAGCCACGAGGATCCAGAAGTACAATTCAATTGGTATGTAGACGGG  
 GTCGAGGTCCATAACGCAAAGACGAAACGAGGGAAAGAGCAGTTCAATTGACTTCCGG  
 -----

GTGGTGTGGTCTTACAGTCGTACATCAGGACTGGTGAACGGGAAGGAGTACAAGTGT  
 AAGTATCGAATAAGGCCCTCCAGGCCGATGAAAAGACCATCTCAAGACCAAAGGA  
 CAGCCACGAGAGCCGCAAGTCTATACGCTTCCCTCCAGCCGAGAAAAGATGACTAAAAAC  
 CAGGTATCGTTACGTTCTCGTCAAGGGTTCTACCCCTCGGACATCGCGGTGGAATGG  
 GAGAGCAATGGACAACCGGAAACAACTACAAGACGACACCGCCTATGTTGAAAAGCGAT  
 GGATCGTTTCTCTATTGAAACTCACGGTCGATAAGTCACGGTGGCAGCAGGGGAAT  
 GTGTTCTCTGTTAGTGTGACGAGGCCTCCACAATCACTATACCCAGAAAAGCCTG  
 TCACTTCCCCGGGAAATGA

**219R45 Heavy chain nucleotide sequence (13A Version 2) (SEQ ID NO:33)**

ATGAAGCACCTCTGGTTCTTCCCTGCTCCTCGTGGCTGCTCCTCGGTGGTCCCTCCCAA  
 GTGCAGCTGGTCCAGAGCGGGCTGAGGTGAAGAAACCCGGAGCTTCGTCAAAGTCTCC  
 TGTAAAGCTCCGGATACACCTTACCAACTATTGGATGCACTGGTGCAGGCTCCT  
 GGACAAGGGCTGGAATGGATGGAGACATCAATCCTTCCAATGGCAGAACCTCCTACAAG  
 GAAAATTCAAACGGGGTCAACACTCTCGTGGAGACAGTCTAGCTCCACAGCTTACATG  
 GAACTCTCCTCCCTCGGGTCCGAAGACACAGCTGTCTACTTCTGCACCATCCACTACGAC  
 GACAAGTACTACCCCTCTGATGGACTACTGGGGCAGGGAACCCCTGGTACCGTGTCCAGC  
 GCTTCCACAAAAGGACCTCCCGTCTTCCCCCTCGCCCCCTGCTCCGGTCCACATCCGAA  
 TCAACAGCTGCCCTCGGCTGCTGTCAGACTTCCCAGGCTGTACAGTGTCC  
 TGGAACTCCGGAGCTCTCACATCCGGAGTCCACACATTTCTGCTGTGCTCCAATTCGGGACACAGACC  
 GGAAGTGTATTCCCTCTCCCGTGGTACAGTGCCTTCTCCAATTTCGGGACACAGACC  
 TATACATGCAACAGTGGACCACAAAACCTCCAACACCAAAAGTCGATAAGACAGTGGAGCGC  
 AAGTGTGCTGGAGTGTCCCCCTGTCCTGCTCTGCTCCCCCTGTGGCTGGACCTTCCGCTTT  
 CTGTTTCTCTAAACCTAAAGACACCCCTCATGATCTCCGGACCCCCGAGGTACATGC  
 GTGGTGTGATGTGAGGACCGAGGACCCGAAGTCCAATTTAATTGGTATGTGGACGGG  
 GTGGAGGTCTATAACCGTAAGGACAAACCTAGGGAGAGCAGTTCAATTCCACTTCCGG  
 GTGGTGTCCGTGTCACCGTGTGACAGTCAACAGGACTGGCTCAACGGGAAAGAACAAATGC  
 AAAGTCTCTAAAGGCCCTCCCTGCTCTATTGAAAAAACAAATTTCCAACAAACAAAGGA  
 CAACCTCGGAGCCTCAAGTCTACACACTGCCACCTCCGGAAACATGCAAAAGGAAAT  
 CAAGTCTCCCTCACATGTCTCGTCAAGGGATTCTACCCCTCCGACATGCTGTGGAATGG  
 GAATCCAATGGACAACCTGAAAACAACACTACAAGACAAACACCTCCTATGCTCAAAAGCGAT  
 GGGTCTTTCTCTATTCCAAACTCACAGTCGATAAGTCACGGTGGCAGCAGGGGAAT  
 GTGTTCTCTGTTCCGTGATGACGAGGCTCTCCACAATCACTATACCCAGAAAAGCCTG  
 TCCCTCTCCCTGGAAATGA

**Light chain nucleotide sequence (SEQ ID NO:34)**

ATGGTGTGAGACCCAGGTGTTCACTCTCCCTGCTGCTGTGGATCTCCGGCGCTACGGC  
 GACATCGTGTGATGACCCAGTCCCCAGACTCCCTGGCTGTGCTCTGGGAGAGCGGGCCACC  
 ATCTCTTGCAAGAGCCTCCAGTCCGGACAACACTACGGCATCTCCTCATGAGTGGTTC  
 CAGCAGAAGCCCGGCCAGCCCCAAAGCTGCTGATCTACGCCGCTCCAACCAGGGATCT  
 GCCGTGCCGACCGGTTCTCTGGATCCGGCTCTGGCACCGACTTACCCCTGACCATCAGC  
 TCCCTGCAGGCCGAGGACGTGGCGGTGACTACTGCCAGCAGTCAAAGAGGTGCCCTGG  
 ACCTTCCGGAGGCACCAAGGTGAAATCAAGCGGACCGTGGCGCTCCCTCCGGTGTTC  
 ATCTTCCCACCCCTCGACGAGCAGCTGAAGTCCGGAACCGCCTCGTCGTGTGCTGCTG  
 AACAACTTCTACCCCCCGAGGGCAAGGTGCAAGTGGAAAGGTGGACAAACGCCCTGCAGTCC  
 GGCAACTCCAGGAATCGTCACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCC  
 TCCACCCCTGACCCCTGTCACGGCCACTACGAGAAGCACAAGGTGTACGCGTGTGCAAGTG  
 ACCCACCAGGGCCTGTCAGCCCCGTGACCAAGTCCTCAACCGGGCGAGTGTGTTAG

**21M18 Heavy chain variable region nucleotide sequence (SEQ ID NO:35)**

CAGGTGCAGCTGGTGCAGTCTGGGCCGAAGTGAAGAAACCTGGCGCCTCCGTGAAGATC  
 TCCTGCAAGGCCTCCGGTACTCCTTCACCGCTTACTACATCCACTGGTCAAGCAGGCC  
 CCTGGCGAGGCCCTGGAATGGATCGGCTACATCTCCTACAACGGGCCACCAACTAC  
 AACCAAGAAATTCAAGGGCCGCGTGAACCTCACCCGACACCTCCACCTCCACCGCCTAC  
 ATGGAACTCGGGTCCCTCGGGAGCGACGACACCGCCGTGACTACTGCCAGAGACTAC  
 GACTACGACGTGGCATGGACTACTGGGCCAGGGCACCCCTGGTACCGTGTCTCT

**21R79 Heavy chain variable region nucleotide sequence (13B) (SEQ ID NO:36)**

CAGGTGCAGCTGGTGCAGTCTGGGCCGAAGTGAAGAAACCTGGCGCCTCCGTGAAGATC  
 TCCTGCAAGGCCTCCGGTACTCCTTCACCGCTTACTACATCCACTGGTGAACACAGGCC  
 CCAGGCCAGGGACTGGAATGGATCGGCTATACGCAACTACAACCGGGCACCAACTAC  
 AACCAAGAAATTCAAGGGCCGCGTGAACCTCACCCGACACCTCCACCTCCACAGCCTAC

ATGGAACTCGGGTCCCTCGGGAGCGACGACACCGCCGTGACTACTGCCAGAGACTAC  
 GACTACGACGTGGCATGGACTACTGGGCCAGGGCACCCCTGGTACAGTGTCTCC

**21R79 Heavy chain variable region nucleotide sequence (13B Version 2 ) (SEQ ID NO:37)**

CAGGTTCAAGCTAGTTCAGTCTGGAGCGGAAGTTAAGAAACCTGGAGCATTGGTAAAATA  
AGTTGCAAGGCATCCGGTTACTCGTTACCGCATACTATATCCACTGGGTTAACAGGCA  
CCAGGACAGGGACTTGAATGGATCGGATATATCGCTAATTATAATAGAGCTACAAACTAT  
AACCAAAAATTCAAAAGGACCGGTGACTTTCAAACTGACACCTCAACCTGACAGCATAAC  
ATGGAATTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTGCGCTAGAGATTAT  
GATTATGATGTTGGAATGGACTATTGGGCCAGGGAACACTGGTACAGTGTCTTCT

**219R45 Heavy chain variable region nucleotide sequence (13A version 1) (SEQ ID NO:38)**

CAAGTGCAGCTGGTCCAGAGCGGGCTGAGGTGAAGAAACCCGGAGCAAGCGTAAAAGTA  
TCGTGTAAGGCCTCGGGTACACGTTACAAACTACTGGATGCAATTGGGTGCGGCAGGCT  
CCGGGACAGGGTTGGAATGGATGGGTGACATAACCCCTCAAATGGCAGAACATCATAT  
AAGGAAAAGTCAAACGCCCGTACACTCTCCGTGGACAAGTCAGCTCGACTGCCGTAC  
ATGGAACTTCGTCGCTGAGGTGCGAGGACACGGCAGTGTACTTTGACCATCATTAT  
GATGACAAGTATTACCCCTGTATGGATTATTGGGTCAGGGTACGGTACCGTCTCC  
AGC

**219R45 Heavy chain variable region nucleotide sequence (13A Version 2) (SEQ ID NO:39)**

CAAGTGCAGCTGGTCCAGAGCGGGCTGAGGTGAAGAAACCCGGAGCTTCCGTCAAAGTC  
TCCTGTAAGGCTTCCGGATAACACCTTACCAACTATTGGATGCACTGGGTGCGGCAGGCT  
CCTGGACAAGGGCTGGAATGGATGGAGACATCAATCCTCCAATGGCAGAACCTCCTAC  
AAGGAAAAGTCAAACGCCGGGTACACTCTCCGTGGACAAGTCAGCTCCACAGCCTAC  
ATGGAACTCCCTCCCTGCGGTCCGAAGACACAGCTGTCTACTTCTGACCATCCACTAC  
GACGACAAGTACTACCCCTGTATGGACTACTGGGCCAGGGAACCTGGTACCGTGTCC  
AGC

**Light chain variable region nucleotide sequence (SEQ ID NO:40)**

GACATCGTGTATGACCCAGTCCCCAGACTCCCTGGCTGTGTCCTGGGAGAGCGGGCCACC  
ATCTCTTGAGAGCCTCCGAGTCGGACAACACTACGGCATCTCCTCATGAAGTGGITC  
CAGCAGAAGCCCGGCCAGCCCCAAAGCTGCTGATCTACGCCGCTCCAACCAGGGATCT  
GGCGTGCAGCCGACCGGTTCTGGATCCGGCTCTGGCACCGACTTACCTGACCATCAGC  
TCCCTGCAGGCCGAGGAACGTGGCCGTACTACTGCCAGTCAGTCAAAGAGGTGCCCTGG  
ACCTTCGGCGGAGGCACCAAGGTGAAATCAAG

**Human IgG1 Heavy chain constant region (SEQ ID NO:41)**

ASTKGPSVFPLA~~SSK~~STSGGTAALGCLVKDYFPEPVT~~V~~WNSGALTSGVHTFP~~A~~VLQSS  
GLYSI~~LSS~~VVTVP~~SS~~SLGTQTYICNVN~~H~~KPSNTKVDK~~V~~E~~P~~KSCDK~~T~~H~~T~~C~~PP~~C~~A~~PE~~L~~GG  
PSVFLFPPKPKDTLMISRTPEVTCVV~~D~~V~~S~~HEDPEV~~K~~FN~~W~~Y~~D~~G~~V~~E~~V~~HNAK~~T~~KP~~R~~E~~Q~~YN  
STY~~R~~V~~V~~SL~~T~~VL~~H~~QDWL~~N~~GKEY~~K~~C~~V~~SN~~K~~ALP~~A~~PIE~~K~~T~~I~~SK~~A~~K~~G~~Q~~P~~RE~~P~~Q~~V~~Y~~T~~L~~F~~PSR~~D~~E  
LTKNQ~~V~~SL~~T~~CL~~V~~K~~G~~F~~Y~~PSD~~I~~AV~~E~~W~~E~~S~~N~~G~~Q~~P~~E~~NNY~~K~~TP~~V~~L~~D~~G~~S~~F~~F~~LY~~S~~K~~L~~T~~V~~D~~K~~SR~~W~~  
QQGNF~~S~~CSVM~~H~~EA~~L~~H~~N~~Y~~T~~Q~~K~~SL~~S~~PGK

