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).

[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.

[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 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 embodiments, the binding agents are novel polypeptides, such as antibodies, antibody fragments, and other polypeptides related to such antibodies. In certain embodiments, the binding agents are antibodies that specifically bind human VEGF. In some embodiments, the binding agents are antibodies that specifically bind human DLL4. In some embodiments, the binding agents are bispecific antibodies that specifically bind human VEGF and human DLL4. The ~~invention~~ present disclosure further provides methods of inhibiting the growth of a tumor by administering the binding agents to a subject with a tumor. The ~~invention~~ present disclosure further provides methods of treating cancer by administering the binding agents to a subject in need thereof. In some ~~embodiments~~examples, the methods of treating cancer or inhibiting tumor growth comprise targeting cancer stem cells with the binding agents. In certain ~~embodiments~~examples, 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.

[0013] In one ~~aspect~~example, the ~~invention~~ present invention provides a binding agent, such as an antibody, that specifically binds human VEGF. In some ~~embodiments~~examples, the binding agent inhibits binding of VEGF to at least one VEGF receptor. In some ~~embodiments~~examples, the binding agent inhibits binding of VEGF to VEGFR-1 and/or VEGFR-2. In some ~~embodiments~~examples, the binding agent modulates angiogenesis. In certain ~~embodiments~~examples, the antibody or other binding agent further specifically binds to and/or inhibits human DLL4 in addition to human VEGF.

[0014] In some ~~embodiments~~examples, 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).

[0015] In certain ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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.

[0016] In some ~~embodiments~~examples, the binding agent is antibody 219R45, 219R45-MB-21M18, 219R45-MB-21R79, 219R45-MB-21R75, or 219R45-MB-21R83.

[0017] In another ~~aspect~~example, the ~~invention~~ present disclosure provides a binding agent, such as an antibody, that specifically binds human DLL4. In some ~~embodiments~~examples, the binding agent inhibits binding of DLL4 to at least one Notch receptor. In some ~~embodiments~~examples, the binding agent inhibits binding of DLL4 to Notch1, Notch2, Notch3, and/or Notch4. In some ~~embodiments~~examples, the binding agent inhibits Notch signaling. In some ~~embodiments~~examples, the binding agent promotes unproductive angiogenesis. In certain ~~embodiments~~examples, the antibody or other binding agent further specifically binds to and/or inhibits human VEGF in addition to human DLL4.

[0018] In some ~~embodiments~~examples, 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 $YIX_1X_2YX_3X_4ATNYNQKFKG$ (SEQ ID NO:80), wherein X_1 is serine or alanine, X_2 is serine, asparagine, or glycine, X_3 is asparagine or lysine, and X_4 is glysine, 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 ~~embodiments~~examples, 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).

[0019] In certain ~~embodiments~~examples, 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 ~~embodiments~~examples, 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.

[0020] In some ~~embodiments~~examples, the binding agent is antibody 21R79 or antibody 219R45-MB-21R79.

[0021] In some ~~embodiments~~examples, 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).

[0022] In certain ~~embodiments~~examples, 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 ~~embodiments~~examples, 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.

[0023] In some ~~embodiments~~examples, the binding agent is antibody 21R75 or antibody 219R45-MB-21R75.

[0024] In some ~~embodiments~~examples, 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).

[0025] In certain ~~embodiments~~examples, 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

~~embodiments~~examples, 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.

[0026] In some ~~embodiments~~examples, the binding agent is antibody 21R83 or antibody 219R45-MB-21R83.

[0027] In certain ~~embodiments~~examples of each of the aforementioned aspects or ~~embodiments~~examples, as well as other aspects and/or ~~embodiments~~examples described elsewhere herein, the binding agent is a bispecific antibody. In some ~~embodiments~~examples, the bispecific antibody specifically binds human VEGF and a second target. In some ~~embodiments~~examples, the bispecific antibody specifically binds human DLL4 and a second target. In some ~~embodiments~~examples, the bispecific antibody specifically binds both human VEGF and human DLL4. In some ~~embodiments~~examples, the bispecific antibody modulates angiogenesis. In certain ~~embodiments~~examples, the bispecific antibody inhibits Notch signaling. In some ~~embodiments~~examples, the bispecific antibody modulates angiogenesis and inhibits Notch signaling. In some ~~embodiments~~examples, the bispecific antibody reduces the number of frequency of cancer stem cells. In certain ~~embodiments~~examples, the bispecific antibody comprises two identical light chains. In certain ~~embodiments~~examples the bispecific antibody is an IgG antibody (e.g., IgG2).

[0028] In some ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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).

[0029] In ~~certain embodiments~~another aspect, 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 $YIX_1X_2YX_3X_4ATNYNQKFKG$ (SEQ ID NO:80), wherein X_1 is serine or alanine, X_2 is asparagine, serine, ~~asparagine,~~ or glycine, X_3 is asparagine or lysine, and X_4 is ~~glysine,~~ arginine, glycine, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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).

[0030] In ~~some~~ one aspect~~embodiments~~, there is provided ~~the~~ a bispecific antibody ~~comprises~~comprising: 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 $YIX_1X_2YX_3X_4ATNYNQKFKG$ (SEQ ID NO:80), wherein X_1 is serine or alanine, X_2 is serine, asparagine, or glycine, X_3 is asparagine or lysine, and X_4 is glysine, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 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).

[0031] In some ~~embodiments~~examples, 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 ~~embodiments~~examples, 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.

[0032] In some ~~embodiments~~examples, 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 ~~embodiments~~examples, 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.

[0033] In some ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 ~~embodiments~~examples, 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 some ~~embodiments~~examples, 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 some ~~embodiments~~examples, 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.

[0034] In some ~~embodiments~~examples, 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 0.1 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. In certain ~~embodiments~~examples, the bispecific antibody comprises two identical light chains.

[0034a] In another aspect, there is provided a bispecific antibody that specifically binds human VEGF and human DLL4, comprising: (a) a heavy chain of SEQ ID NO: 7; (b) a heavy chain of SEQ ID NO: 62, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 56; and (c) two light chains of SEQ ID NO: 8.

[0035] In ~~some~~ one aspect~~embodiments~~, 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.

[0035a] In another aspect, there is provided an isolated antibody that specifically binds human VEGF, which 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).

[0036] In certain ~~embodiments~~examples of each of the aforementioned aspects, as well as other aspects and/or ~~embodiments~~examples described elsewhere herein, the binding agent or antibody is isolated.

[0037] In another aspect, the invention provides a polypeptide selected from the group consisting of: SEQ ID NO:12, 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 ~~embodiments~~examples, the polypeptide is isolated. In certain ~~embodiments~~examples, the polypeptide is substantially pure. In certain ~~embodiments~~examples, the polypeptide is an antibody or part of an antibody, such as an antibody fragment.

[0038] In another aspect, the ~~invention~~ present disclosure provides isolated polynucleotide molecules comprising a polynucleotide that encodes the binding agents and/or polypeptides of each of the aforementioned aspects, as well as other aspects and/or ~~embodiments~~examples described herein. In some ~~embodiments~~examples, 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 invention further provides expression vectors that comprise the polynucleotides, as well as cells that comprise the expression vectors and/or the polynucleotides. In some ~~embodiments~~examples, the cell is a prokaryotic cell or a eukaryotic cell.

[0039] ~~In other aspects, the invention provides~~ Also disclosed are 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.

[0040] ~~In another aspect