**Human IgG2 Heavy chain constant region (SEQ ID NO:42)**

ASTKGPSVFPLA~~PC~~RS~~T~~SE~~TA~~ALGCLVKDYFPEPVT~~V~~WNSGALTSGVHTFP~~A~~VLQSS  
GLYSI~~LSS~~VVTVP~~SS~~NGTQTYTCNV~~D~~H~~K~~PSNTKVDK~~T~~VERKCC~~V~~E~~C~~PPC~~A~~PPVAGPSV~~F~~  
LFPPKPKDTLMISRTPEVTCVV~~D~~V~~S~~HEDPEV~~Q~~FN~~W~~Y~~D~~G~~V~~E~~V~~HNAK~~T~~KP~~R~~E~~Q~~FN~~S~~T~~F~~R  
V~~V~~SL~~T~~VL~~V~~HQDWL~~N~~GKEY~~K~~C~~V~~SN~~K~~GLP~~A~~PIE~~K~~T~~I~~SK~~T~~K~~G~~Q~~P~~RE~~P~~Q~~V~~Y~~T~~L~~F~~PSREEMTKN  
Q~~V~~SL~~T~~CL~~V~~K~~G~~F~~Y~~PSD~~I~~AV~~E~~W~~E~~S~~N~~G~~Q~~P~~E~~NNY~~K~~TP~~V~~M~~L~~D~~G~~S~~F~~FLY~~S~~K~~L~~T~~V~~D~~K~~SR~~W~~Q~~G~~N  
VF~~S~~CSVM~~H~~EA~~L~~H~~N~~Y~~T~~Q~~K~~SL~~S~~PGK

**Human IgG3 Heavy chain constant region (SEQ ID NO:43)**

ASTKGPSVFPLA~~PC~~RS~~T~~SE~~TA~~ALGCLVKDYFPEPVT~~V~~WNSGALTSGVHTFP~~A~~VLQSS  
GLYSI~~LSS~~VVTVP~~SS~~SLGTQTYTCNV~~D~~H~~K~~PSNTKVDK~~R~~VELK~~T~~PLGDT~~H~~TC~~P~~RC~~P~~E~~K~~  
D~~T~~PP~~P~~RC~~P~~E~~K~~SCD~~T~~PP~~P~~RC~~P~~E~~K~~SCD~~T~~PP~~P~~RC~~P~~A~~P~~E~~L~~GGPSV~~F~~L~~F~~PPKPKDT  
LMISRTPEVTCVV~~D~~V~~S~~HEDPEV~~Q~~K~~W~~Y~~D~~G~~V~~E~~V~~HNAK~~T~~KP~~R~~E~~Q~~YN~~S~~T~~F~~R~~V~~V~~S~~L~~T~~VL~~H~~  
Q~~D~~W~~L~~~~N~~GKEY~~K~~C~~V~~SN~~K~~ALP~~A~~PIE~~K~~T~~I~~SK~~T~~K~~G~~Q~~P~~RE~~P~~Q~~V~~Y~~T~~L~~F~~PSREEMTKNQ~~V~~SL~~T~~CL~~V~~  
G~~F~~Y~~P~~PSD~~I~~AV~~E~~W~~E~~S~~N~~G~~Q~~P~~E~~NNY~~K~~TP~~V~~M~~L~~D~~G~~S~~F~~FLY~~S~~K~~L~~T~~V~~D~~K~~SR~~W~~Q~~G~~N~~I~~F~~S~~CSVM~~H~~  
ALHN~~R~~F~~T~~Q~~K~~SL~~S~~PGK

**Human IgG4 Heavy chain constant region (SEQ ID NO:44)**

ASTKGPSVFPLA~~PC~~RS~~T~~SE~~TA~~ALGCLVKDYFPEPVT~~V~~WNSGALTSGVHTFP~~A~~VLQSS  
GLYSI~~LSS~~VVTVP~~SS~~SLGTQTYTCNV~~D~~H~~K~~PSNTKVDK~~R~~VES~~K~~Y~~G~~PPC~~P~~C~~P~~A~~P~~E~~F~~IGG~~P~~SV~~F~~  
FL~~F~~PPKPKDTLMISRTPEVTCVV~~D~~V~~S~~OEDPEV~~O~~FN~~W~~Y~~D~~G~~V~~E~~V~~HNAK~~T~~KP~~R~~E~~Q~~FN~~S~~TY

RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK  
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDGSFFLYSRLTVDKSRWQEG  
NVSFCSCVMHEALHNHYTQKSLSLSLGK

**FLAG peptide (SEQ ID NO:45)**

DYKDDDDK

**Parental 21R79 Heavy chain with signal sequence underlined unmodified chain (SEQ ID NO:46)**

MKHLWFFLLVAAPRWVLSQVQLVQSGAEVKKPGASVKISCKASGYSTAYYIHWVKQAP  
GQGLEWIGYIANYN RATNYNQKFGRVTFTTDSTSTAYMELRSLSRSDDTAVYYCARDYD  
YDVGMDYWGQGT LTVSSASTKGPSVFLAPCSRSTSESTAALGCLVKDYFPEPVTVSN  
SGALTSGVHTEPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKC  
CVC C P C P A P P V A G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V Q F N W Y V D G V E  
V H N A K T K P R E E Q F N S T F R V V S V L T V V H Q D W L N G K E Y K C K V S N K G L P A P I E K T I S K T K G P  
R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P M I L D S G S  
F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G K

**Parental 219R45 Heavy chain with signal sequence underlined (SEQ ID NO:47)**

MKHLWFFLLVAAPRWVLSQVQLVQSGAEVKKPGASVKISCKASGYTFTNYWMHWVRQAP  
GQGLEWMGDINPSNGRTSYKEKFRRVTLSVDKSSSTAYMELSSLRSEDTAVYFCTIHYD  
DKYYPLMDYWGQGT LTVSSASTKGPSVFLAPCSRSTSESTAALGCLVKDYFPEPVTVS  
WNSGALTSGVHTEPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVER  
KCCVEC C P C P A P P V A G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V Q F N W Y V D G  
V E V H N A K T K P R E E Q F N S T F R V V S V L T V V H Q D W L N G K E Y K C K V S N K G L P A P I E K T I S K T K G  
Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P M I L D S D  
G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G K

**Parental 21R79 Heavy chain without predicted signal sequence (SEQ ID NO:48)**

QVQLVQSGAEVKKPGASVKISCKASGYSTAYYIHWVKQAPGQGLEWIGYIANYN RATNY  
NQKFGRVTFTTDSTSTAYMELRSLSRSDDTAVYYCARDYD YDVGMDYWGQGT LTVSSA  
STKGPSVFLAPCSRSTSESTAALGCLVKDYFPEPVTVSNNSGALTSGVHTEPAVLQSSG  
LYS LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVEC C P C P A P P V A G P S V F L  
F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V Q F N W Y V D G V E V H N A K T K P R E E Q F N S T F R V  
V S V L T V V H Q D W L N G K E Y K C K V S N K G L P A P I E K T I S K T K G Q P R E P Q V Y T L P P S R E E M T K N Q  
V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P M I L D S G S F F L Y S K L T V D K S R W Q Q G N V  
F S C S V M H E A L H N H Y T Q K S L S L S P G K

**Parental 219R45 Heavy chain without signal sequence (SEQ ID NO:49)**

QVQLVQSGAEVKKPGASVKISCKASGYTFTNYWMHWVRQAPGQGLEWMGDINPSNGRTSY  
KEKFRRVTLSVDKSSSTAYMELSSLRSEDTAVYFCTIHYDDKYYPLMDYWGQGT LTV S  
S A S T K G P S V F L A P C S R T S E S T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S  
  
S G L Y S L S S V V T V P S S N F G T Q T Y T C N V D H K P S N T K V D K T V E R K C C V E C C P C P A P P V A G P S V  
F L F P P K P K D T L M I S R T P E V T C V V D V S H E D P E V Q F N W Y V D G V E V H N A K T K P R E E Q F N S T F  
R V V S V L T V V H Q D W L N G K E Y K C K V S N K G L P A P I E K T I S K T K G Q P R E P Q V Y T L P P S R E E M T K N Q  
N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P M I L D S G S F F L Y S K L T V D K S R W Q Q G  
N V F S C S V M H E A L H N H Y T Q K S L S L S P G K

**Parental 21R79 Heavy chain variable region nucleotide sequence (SEQ ID NO:50)**

CAAGTGCAGCTCGTCAGTCAGGGCGGAGGTCAAGAACGCCGGGAGCATCGGTCAAAATC  
TCGTGTAAGGCCTCGGGTACTCCTTACTCGCTATTACATCCATTGGTAAAGCAGGCG  
CCAGGGCAGGGATTGGAGTGGATTGGTATATGCCAATTACAATCGCGCACGAACTAT  
AACCAAGAAATTCAAGGGAGGGTGACCTTCACAACGGATACATCGACATCGACGGCTAC  
ATGGAACTTCGCAGCCTGGCATCAGATGACACGGCGTATACTATTGGCAAGAGATTAC  
GACTATGATGTGGAAATGGACTATTGGGTCAAGGTACTCTGGTACAGTCTCC

**Parental 219R45 Heavy chain variable region nucleotide sequence (SEQ ID NO:51)**

CAGGTACAGCTCGTCATCGGGGGCAGAGGTCAAAAGCCGGTGCCTCGGTAAAGGTC  
AGCTGCAAAGCGTCAGGTTATACATTACGAAATTACTGGATGCATTGGTCAGACAGGCC  
CCTGGACAAGGGCTTGAATGGATGGAGATATCGCAATCCGTCAACGGACGGACTAGCTAT  
AAGGAGAAGTTAAGAGGGCGCTAACACTGTGGTGGACAAATCGTCTCAACGGCCTAC  
ATGGAGTTGCATCCCTGGCGGTCAAAGATAACGGCGGTCTACTTCTGACTATCCACTAT  
GAGCTATGATGTGGAAATGGACTATTGGGTCAAGGTACTCTGGTACAGTCTCC

AGC  
AGC

**Parental 21R79 Heavy chain nucleotide sequence with signal sequence (SEQ ID NO:52)**

ATGAAACACTTGTGGTTTTCTCTTGCTCGTGGCAGCTCCTCGGTGGGTACTTCACAA  
GTGCAGCTCGTGCAGTCAGGGCGGAGGTCAAGAAGCCGGGAGCATCGTCAAAATCTCG  
TGTAAAGGCTCGGGTACTCCTTACTCGTATTACATCCATTGGTAAAGCAGGCCA  
GGCAGGGATTGGAGTGGATGGTATATGCCAATTACAATCGCGCACGAACTATAAC  
CAGAAATTCAAGGAAAGGGTGACCTCACAAACGGATACATCGACATCGACGGCCTACATG  
GAACCTCGCACGCTCGCATCAGATGACAGCAGGGTATACTATTGCCAAGAGATTACGAC  
TATGATGTGGAATGGACTATTGGGTCAAGGTACTCTGGTCACAGTCTCCTCCGCCAGC  
ACCAAGGGCCCTAGCGTCTCCCTCGGCTCCCTGCAGCAGGAGCACAGCGAGAGCACA  
GCCGCCCTGGGCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTGGAAAC  
TCAGGCCTCTGACCAGCGCGTGCACCTTCCCAGCTGTCTACAGTCTCAGGACTC  
TACTCCCTCAGCAGCGTGGTGACCGTCCAGCAACTTCCGACCCAGACCTACACC  
TCAACAGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTGTAGCGCAAATGT  
TGTGTCAGTGCCCACCGTGGCACCACCTGTGGCAGGACCGTCAGTCTCCTCTTC  
CCCCCAAAACCAAGGACACCCCTCATGATCTCCGGACCCCTGAGGTACGTGCGTGGTG  
GTGGACGTGAGCCACGAAGACCCCAGGTCCAGTTCAACTGGTACGGACGGCGTGGAG  
GTGCAATAATGCAAGACAAGCCACGGGAGGAGCAGTCAACAGCACGTTCCGTGTGGTC  
AGCGCTCTCACCGTTGTGACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAGGTC  
TCCAACAAAGGCTCCAGCCCCATCGAGAAAACCATCTCAAAACCAAAGGGCAGCCC  
CGAGAACCCACAGGTGTACACCCCTGGCCCATCCGGGAGGAGATGACAAGAACAGGTC  
AGCCTGACCTGGCTGGTCAAAGGCTTCTACCCCAAGCAGCACATGCCGTGGAGTGGGAGAGC  
AATGGGCAGCCGGAGAACAACTACAAGACCACCTCCATGCTGGACTCCGACGGCTTTC  
TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGAACGTCTTC  
TCATGCTCCGTGATGCATGAGGCTCTGCACAACCAACTACACGCAGAAGAGCCTCTCCCTG  
TCTCCGGTAAA

**Parental 219R45 Heavy chain nucleotide sequence with signal sequence (SEQ ID NO:53)**

ATGAAACACCTCTGGTTCTTTGCTCCTGGTGGCAGCTCCCGATGGGTGCTTAGCCAG  
GTACAGCTCGTGAATCGGGGGCAGAGGTCAAAAAGCCCGTGCCTGGTAAAGGTGAGC  
TGCAAAAGCGTCAGGTTATACATTACGAATTACTGGATGCAATTGGTCAGACAGGCCCT  
GGACAAGGGCTTGAATGGATGGAGATATCAATCCGTGAAACGGACGGACTAGCTATAAG  
GAGAAGTTAACAGAGGCCGTAAACACTGTCGGTGGACAAATGTCCTCAACGGCCTACATG  
GAGTTGTATCCCTCGCGTCGGAAGATAACGGCGGTCTACTTCTGTACTATCCACTATGAC  
GATAAGTACTACCCGTTATGGACTACTGGGTCAAGGGAACATTGGTAACCGTGAGCAGC  
GGTCCACAAAGGGCCCTAGCGTCTTCCCTCGGCTCCCTGCAGCAGGAGCACAGCGAG  
AGCACAGCCCTGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTGCG  
TGGAACTCAGGCCTCTGACCAGCGCGTGCACACCTTCCCAGCTGTCTACAGTCTCA  
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAACTTCGGCACCCAGACC  
TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGC  
AAATGTGTGTCGAGTCCCCACCGTGGCCAGCACCACCTGTGGCAGGACCGTCAGTCTTC  
CTCTTCCCCCAAACCAAGGACACCCCTCATGATCTCCGGACCCCTGAGGTACGTG  
GTGGTGGGACGTGAGGCCACGAAGACCCAGGTCCAGTTCAACTGGTACGTGGACGGC  
GTGGAGGTGATAATGCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGT  
GTGGTCAAGCGTCTCACCGTTGTGACCCAGGACTGGCTGAACGGCAAGGAGTACAAGTGC  
AAAGGTCTCAAACAAAGGCCTCCCAGCCCCATCGAGAAAACCATCTCAAAACCAAAGGG  
CAGCCCCGAGAACCCACAGGTGTACACCCCTGGCCCATCCGGGAGGAGATGACCAAGAAC  
CAGGTCAAGCCTGACCTGGCTGGTCAAAGGCTTCTACCCCAAGCAGCACATGCCGTGGAGTGG  
GAGAGCAATGGGAGCCGGAGAACAACTACAAGACCACACCTCCATGCTGGACTCCGAC  
GGCTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC  
GTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAACTACACGCAGAAGAGCCTC  
TCCCTGTCTCCGGTAAA

**Parental 21R79 and 219R45 light chain variable region nucleotide sequence (SEQ ID NO:54)**

GACATCGTGTGATGACCCAGTCCCTGACTCCCTGGCTGTGTCCTGGCGAGAGGGCCACC  
ATCTCTGCAAGAGCCAGCGAATCGTCGATAATTATGGCATTTCCTTATGAAGTGGTTC  
CAGCAGAAACCAGGACAGCCTCTAAAGCTGCTATTACGCTGCATCAACCAAGGGTCC  
GGGGTCCCTGACAGGTTCTCGGCAGCGGGTCCGGAACAGATTCTACTCTCACCATCAGC  
AGCCTGCAGGCTGAAGATGTGGCTGTCTATTACTGTCAGCAAAGCAAGGAGGTGCCCTGG  
ACATTGGAGGAGGGACCAAGGTGGAAATCAAA

**Parental 21R79 and 219R45 light chain nucleotide sequence (SEQ ID NO:55)**

ATGGTGCTCCAGACCCAGGTCTCATTTCCTGCTGCTCTGGATCAGCGGAGCCTACGGG

GACATCGTGTGATGACCCAGTCCCCGACTCCCTGGCTGTGTCCCTGGGCAGAGGGCCACC  
 ATCTCTCGAGGCCAGCGAATCCGTCGATAATTATGGCATTCCTTATGAAGTGGTTC  
 CAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTACGCTGCATCCAACCAAGGGTCC  
 GGGGTCCCTGACAGGTTCTCCGGCAGCGGGTCCGGAACAGATTTCACCTCTCACCATCAGC  
 AGCCTGCAGGCTGAAGATGTGGCTGTCTATTACTGTCAAGCAAAGCAAGGGAGGTGCCCTGG  
 ACATTGGAGGAGGGACAAGGTGAAATCAAACGTACGGTGGCTGCCCTCCGTCTTC  
 ATCTCCCCCCCAGCGATGAGCAGCTGAAAGCGGCAGCTGCCAGCGTGGTGTGCCCTGCTG  
 ATAACCTCTATCCCCGGGAGGCCAAAGTGCAGTGGAAAGGTGGATAACGCCCTCCAAAGC  
 GGCAACTCCCAGGAGAGCGTCAACAGAGCAGCAAGGACAGCACCTACAGCCTCAGC  
 AGCACCCCTGACCCCTGAGCAAAGCCACTACGAGAAACACAAAGTCTACGCCCTGCCAGT  
 ACCCATCAGGGCCTGAGCAGCCCCGTACAAAGAGCTAACAGGGCGAGTGTGA

**21R75 Heavy chain without predicted signal sequence (SEQ ID NO:56)**

QVQLVQSGAEVKKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYIAGYKDATNY  
 NQKFKGRVTFTDTSTSTAYMELRSLSRSDDTAVYYCARDYDYDVGMDYWGQGTLLTVSSA  
 STKGPSVFLAPCSRSTSESTAAAGCLVKDYFPEPVTVWSNSGALTSGVHTFPAVLQSSG  
 LYSLSSVVTPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPVAPPVAGPSVFL  
 FPPKPKDTLMISRPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRV  
 VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTIKTKQGPREPQVYTLPPSREEMTKNQ  
 VSILTCLVEGFYPSDIAVEWESENQGPENNYKTTPPMLSDGSFFYSELTVDKSRWQQGNV  
 FSCSVMHEALHNHYTQKSLISLSPGK

**21R75 Heavy chain with predicted signal sequence (underlined) (SEQ ID NO:57)**

MKHLWFFLLVAAPRWVLSQVQLVQSGAEVKKPGASVKISCKASGYSFTAYYIHWVKQAP  
GOOGLEWIGYIAGYKDATNYNQKFKGRVTFTDTSTSTAYMELRSLSRSDDTAVYYCARDYD  
YDVGMDYWGQGTLLTVSSASTKGPSVFLAPCSRSTSESTAAAGCLVKDYFPEPVTVWSN  
SGALTSGVHTFPAVLQSSGLYSLSSVVTPSSNFGTQTYTCNVDHKPSNTKVDKTVERKC  
CVECPVAPPVAGPSVFLFPPKPKDTLMISRPEVTCVVVDVSHEDPEVQFNWYVDGVE  
VHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTIKTKQP  
REPQVYTLPPSREEMTKNQVSLTCLVEGFYPSDIAVEWESENQGPENNYKTTPPMLSDGS  
FFLYSELTVDKSRWQQGNVFSVMHEALHNHYTQKSLISLSPGK

**21R75 Heavy chain variable region (SEQ ID NO:58)**

QVQLVQSGAEVKKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYIAGYKDATNY  
 NQKFKGRVTFTDTSTSTAYMELRSLSRSDDTAVYYCARDYDYDVGMDYWGQGTLLTVSS

**21R75 Heavy chain CDR2 (SEQ ID NO:59)**

YIAGYKDATNYNQKFKG

**21R75 Heavy chain nucleotide sequence with signal sequence (13B Version 1) (SEQ ID NO:60)**

ATGAAGCACCTGTGGTCTTCTGCTGGTGGCGCTCCAGATGGGTGCTGTCCCG  
GTGCAGCTGGTGCAGTCGGCGCCAAAGTGAAGAAACCTGGCGCTCCGTGAAGATCTCC  
TGCAAGGCCCTCCGGCTACTCCTTCACCGCTACTACATCCACTGGTCAAGCAGGCCCT  
GGACAGGGCTCTGGAATGGATCGGCTATATCGCCGGCTACAAGGACGCCACCAACTACAA  
CAGAAATTCAAGGGCAGAGTGACCTTCACCACCGACACCTCCACCTCACCGCCTACATG  
GAACTCGGTCCCTGCGGAGCGACACCCGGCGTGTACTACTGCGCCAGAGACTACGAC  
TACGACGTGGCATGGACTACTGGGCCAGGGCACACTCGTGACCGTGTCTCTGCTTCC  
ACCAAGGGCCCTCCGTGTTCTCTGCCCCCTTGCTCCAGATCCACCTCCGACTCTACC  
GCGCTCTGGCTGCCCTCGTGAAAGACTACTTCCCCGAGCCCGTGACAGTGTCTTGGAA  
TCTGGCGCCCTGACCTCCGGCGTGACACCTTCCAGCTGTGCTGCAAGTCTCCGGCCTG  
TACTCCCTGTCTCCGCGTGACTGTGCCCTCTCAACTCGGCACCCAGACCTACACC  
TGTAACGTGACCACAAGCCCTCAACACCAAGGTGGACAAGACCGTGGAACCGGAAGTGC  
TGCGTGAATGCCCTTGTCCTGCCCCCTCTGTGGCTGGCCCTAGCGTGTCTCTGTT  
CCCCAAAGCCCAAGGACACCCCTGATGATCTCCGGACCCCCGAAGTGAACCTGCCGTGG  
GTGGATGTGCCCACGAGGACCCCGAGGTGCAAGTCAATTGGTACGTGGACGGCGTGGAA  
GTGCACAAAGCCAAGACCAAGGCCAGAGAGGAACAGTCAACTCCACCTCCGGGTGGT  
TCCGTGCTGACCGTGGTGATCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAGGTG  
TCCAACAAGGGCCTGCCCTGCCCCCATGAAAAGACCATCTCTAACGACCAAGGGACAGCC  
CGCGAGCCCCAGGTGTACACACTGCCCTCATCCGGGAAGAGATGACCAAGAACCGGTG  
TCCCTGACCTGTCTGGTGGAGGGCTTCTACCCCTCCGATAATGCCGTGGAAATGGGAGTCC  
AACGGCCAGCCGAGAACAACTACAAGACCAACCCCCCCCCATGCTGGACTCCGACGGCTCA  
TTCTTCTGTACAGCGAGCTGACAGTGGACAAGTCCCGTGGCAGCAGGGCAACGTGTTC  
TCTGTCCGTGATGCACGAGGCCCTGCACAAACCACTACACCCAGAACGTCCCTGTCCCTG  
AGCCCCGGCAAG

## 21R75 Heavy chain nucleotide sequence with signal sequence (13B Version 1T) (SEQ ID NO:77)

ATGAAGCACTGTGGTCTTCTGCTGGTGGCCGCTCCAGATGGGTGCTGTCTCAG  
GTGCAGCTGGTGCAGTCGGCGCGAAGTGAAGAAACCTGGCGCTCCGTGAAGATCTCC  
 TGCAAGGCTCCGGCTACTCCTCACCGCCTACTACATCCACTGGGTCAGCAGGCCCT  
 GGACAGGGCTTGGAAATGGATCGGCTATATCGCCGGCTACAAGGACGCCACCAACTACAAAC  
 CAGAAAATTCAAGGGCAGAGTGACCTTCACCACCGACACCTCCACCTCACCGCCTACATG  
 GAACTGCGGTCCCTGCGGAGCGACCGACACCGCCGTGTACTACTGCGCCAGAGACTACGAC  
 TACGACGTGGGATGGACTACTGGGGCAGGGCACACTCGTACCGTGTGCTCTGCTTCC  
 ACCAAGGGCCCTCGTGTTCCTCTGGCCCTTGCTCCAGATCCACCTCGAGTCTACC  
 GCCGCTCTGGCTGCCTCGTGAAGGACTACTTCCCCGAGCCCGTGACAGTGTCTTGAAC  
 TCTGGCGCCCTGACCTCCGGCGTGCACACCTTCCAGCTGTGCTGAGTCCCTCCGGCCTG  
 TACTCCCTGTCCCTCGTGTACTGTGCCCTCTCCAACCTCGCACCCAGACCTACACC  
  
 TCTAACGTGGACCACAAGCCCTCCAACACCAAGGTGGACAAGACCGTGGAACGGAAGTGC  
 TCGGTGAATGCCCTTGTCTGCCCCTCTGTGGCTGGCCCTAGCGTGTCTGTT  
 CCCCCAAAGCCCAAGGACACCCGTATGATGATCTCCGGACCCCCGAAGTGAACCTGCCGTGGT  
 GTGGATGTGCCCACGAGGACCCCGAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAA  
 GTGCACAACGCCAAGACCAAGCCCAGAGAGAACAGTCAACTCACCTCCGGGTGGT  
 TCCGTGCTGACCGTGGTGCATCAGGACTGGTGAACGGCAAAGAGTACAAGTCAAGGTG  
 TCCAACAAGGGCTGCCCTGCCCTCATGAAAAGACCATCTCTAACGACCAAGGGACAGCCC  
 CGCGAGCCCCAGGTGTACACACTGCCCTCATCCGGAAAGAGATGACCAAGAACCAAGGTG  
 TCCCTGACCTGTCTGGTGAAGGCTTCTACCCCTCCGATATGCCGTGGAAATGGGAGTCC  
 AACGGCCAGCCGAGAACAACTACAAGACCAACCCCCCCCAGCTGGACTCCGACGGCTCA  
 TTCTCCTGTACAGCGAGCTGACAGTGGACAAGTCCCGTGGCAGCAGGGCACCGTGTTC  
 TCTGCTCCGTATGCAACGAGGCCCTGCACAACCAACTACACCCAGAACGTCCCTGCTCG  
 AGCCCCGGCAAG

## 21R75 Heavy chain nucleotide sequence with signal sequence (13B Version S1-2) (SEQ ID NO:61)

ATGAAGCACCTGTGGTCTTCTGCTGGTGGCCGCTCCAGATGGGTGCTGTCCAG  
GTCAGCTAGTCAGTCGGAGCGGAAGTTAAAGAAACCTGGAGCATCCGTGAAAATAAGT  
 TGCAAGGCACTCCGGTTACTCGTTCACCGCATACTATATCCACTGGTAAACAGGCCACCA  
 GGACAGGGACTTGAATGGATCGGATATATCGCTGGATATAAGATGCTACAAACATATAAC  
 CAAAAATTCAAAGGACCGGTGACTTCACAACCTGACACCTCAACCTCGACAGCATACTG  
 GAATTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTGCGCTAGAGATTATGAT  
 TATGATGTGGAATGGACTATTGGGCCAGGGAACACTGGTACAGTGTCTCTGCATCC  
 ACTAAGGGACCATCCGTGTTCCCTTGCCCTTGCTCTCGTGCACCTCTGAATCGACT  
 GCCGCTCTGGATGCCCTCGTGAAGGATTACTTCCCTGAGCCTGTGACCGTTCTGGAAAC  
 TCGGGCGCCCTAACCTCTGGCGTGCACACATTCCCTGCCGTCTACAGTCTCTGGCCTA  
 TACTCTTATCTCGGTGTACCGTACCTCTCTAACCTCGAACCCAAACTACACC  
 TGTAAACGTAGACCACAAGCCTCGAACACCAAGGTGGACAAGACTGTTGAGCGAAAGTGC  
 TCGGTTGAGTGCCTCCATGTCCTGCACCTCTGTGGCTGGCCCTTCTGTGTTCTGT  
 CCTCCAAAACCTAAGGACACTCTAACATGATCTCTCGGACTCTGAGGTGACTTGCCTGGT  
 GTGGACGTGCCCACGAGGACCCCTGAGGTGCAGTTCAATTGGTACGTGGACGGAGTCGAG  
 GTGCACAATGCAAAGACCAAGCCTCGGGAGGAACAGTCAACTCACCTCCGGGTGGTT  
 TCTGTGTTGACCGTTGTCACCAAGACTGGTGAACGGCAAAGAAATACAAGTCAAGGTG  
 TCCAACAAGGGCTGCCCTGCCCCATCGAAAAGACCATCAGCAAGACCAAGGGCCAGCCT  
 CGCGAGCCTCAGGTGTACACCCCTGCCCTCCAGCCGGAAAGAAATGACCAAGAACCAAGGTG  
 TCCCTGACCTGTCTGGTGGAGGGCTTCTACCCCTCCGACATGCCGTGAGTGGAGTCT  
 AACGGACAGCCGAGAACAACTACAAGACTACGCCCTCAATGCTGGACTCCGACGGCTCC  
 TTCTCCTGTACTCCGAACTGACCGTGGACAAGTCCCGTGGCAGCAGGGCACCGTGTTC  
 TCATGCTCCGTAATGCAACGAAAGCCTTGACAACTACACTCAAAGTCCCTATCCTTA  
 TCTCCTGGCAAG

## 21R83 Heavy chain without predicted signal sequence (SEQ ID NO:62)

QVQLVQSGAEVKPGASVKISCKASGYSFTAYIHWVKQAPGQLEWIGYISNYN RATNY  
 NQKFKGRVTFTTDSTSTAYMELRSLRSDDTAVYYCARDYDYDVGMDYWGQGTLVTVSSA  
 STKGPSVFPLAPCSRSTSESTAAAGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG  
 LYSLSSVTVPSNSFGTQTYTCNVDHKPSNTKVDKTVERKCCVEPPCPAPPVAGPSVFL  
 FPPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTPREEQFNSTFRV  
 VSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTIKSKQPREPQVYTLPPSREEMTKNQ  
 VSILTCLVEGFYPSDIAVEWESNGQPENNYKTPPMILSDGSFFLYSELTVDKSRWQQGNV  
 FSCSVMHEALHNHYTQKSLSLSPGK

## 21R83 Heavy chain with predicted signal sequence (underlined) (SEQ ID NO:63)

MKHLWFFLLLVAAPRWVLSQVQLVQSGAEVKKPGASVKISCKASGYSFTAYYIHWVKQAP  
GQGLEWIGYISNYNRATNYNQKFKGRVTFTDTSTSTAYMELRSLSRSDDTAVYYCARDYD  
YDVGMDYWGQGTLVTVSSASTKGPSVPLAPCSRSTSESTAALGCLVKDYFPEPVTVSN

SGALTSGVHTFPAVLQSSGLYSLSSVVTPSSNFGTQTYTCNVDHKPSNTKVDKTVERKC  
CVECPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVE  
VHNALKPREEQFNSTFRVVSILTVVHQDWLNGKEYKCKVSNKGLPAPIEKTIKTKQD  
REFQVYTLPPSREEMTKNQVSLTC1VEGFYPSDIAVEWESNGQPENNYKTTPPMLSDGS  
FFLYSELTVDKSRWQQGNVFSCSVMHEALHNHTQKSLSLSPKG

## 21R83 Heavy chain variable region (SEQ ID NO:64)

QVQLVQSGAEVKKPGASVKISCKASGYSFTAYYIHWVKQAPGQGLEWIGYISNYNRATNY  
NQKFKGRVTFTDTSTSTAYMELRSLSRSDDTAVYYCARDYDYGMDYWGQGTLVTVSS

## 21R83 Heavy chain CDR2 (SEQ ID NO:65)

YISNYNRATNYNQKFKG

## 21R83 Heavy chain nucleotide sequence with signal sequence underlined (13B Version 1) (SEQ ID NO:66)

ATGAAGCACCTGTGGTCTTCTGCTGCTGGTGGCCGCTCCAGATGGGTGCTGTCCCAG  
GTGCAGCTGGTGCAGTCGGCGCGAAGTGAAGAAACCTGGCGCCTCCGTGAAGATCTCC  
TGCAAGGCTCCGGCTACTCCTTACCGCCTACTACATCCACTGGGTCAGCAGGCCCC  
GGACAGGGCTTGAATGGATCGGCTACATCTCCAACGACACCCGACACCTCTACCGCCTACATG  
CAGAAATTCAAGGGCCCGCGTGACCTTACCGACACCTCTACCGCCTACATG  
GAACTCGGTCCCTGCGAGCGACACCGCCGTGTACTACTGCGCAGAGACTACGAC  
TACGACGTGGGATGGACTACTGGGCCAGGGCACACTCGTGACCGTGTCTAGCGCTTCC  
ACCAAGGGCCCTCCGTGTTCTGGCCCTTGCTCCAGATCCACCTCCGAGTCTACC  
GCCGCTCTGGCTGCCTCGTGACAGACTACTTCCCAGGGCCGTGACAGTGTCTGGAAAC  
TCTGGCGCTGACCTCCGGCGTGACACCTTCCAGCTGTGCTGCAGTCCTCCGGCCTG  
TACTCCCTGTCCCTCGTGTACTGTGCCCTCTCCAACCTCGCACCCAGACACCTACACC  
TGTAAACGTGGACCACAAGCCCTCAACACCAAGGTGGACAAGACCGTGGAACCGAAAGTGC  
TGCCTGGAATGCCCCCTTGTCTGCCCCCTCTGTTGGCTGGCCCTAGCGTGTCTGGT  
CCCCAAAGCCAAGGACACCCGTGATGATCTCCGGACCCCCGAAGTGAACCTGCGTGGT  
GTGGATGTGCCCACGAGGACCCCGAGGTGCACTTCATGGTACCTGGGACGGCGTGGAA  
GTGCACAACGCCAAGACCAAGGCCAGAGAGAACAGTCAACTCCACCTTCCGGGTGGT  
TCCGTGCTGACCGTGGTGCATCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAGGTG  
TCCAACAAGGGCTGCCTGCCCCATCGAAAGACCATCTCTAAGACCAAGGGACAGCCC  
CGCGAGCCCCAGGTGTACACACTGCCCATCCGGAAAGAGATGACAAGAACCAAGGAGT  
TCCCTGACCTGTCTGGTGGAGGCTTCTACCCCTCCGATACTGCCGTGGAAATGGGAGTCC  
AACGGCCAGCCGAGAACAACTACAAGACCAACCCCCCCCAGCTGGACTCCGACGGCTCA  
TTCTCCGTACAGCGAGCTGACAGTGGACAAGTCCCGTGGCAGCAGGGCAACGGTGTTC  
TCTGGCTCCGTGATGCACGAGGCCCTGCACAACCAACTACACCCAGAAGTCCCTGCTCC  
AGCCCCGGCAAG

## 21R83 Heavy chain nucleotide sequence with signal sequence underlined (13B Version 1T) (SEQ ID NO:78)

ATGAAGCACCTGTGGTCTTCTGCTGCTGGTGGCCGCTCCAGATGGGTGCTGTCTCAG  
GTGCAGCTGGTGCAGTCGGCGCGAAGTGAAGAAACCTGGCGCCTCCGTGAAGATCTCC  
TGCAAGGCTCCGGCTACTCCTTACCGCCTACTACATCCACTGGGTCAGCAGGCCCC  
GGACAGGGCTTGAATGGATCGGCTACATCTCCAACGACACCCGACACCTCTACCGCCTACATG  
CAGAAATTCAAGGGCCCGCGTGACCTTACCGACACCTCTACCGCCTACATG  
GAACTCGGTCCCTGCGAGCGACACCGCCGTGTACTACTGCGCAGAGACTACGAC  
TACGACGTGGGATGGACTACTGGGCCAGGGCACACTCGTGACCGTGTCTAGCGCTTCC  
ACCAAGGGCCCTCCGTGTTCTGGCCCTTGCTCCAGATCCACCTCCGAGTCTACC  
GCCGCTCTGGCTGCCTCGTGACAGACTACTTCCCAGGGCCGTGACAGTGTCTGGAAAC  
TCTGGCGCTGACCTCCGGCGTGACACCTTCCAGCTGTGCTGCAGTCCTCCGGCCTG  
TACTCCCTGTCCCTCCGTGTACTGTGCCCTCTCCAACCTCGCACCCAGACACCTACACC  
TGTAAACGTGGACCACAAGCCCTCAACACCAAGGTGGACAAGACCGTGGAACCGAAAGTGC

TGCCTGGAATGCCCCCTTGTCTGCCCCTCTGTGGCTGGCCCTAGCGTGTCTGGT  
CCCCAAAGCCAAGGACACCCGTGATGATCTCCGGACCCCCGAAGTGAACCTGCGTGGT  
GTGGATGTGCCCACGAGGACCCCCGAAGTGAACCTGCGTGGAAAGGCGTGGAA

GTGCACAAAGCCAAGACCAAGGCCAGAGAGGAACAGTTCAACTCCACCTTCCGGGTGGTG  
 TCCGTGCTGACCGTGGTGCATCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTG  
 TCCAACAAGGGCCTGCCATCGAAAAGACCATCTCTAAGACCAAGGGACAGCCC  
 CGCGAGCCCCAGGTGTACACACTGCCATCCGGGAAGAGATGACCAAGAACCCAGGTG  
 TCCCTGACCTGTCTGGTGGAGGCTTCTACCCCTCCGATATCGCGTGGAAATGGGAGTCC  
 AACGGGCCAGCCGAGAACAACTACAAGACCAACCCCCCCCAGTGTGGACTCCGACGGCTCA  
 TTCTTCTGTACAGCGAGCTGACAGTGGACAAGTCCCGTGGCAGCAGGGCAACGTGTT  
 TCCTGCTCCGTGATGCACGAGGCCCTGCACAACCAACTACACCCAGAAGTCCCTGTCCCTG  
 AGCCCCGGCAAG

**21R75 Heavy chain nucleotide sequence with signal sequence underlined (13B Version S1-2)  
 (SEQ ID NO:67)**

AIGAAGCACCTGTGGTTCTTCTGCTGCTGGTGGCCGCTCCAGATGGGTGCTGTCCCAG  
 GTTCAGCTAGTTCACTGCTGGAGCGGAAGTTAAGAAACCTGGAGCATCGTGAAGGATAAGT  
 TCGAAGGCATCCGGTTACTCGTTACCGCATACTATATCCACTGGGTAAACAGGCACCA  
 GGACAGGGACTTGAATGGATCGGATATATCGCTGGATATAAGATGCTACAAACTATAAC  
 CAAAATTCAAAGGACGCGTGACTTTACAACGACACCTCAACCTCGACAGCATACTG  
 GAATTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTGCGCTAGAGATTATGAT  
 TATGATGTTGAATGGACTATTGGGCCCAGGGAAACACTGGTGTGACAGTGTCTCTGCATCC  
 ACTAAGGGACCATCCGTGTTCCCTTGGCCCTTGCTCTCGTGCACCTCTGAATCGACT  
 GCGCTCTGGATGCCCTGTAAGGATAACTTCCCTGAGCCTGTGACCGTTCTGGAAC  
 TCGGGCGCCCTAACCTCTGGCGTGCACACATTCCCTGCCGTCTACAGTCTCTGGCCTA  
 TACTCTTATCTCGGTGTTACCGTACCTCTCTAACCTCGAACCCAAACTTACACC  
 TGTAACGTAGACCACAAGCCTCGAACACCAAGGTGGACAAGACTGTTGAGCGAAAGTGC  
 TCGGTGAGGCCCCATGCTCCTGACCTCTGTGGCTGGGCTTCTGTGTTCTGTTC  
 CCTCCAAAACCTAACGGACACTCTAATGATCTCTGGACTCCTGAGGTGACTTGCCTGGTT  
 GTGGACGTGCCCACGAGGACCCCTGAGGTGCAATTGGTACGGTGGACGGAGTCGAG  
 GTGCACAATGCAAAGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTTCCGGGTGGTT  
 TCTGTGTTGACCGTTGTCACCAAGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTG  
 TCCAACAAGGGCCTGCCCTATCGAAAAGACCATCAGCAAGACAAGGGCAGCCT  
 CGCGAGCCTCAGGTGTACACCCCTGCCCTCAGGGAAAGAAATGACCAAGAACCCAGGTG  
 TCCCTGACCTGTCTGGTGGAGGCTTCTACCCCTCCGACATGCCGTGAGTGGAGTCT  
 AACGGACAGCCGGAGAACAACTACAAGACTACGCCCTCAATGCTGGACTCCGACGGCTCC  
 TTCTCCTGTAATGCACGAAGCCTTGACAACTACACTACACTCAAAGTCCCTATCCTTA  
 TCTCCTGGCAAG

**21R75 Heavy chain variable region nucleotide sequence (13B Version 1) (SEQ ID NO:68)**

CAGGTGCAGCTGGTGCAGTCTGGGCCGAAGTGAAAGAAACCTGGCGCTCCGTGAAGATC  
 TCCTGCAAGGCCTCCGGTACTCCTCACCGCTACTACATCCACTGGGTCAAGCAGGCC  
 CCTGGACAGGGCCTGGATGGATCGGCTATATCGCGGCTACAAGGACGCCACCAACTAC  
 AACCAAGAAATTCAAGGGCAGAGTGACCTCACCCACACCTCCACCTTACCGCCTAC  
 ATGGAACTGCCCTGCCCTGGGAGCGACGACACCCTGTACTACTGCCAGAGACTAC  
 GACTACGACGTGGCATGGACTACTGGGCCAGGGCACACTCGTGACCGTGTCCCT

**21R75 Heavy chain variable region nucleotide sequence (13B Version 2) (SEQ ID NO:69)**

CAGGTCAAGCTAGTTCACTGCTGGAGCGGAAGTTAAGAAACCTGGAGCATCGTGAAGGATA  
 AGTTGCAAGGCATCCGGTTACTCGTTACCGCATACTATATCCACTGGGTAAACAGGCA  
 CCAGGACAGGGACTTGAATGGATCGGATATATCGCTGGATATAAGATGCTACAAACTAT  
 AACCAAAATTCAAAGGACGCGTGACTTTACAACGACACCTCAACCTCGACAGCATAAC  
 ATGGAATTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTGCGCTAGAGATTAT

GATTATGATGTTGGAATGGACTATTGGGCCAGGGAACACTGGTGACAGTGTCTTCT

**21R83 Heavy chain variable region nucleotide sequence (13B Version 1) (SEQ ID NO:70)**

CAGGTGCAGCTGGTGCAGTCTGGGCCGAAGTGAAAGAAACCTGGCGCTCCGTGAAGATC  
 TCCTGCAAGGCCTCCGGTACTCCTCACCGCTACTACATCCACTGGGTCAAGCAGGCC  
 CCTGGACAGGGCCTGGATGGATCGGCTACATCTCAACTACAACCGGGCACCAATTAC  
 AACCAAGAAATTCAAGGGCCCGTGAACCTCACCCACACCTTACCTCTACCCCTAC  
 ATGGAACTGCCCTGCCCTGGGAGCGACGACACCCTGTACTACTGCCAGAGACTAC  
 GACTACGACGTGGCATGGACTACTGGGCCAGGGCACACTCGTGACCGTGTCTAGC

**21R75 Heavy chain variable region nucleotide sequence (13B Version 2) (SEQ ID NO:71)**

CAGGTCAAGCTAGTTCACTGCTGGAGCGGAAGTTAAGAAACCTGGAGCATCGTGAAGGATA

AGTTGCAAGGCATCCGGTTACTCGTTACCCGATACTATATCCACTGGGTTAACAGGCA  
CCAGGACAGGGACTTGAATGGATCGGATATATCGCTGGATATAAAGATGCTACAAACTAT  
AACCAAAAATTCAAGGACGCGTGACTTTACAACTGACACCTCACACCTGACAGCATACT  
ATGGAATTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTCGCGCTAGAGATTAT  
GATTATGATGTTGAATGGACTATTGGGGCCAGGGAACACTGGTGACAGTGTCTTCT

21R83 Heavy chain nucleotide sequence with signal sequence underlined (13B Version 2) (SEQ ID NO:72)

ATGAAAGCACCTATGGTCTTCTATTATTAGTGGCCGCTCCCGTGGGTGTTATCGCAG  
GTTCAGCTAGTTCAGTCTGGAGCGGAAGTAAAGAAACCTGGAGCATCCGTGAAAATAAGT  
TGCAAGGGCATCCGGTTACTCGTCACCGCATACTATATCCACTGGGTTAACAGGCCACCA  
GGACAGGGACTTGAATGGATCGGATATATCTCCAATTATAATAGAGCTACAAACTATAAC  
CAAAAATTCAAAGGACCGCGTGAATTTCACAACCTGACACCTCAACCTCGACAGCATACTG  
GAATTACGGTCCCTACGGTCTGACGACACTGGCGTTACTATTGCGCTAGAGATTATGAT  
TATGATGTTGAATGGACTATTGGGCCAGGGAACACTGGTACAGTGTCTCTGCATCC  
ACTAAGGGACCATCCGTGTTCCCTTGGCCCTTGCCTCGTGCACCTCTGAATCGACT  
GCCGCTCTGGGATGCCTCGTGAAGAATTACTTCCCTGAGCCTGTGACCGTTCTGGAAC  
TCGGGCGCCCTAACCTCTGGCGTGCACACATTCCCTGCCGTCTACAGTCTCTGGCCTA  
TACTCTTATCTTCGGTTACCGTACCTCTCTAACTTCGGAACCCAAACTTACACC  
TGTAACGTAGACCACAAGCCTCGAACACCAAGGTGGACAAGACTGTTGAGCGAAAGTGC  
TGCCTTGAGTGCCTCCATGTCCTGCACCTCTGGCTGGCCCTCTGTGTTCTGTT  
CCTCCAAAACCTAACGGACACTCTAATGATCTCGGACTCTGAGGTGACTTGCCTGGTT  
GTGGACGTGCTCCACGAGGACCTGAGGTGCGATTCAATTGGTACGTGGACGGAGTCGAG  
GTGCACAATGCAAAGACCAAGCCTCGGGAGGAACAGTCAACTCCACCTCCGGTGGTT  
TCTGTGTGACCGTTGTGACCAAGACTGGCTGAACGGCAAAGAATACAAGTCAAGGTG  
TCCAACAAGGGCCTGCCCTGCCCTATCGAAAAGACCATCAGCAAGACCAAGGGCCAGCCT  
CGCGAGCCTCAGGTGTACACCCCTGCCCTCCAGCCGGGAAGAAATGACCAAGAACAGGTG  
TCCCTGACCTGTCTGGTGGAGGGCTTCTACCCCTCCGACATGCCGTGAGTGGAGTCT  
AACGGACAGCCGGAGAACAACTACAAGACTACGCCCTCAATGCTGGACTCCGACGGCTC  
TTCTCCGTACTCCGAACTGACCGTGGACAGTCCGGTGGCAGCAGGGCAACGTGTT  
TCATGCTCCGTAATGCACGAAGCCTTGCACAATCACTACACTCAAAAGTCCCTATCCTTA  
TCTCCTGGCAAGTAG

21R83 Heavy chain variable region nucleotide sequence (13B Version 2) (SEQ ID NO:73)

CAGGGTTCAGCTAGTCAGTCTGGAGCGGAAGTTAAGAAACCTGGAGCATCCGTGAAAATA  
AGTTGCAAGGCATCCGTTACTCGTCACCCATACTATATCCACTGGGTAAACAGGCA  
CCAGGACAGGGACTTGAATGGATCGGATATATCTCAAATTATAATAGAGCTACAAACTAT  
AACCAAAAATTCAAGGACGCGTGACTTTACAACTGACACCTCACCTCGACAGCATAAC  
ATGGAATTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTGCGCTAGAGATTAT  
GATTATGATGTTGGAATGGACTATTGGGGCAGGGAACACTGGTACAGTGTCTTGT

21R75 Heavy chain nucleotide sequence with signal sequence underlined (13B Version 2) (SEQ ID NO:74)

ATGAAAGCACCTATGGTTCTTCTATTATTAGTGGCCGCTCCCGTGGGTGTTATCGCAG  
GTTCAGCTAGTTCAGTCTGGAGCGAAGTTAAGAAACCTGGAGCATCCGTGAAAATAAGT  
TGCAAGGCATCCGGTTACTCGTCACCGCATACTATATCCACTGGTTAACAGGCACCA  
GGACAGGGACTTGAATGGATCGGATATATCGCTGGATATAAGATGCTACAAACTATAAC  
CAAAAATTCAAAGGACCGTGACTTCACAACGTACACCTCACACCTGACAGCATACATG  
GAATTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTGCGCTAGAGGATTATGAT  
TATGATGTTGGAATGGACTATTGGGCCAGGGAACACTGGTGACAGTGTCTCTGCATCC  
ACTAAGGGACCATCCGTGTTCCCTTGGCCCCCTGCTCTGTCGACCTCTGAATGACT  
GCCGCTCTGGGATGCCTCGTGAAGATTACTTCCCTGAGCCTGTGACCCTTCTGGAAC  
TCGGGGCGCCCTAACCTCTGGCGTGACACATTCCCTGCCGTCTACAGTCTCTGGCTA  
TACTCTTATCTCGGTTGTTACCGTACCTCTTAACCTCGGAAACCCAAACTTACACC  
TGTAACCTAGACCAACGGCTTGGACACCAAGGGTGGACAAGACTGTTGACCGAAAGTGC  
TGCCTGAGTGCCTCCATGTCCTGCACCTCTGTGGCTGGCCCTCTGTGTTCTGTT  
CCTCCAAAACCTAAGGACACTCTAATGATCTCTCGGACTCCTGAGGTGACTTGCCTGGTT  
GTGGACGTGTCCTCACGAGGACCTGTGGCTGAGTTCAATTGGTAGCTGGACGGAGTCGAG  
GTGCAACATGCAAAGACCAAGGCTCGGGAGGAACAGTCAACTCCACCTCCGGGTGGTT  
TCTGTGTTGACCGTTGACCAAGACTGGCTGAACGGCAAAGAAATACAAGTGCACAGGTG  
TCCAACAAGGGCCTGCCTGCCCTATCGAAAAGACCATCAGCAAGGACCAAGGGCCAGCCT  
CGCGAGCCTCAGGTGTACACCCCTGCCCTCCAGGCCGGAAAGAAATGACCAAGAACCCAGGTG  
TCCCTGACACTGTCGGTGGAGGGCTCTACCCCTCCGACATGCCGTTGACTGGGAGTCT  
AACGGACAGCCGGAGAACAACTACAAAGACTACGCCCTCAATGCTGGACTCCGACGGCTCC

TCTTCCUTGTAUCUCGAACUTGACUGTGGACAAAGTCCCCGTTGGCAGCAGGGCAACGTGTT  
TCATGCTCCGTAATGCACGAAGCCTGCACAATCACTACACTCAAAAGTCCCTATCCTTA  
TCTCCTGGCAAGTAG

**21M18 Heavy chain nucleotide sequence (version 2) (SEQ ID NO:75)**

ATGAAGCACCTATGGTTCTTCTATTATTAGTGGCCGCTCCCGTTGGGTGTTATCGCAG  
GTCAGCTAGTTCAGTCAGTCAGGAGCGGAAGTTAAGAAACCTGGAGCATCCGTGAAAATAAGT  
TGCAAGGCATCCGGTTACTCGTCACCGCATACTATATCCACTGGGTAAACAGGCACCA  
GGACAGGGACTTGAATGGATCGGAATATACTCTCTTATAATGGAGCTACAAACTATAAC  
CAAATTCAAAGGACGCGTGACTTCACAACCTGACACCTCAACCTGACAGCATACTG  
GAATTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTGCGCTAGAGATTATGAT  
TATGATGTTGGAATGGACTATTGGGCCAGGGAAACACTGGTGACAGTGTCTCTGCATCC  
ACTAAGGGACCATCGTGTCCCTTGGCCCTTGCTCTCGTTGACACTCTGAATCGACT  
GCCGCTCTGGATGCCCTCGTGAAGAATTACTCCCTGAGCCTGTGACCGTTCTGGAAC  
TCGGGCGCCCTAACCTCTGGCGTGCACACATTCCCTGCCGTGCTACAGTCTCTGGCCTA  
TACTCTTATCTCGGTTGTTACCGTACCTCTCTAACCTCGAACCCAAACTACACC  
TGTAACTGAGACCAAGCCTCGAACACCAAGGTGGACAAAGACTGTTGAGCGAAAGTGC  
TGCCTTGAGTGGCCCTCATGTCCTGCACTCCCTGTGGCTGGCCCTTCTGTGTTCTGTT  
CCTCCAAAACCTAAGGACACTCTAAATGATCTCGGACTCCTGAGGTGACTTGCCTGGTT  
GTGGACGTGCCCACGAGGACCTGAGGTGCAATTGGTACGGTGGACGGAGTCGAG  
GTGCACAATGCAAAGGACCAAGCCTCGGGAGGAACAGTTCAACTCCACCTCCGGTGGTT  
TCTGTGTTGACCGGTTGTCACCAAGACTGGCTGAACGGCAAAGAAATACAAGTGCAGGTG  
TCCAACAAGGGCCTGCCCTGCTGCCCCCTATCGAAAAGACCATCAGCAAGACCAAGGGCAGCCT  
CGCGAGCCTCAGGTGTACACCCTGCCCTGCCAGGGAAAGAAATGACCAAGAACCCAGGTG  
TCCCTGACCTGTCTGGGGAGGCTCTACCCCTCCGACATGCCGTGAGTGGAGTCT  
AACGGACAGCGGAGAACAACTACAAGACTACGCCCTCAATGCTGGACTCCGACGGCTCC  
TCTCTCTGACTCCGAACGTGACCGTGGACAAGTCCCCTGGCAGCAGGGCAACGTGTT  
TCATGCTCCGTAATGCACGAAGCCTGCACAATCACTACACTCAAAAGTCCCTATCCTTA  
TCTCCTGGCAAGTAG

**21M18 Heavy chain variable region (version 2) (SEQ ID NO:76)**

CAGCTAGTTAGTCTGGAGCGGAAGTTAAGAAACCTGGAGCATCCGTGAAAATAAGTTGC  
AAGGCATCCGGTTACTCGTTACCGCATACTATATCCACTGGGTAAACAGGCACCGAGGA  
CAGGGACTTGAATGGATCGGATATATCTCTCTTATAATGGAGCTACAAACTATAACCAA  
AAATTCAAAGGACGCGTGACTTCACAACCTGACACCTCAACCTGACAGCATACTGGAA  
TTACGGTCCCTACGGTCTGACGACACTGCCGTTACTATTGCGCTAGAGATTATGATTAT  
GATGTTGGAATGGACTATTGGGCCAGGGAAACACTGGTGACAGTGTCTTCT

**Anti-DLL4 heavy chain CDR2 consensus sequence (SEQ ID NO:80):**

YIX<sub>1</sub>X<sub>2</sub>YX<sub>3</sub>X<sub>4</sub>ATNYNQKFKG, where X<sub>1</sub> is serine or alanine, X<sub>2</sub> is serine, asparagine, or glycine, X<sub>3</sub> is asparagine or lysine, and X<sub>4</sub> is glycine, arginine, or aspartic acid

## REFERENCES CITED IN THE DESCRIPTION

### Cited references

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**Patentkrav****1. Bispecifikt antistof omfattende:**

a) et første antigenbindingssted, der specifikt binder human VEGF, og  
b) et andet antigenbindingssted, der specifikt binder human DLL4,  
5 hvor det første antigenbindingssted omfatter en tungkæde-CDR1 omfattende NYWMH (SEQ ID NO: 17), en tungkæde-CDR2 omfattende DINPSNGRTSYKEKFKR (SEQ ID NO: 18) og en tungkæde-CDR3 omfattende HYDDKYYPLMDY (SEQ ID NO: 19);  
hvor det andet antigenbindingssted omfatter en tungkæde-CDR1 omfattende TAYYIH (SEQ ID NO: 13), en tungkæde-CDR2 omfattende YIX1X2YX3X4ATNYNQKFKG (SEQ ID NO: 80), hvor  $X_1$  er serin eller alanin,  $X_2$  er serin, asparagin eller glycin,  $X_3$  er asparagin 10 eller lysin, og  $X_4$  er glycin, arginin, eller asparaginsyre, og en tungkæde CDR3 omfattende RDYDYDVGMDY (SEQ ID NO: 16); og  
hvor både det første og det andet antigenbindingssted omfatter en letkæde-CDR1 omfattende RASESVDNYGISFMK (SEQ ID NO: 20), en letkæde-CDR2 omfattende AASNQGS (SEQ ID NO: 21) og en letkæde-CDR3 omfattende QQSKEVPWTFGG (SEQ 15 ID NO: 22); og  
hvor det bispecifikke antistof er et IgG-antistof.

**2. Bispecifikt antistof ifølge krav 1, der omfatter:**

(a) en første tungkæde-variabel region med mindst 90 % sekvensidentitet med SEQ ID NO: 11;  
(b) en anden tungkæde-variabel region med mindst 90 % sekvensidentitet med SEQ ID NO: 64 og  
(c) en første og en anden letkæde-variabel region med mindst 90 % sekvensidentitet med 25 SEQ ID NO: 12.

**3. Bispecifikt antistof, der specifikt binder human VEGF og human DLL4, og som omfatter en tungkæde ifølge SEQ ID NO: 7, en tungkæde ifølge SEQ ID NO: 62 og to letkæder ifølge SEQ ID NO: 8.****4. Bispecifikt antistof, der specifikt binder human VEGF og human DLL4, og som omfatter en tungkæde kodet for af nukleotidsekvensen ifølge SEQ ID NO: 33, en tungkæde kodet for af nukleotidsekvensen ifølge SEQ ID NO: 72 og en letkæde kodet for af nukleotidsekvensen ifølge SEQ ID NO: 34.**

5. Bispecifikt antistof ifølge et hvilket som helst af kravene 1 til 4, hvor det andet antigenbindingssted binder et N-terminalt fragment af human DLL4 (aminosyrerne 1-191 ifølge SEQ ID NO: 24).

5 6. Bispecifikt antistof ifølge et hvilket som helst af kravene 1 til 4, hvor det andet antigenbindingssted binder en epitop omfattende aminosyrerne 40-47 og/eller aminosyrerne 113-120 ifølge SEQ ID NO: 25.

10 7. Bispecifikt antistof ifølge et hvilket som helst af kravene 1 til 6, hvor det første antigenbindingssted binder human VEGF med et  $K_D$  på ca. 100 nM eller mindre, og det andet antigenbindingssted binder human DLL4 med et  $K_D$  på ca. 100 nM eller mindre.

15 8. Celle, der omfatter eller frembringer det bispecifikke antistof ifølge et hvilket som helst af kravene 1 til 7.

9. Isoleret polynukleotidmolekyle omfattende et polynukleotid, der koder for et bispecifikt antistof ifølge et hvilket som helst af kravene 1 til 7, en vektor omfattende det isolerede polynukleotid eller en celle omfattende det isolerede polynukleotid eller vektoren.

20 10. Farmaceutisk sammensætning omfattende det bispecifikke antistof ifølge et hvilket som helst af kravene 1 til 7 og en farmaceutisk acceptabel bærer.

25 11. Bispecifikt antistof ifølge et hvilket som helst af kravene 1 til 7 til anvendelse i en fremgangsmåde til modulering af angiogenese hos et individ, hæmning af vækst af en tumor hos et individ, reduktion af frekvensen af cancerstamceller i en tumor hos et individ eller behandling af cancer hos et individ, omfattende indgivelse til individet af en terapeutisk virksom mængde af det bispecifikke antistof.

12. Bispecifikt antistof til anvendelse ifølge krav 11, hvor canceren eller tumoren vælges fra gruppen bestående af colorektalcancer eller -tumor, coloncancer eller -tumor, ovariecancer eller -tumor, pancreascancer eller -tumor, lungecancer eller -tumor, levercancer eller -tumor, brystcancer eller -tumor, nyrecancer eller -tumor, prostatacancer eller -tumor, gastrointestinal cancer eller tumor, melanom, cervixcancer eller -tumor, blærecancer eller -tumor, glioblastom og cancer eller tumor i hoved og hals.

13. Bispecifikt antistof til anvendelse ifølge krav 11 eller krav 12, der endvidere omfatter indgivelse af et andet terapeutisk middel, eventuelt et kemoterapeutisk middel eller et andet antistof.
- 5 14. Farmaceutisk sammensætning til anvendelse i en fremgangsmåde til behandling af cancer og/eller hæmning af tumorvækst hos et individ, hvor den farmaceutiske sammensætning omfatter et bispecifikt antistof, der specifikt binder human VEGF og human DLL4, og som omfatter en tungkæde ifølge SEQ ID NO: 7, en tungkæde ifølge SEQ ID NO: 62 og to letkæder ifølge SEQ ID NO: 8 samt og en farmaceutisk bærer.

# DRAWINGS

Figure 1A

Bispecific Antibody	Anti-VEGF binding region			Anti-DLL4 binding region		
	Heavy Chain			Heavy Chain		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
219R45-MB-21R18	NYWMH (SEQ ID NO:17)	DINPSNNGRTSYKEKFKR (SEQ ID NO:18)	HYDDKRYPLMDY (SEQ ID NO:19)	TAYYTH (SEQ ID NO:13)	YISSYNGATNYNQKFKG (SEQ ID NO:15)	RDYDYYDVGMDY (SEQ ID NO:16)
219R45-MB-21R79	NYWMH (SEQ ID NO:17)	DINPSNNGRTSYKEKFKR (SEQ ID NO:18)	HYDDKRYPLMDY (SEQ ID NO:19)	TAYYTH (SEQ ID NO:13)	YIANYNRAATNYNQKFKG (SEQ ID NO:14)	RDYDYYDVGMDY (SEQ ID NO:16)
219R45-MB-21R75	NYWMH (SEQ ID NO:17)	DINPSNNGRTSYKEKFKR (SEQ ID NO:18)	HYDDKRYPLMDY (SEQ ID NO:19)	TAYYTH (SEQ ID NO:13)	VIAGYKDATNYNQKFKG (SEQ ID NO:59)	RDYDYYDVGMDY (SEQ ID NO:16)
219R45-MB-21R83	NYWMH (SEQ ID NO:17)	DINPSNNGRTSYKEKFKR (SEQ ID NO:18)	HYDDKRYPLMDY (SEQ ID NO:19)	TAYYTH (SEQ ID NO:13)	YISNYNRAATNYNQKFKG (SEQ ID NO:65)	RDYDYYDVGMDY (SEQ ID NO:16)

Bispecific Antibody	Anti-VEGF and Anti-DLL4 binding regions		
	Light Chain		
	CDR1	CDR2	CDR3
219R45-MB-21R18	RASESVVDNYGISFMK (SEQ ID NO:20)	AASNQGS (SEQ ID NO:21)	QQSKEVWPWTGG (SEQ ID NO:22)
219R45-MB-21R79	RASESVVDNYGISFMK (SEQ ID NO:20)	AASNQGS (SEQ ID NO:21)	QQSKEVWPWTGG (SEQ ID NO:22)
219R45-MB-21R75	RASESVVDNYGISFMK (SEQ ID NO:20)	AASNQGS (SEQ ID NO:21)	QQSKEVWPWTGG (SEQ ID NO:22)
219R45-MB-21R83	RASESVVDNYGISFMK (SEQ ID NO:20)	AASNQGS (SEQ ID NO:21)	QQSKEVWPWTGG (SEQ ID NO:22)

Figure 1B

Bispecific Antibody	Anti-VEGF binding region		Anti-DLL4 binding region	
	Heavy Chain Variable Region	Light Chain Variable Region	Heavy Chain Variable Region	Light Chain Variable Region
219R45-MB-21R18	SEQ ID NO:11	SEQ ID NO:12	SEQ ID NO:9	SEQ ID NO:12
219R45-MB-21R79	SEQ ID NO:11	SEQ ID NO:12	SEQ ID NO:10	SEQ ID NO:12
219R45-MB-21R75	SEQ ID NO:11	SEQ ID NO:12	SEQ ID NO:58	SEQ ID NO:12
219R45-MB-21R83	SEQ ID NO:11	SEQ ID NO:12	SEQ ID NO:64	SEQ ID NO:12

Figure 1C

Bispecific Antibody	Anti-VEGF binding region		Anti-DLL4 binding region	
	Heavy Chain	Light Chain	Heavy Chain	Light Chain
219R45-MB-21M18	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:1	SEQ ID NO:4
	SEQ ID NO:7	SEQ ID NO:8	SEQ ID NO:5	SEQ ID NO:8
219R45-MB-21R79	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:2	SEQ ID NO:4
	SEQ ID NO:7	SEQ ID NO:8	SEQ ID NO:6	SEQ ID NO:8
219R45-MB-21R75	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:57	SEQ ID NO:4
	SEQ ID NO:7	SEQ ID NO:8	SEQ ID NO:56	SEQ ID NO:8
219R45-MB-21R83	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:63	SEQ ID NO:4
	SEQ ID NO:7	SEQ ID NO:8	SEQ ID NO:62	SEQ ID NO:8

Figure 2

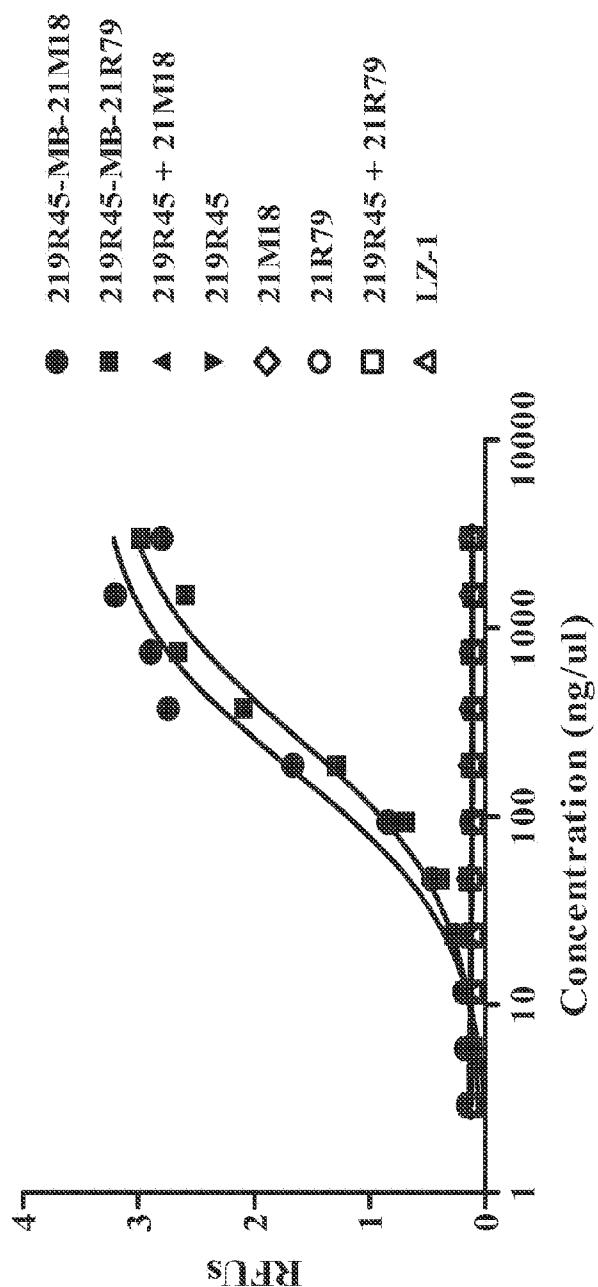


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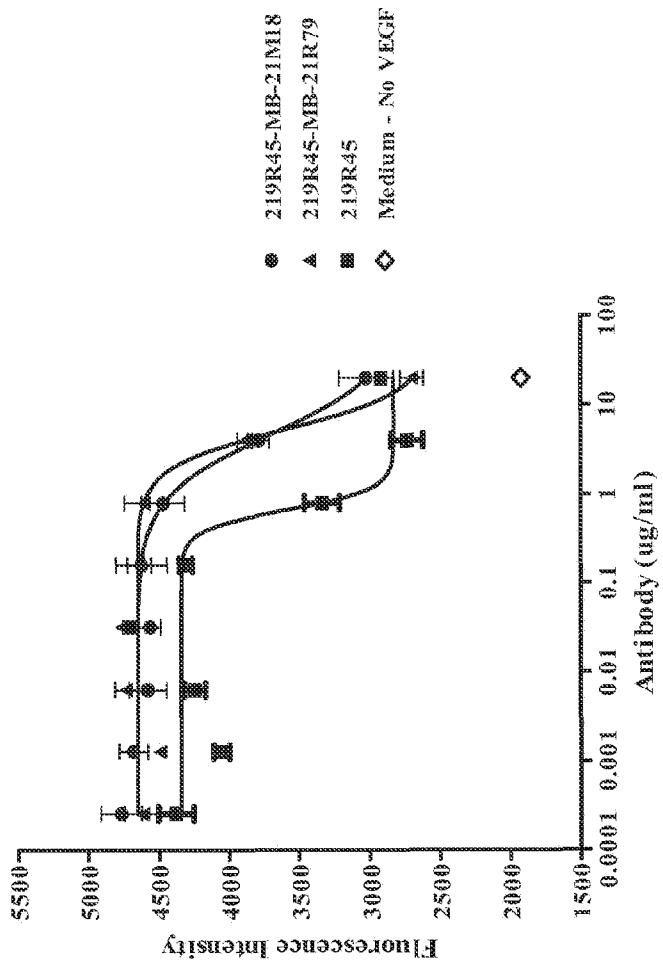


Figure 4

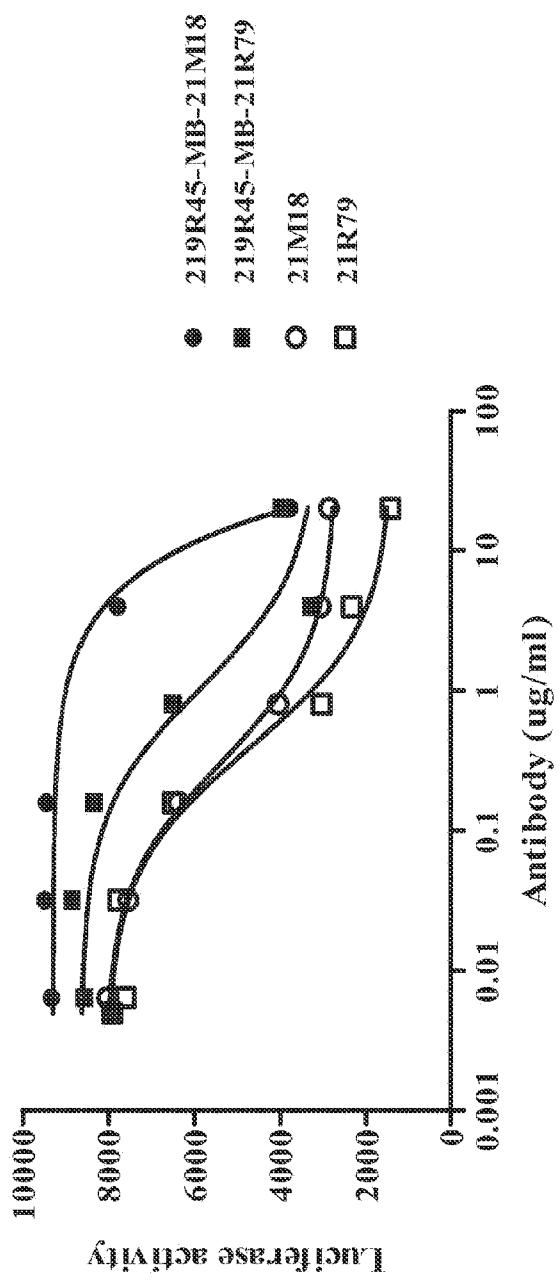


Figure 5

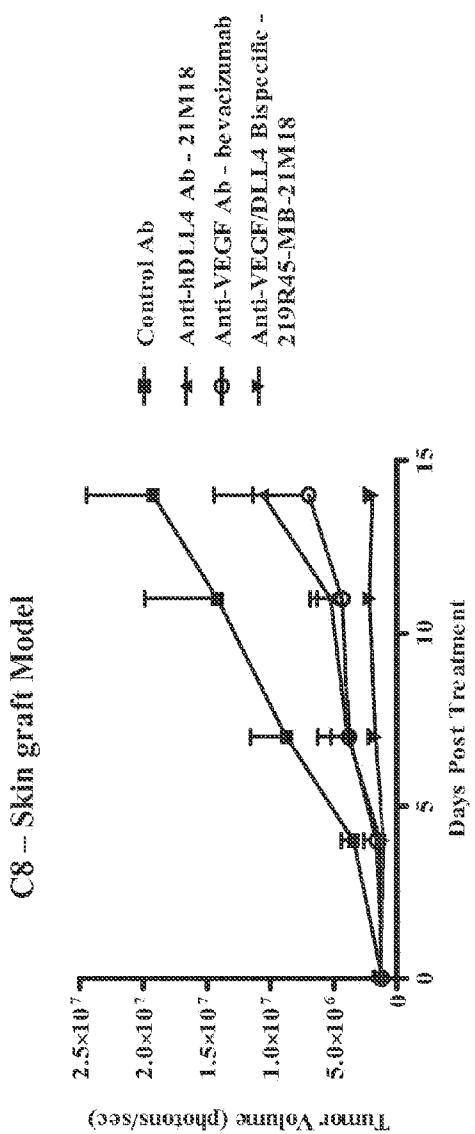


Figure 6

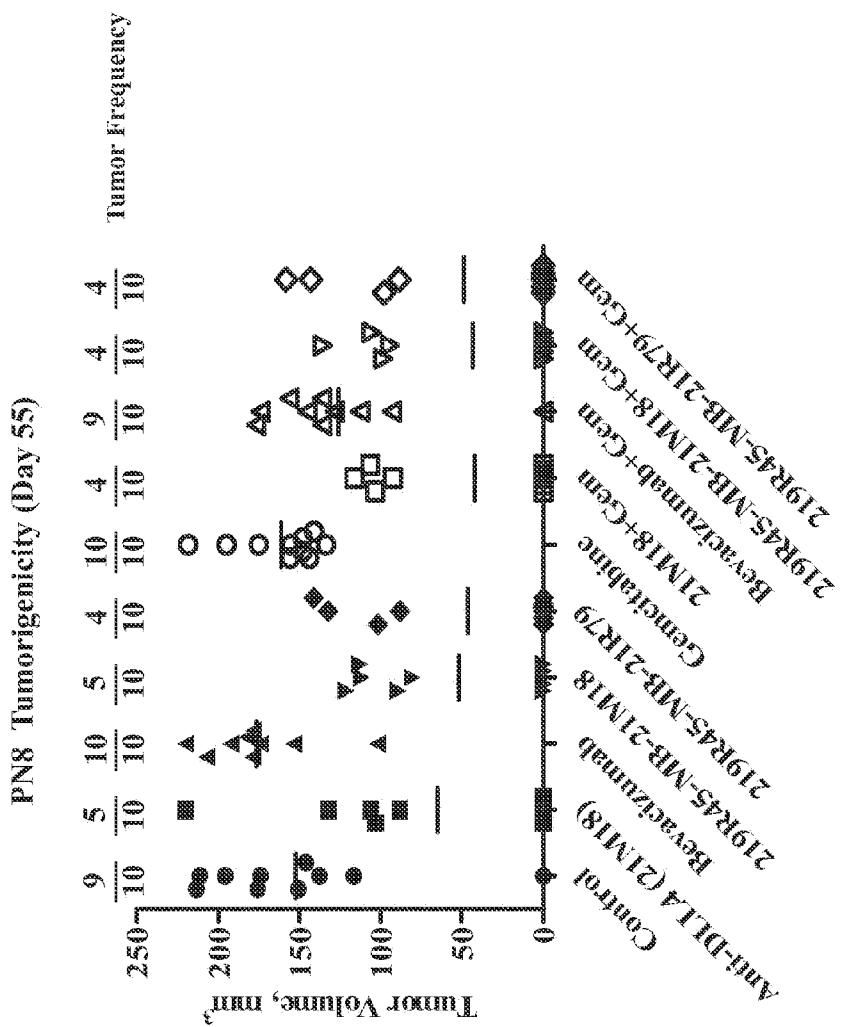


Figure 7

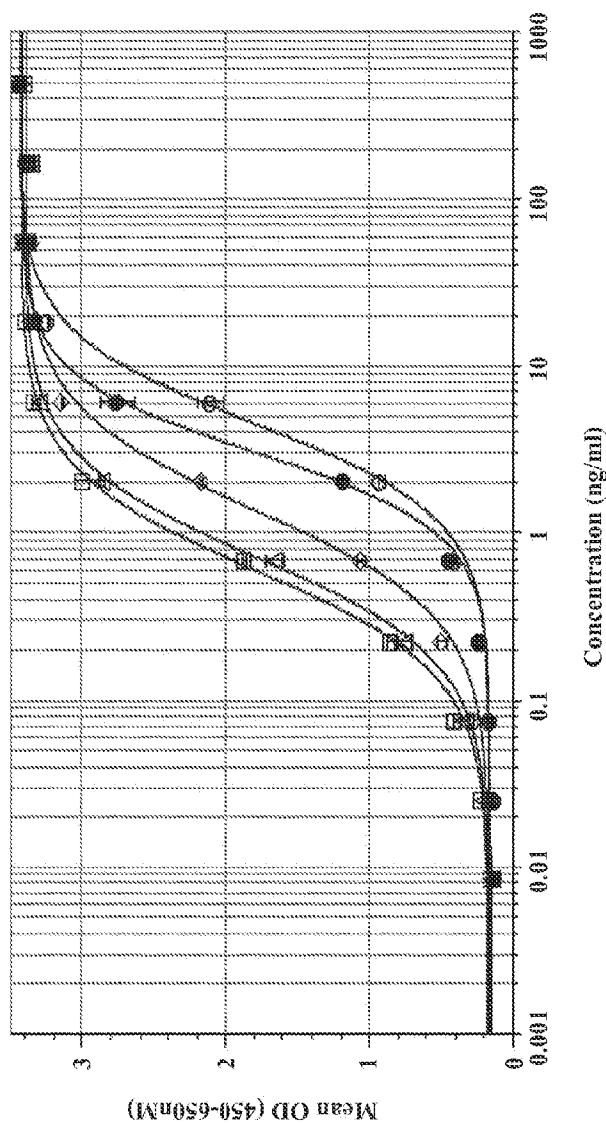


Figure 8

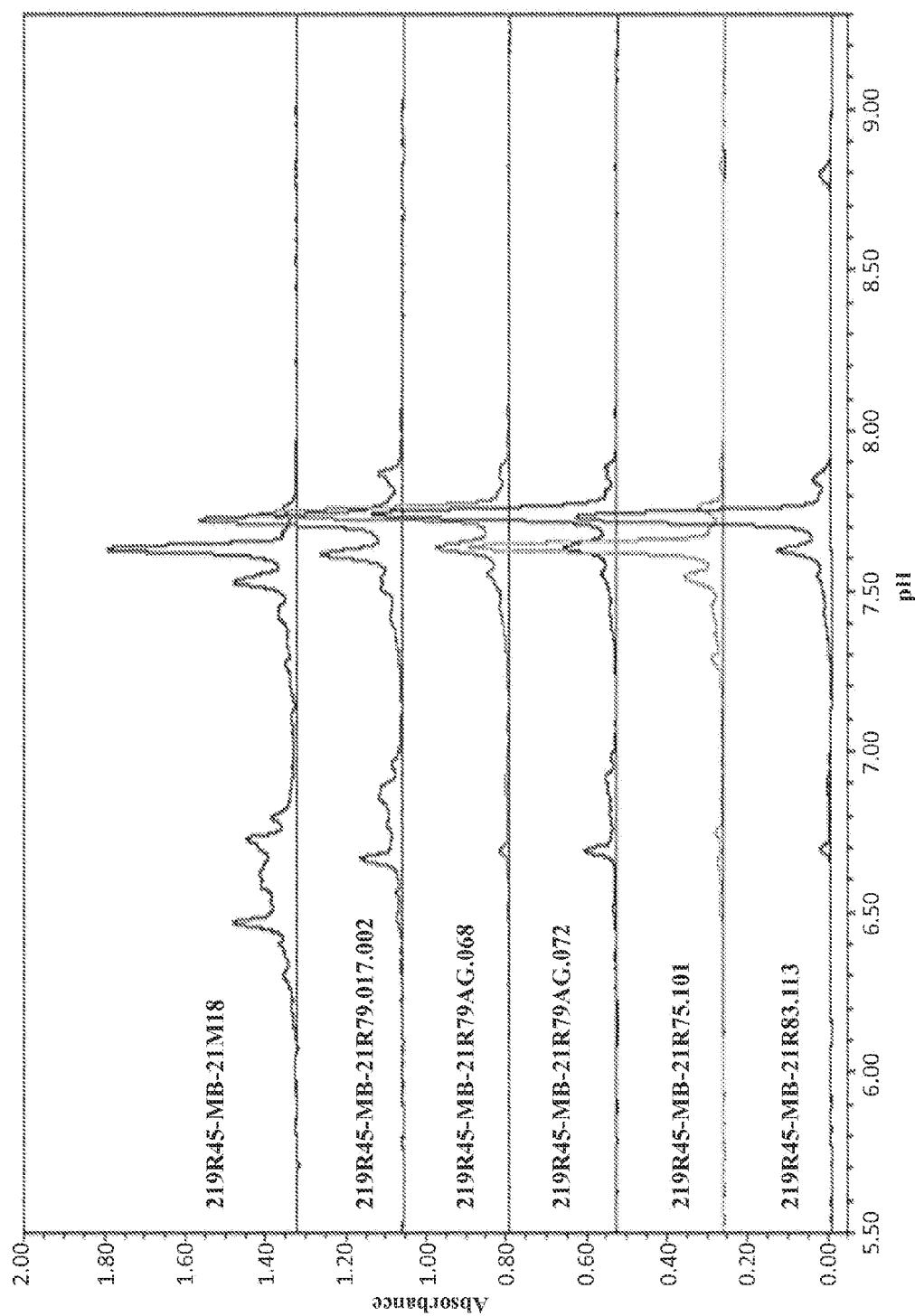
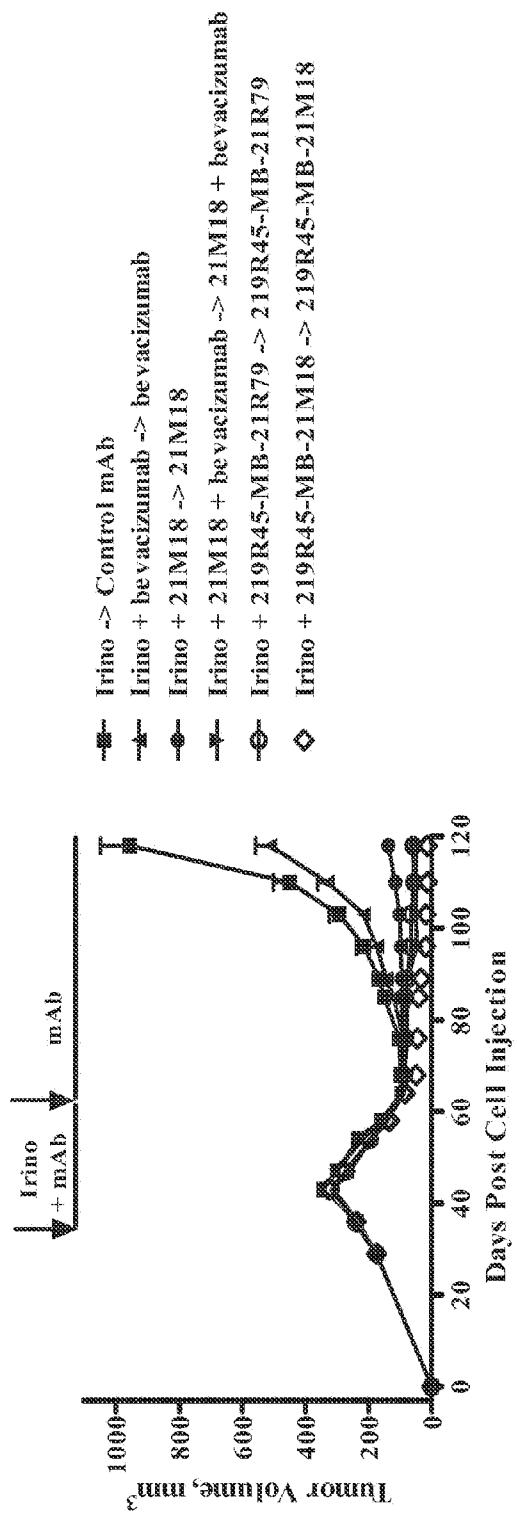


Figure 9



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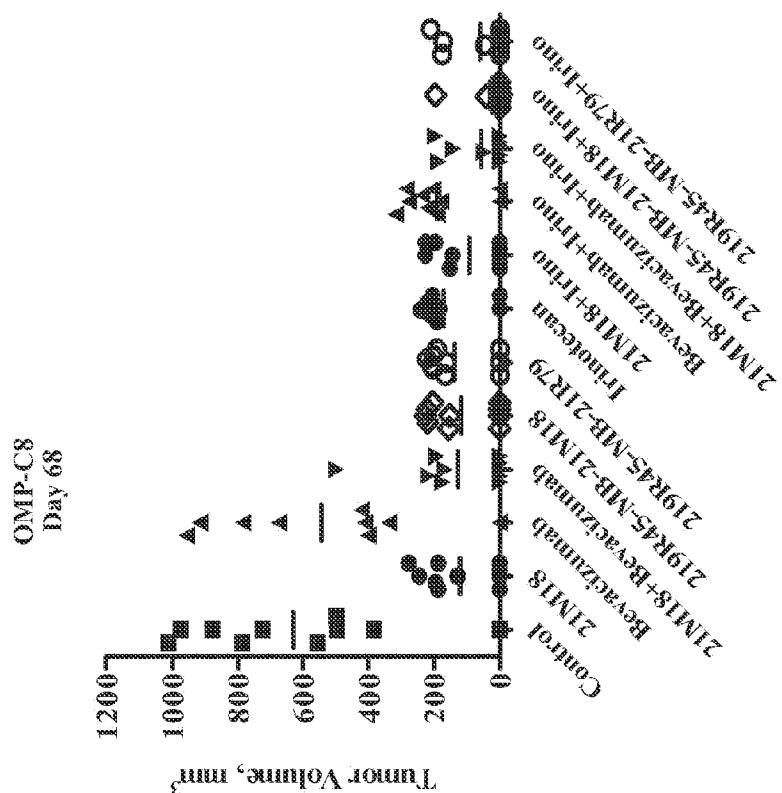
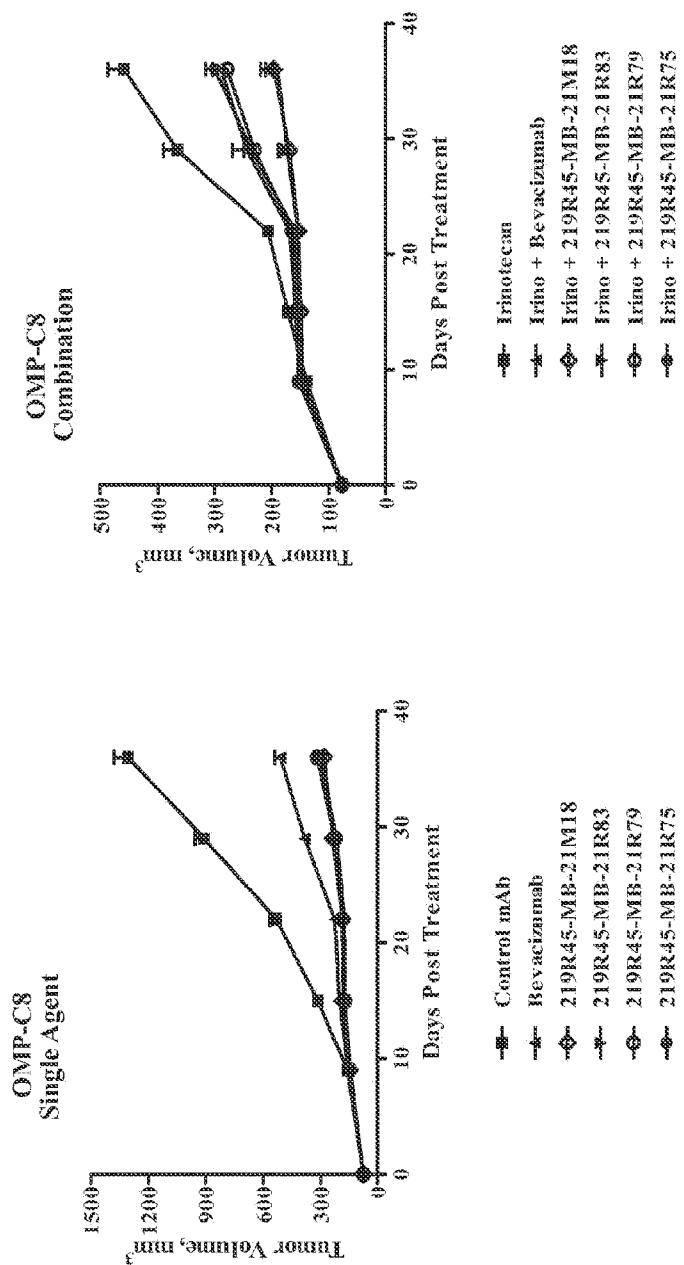


Figure 11



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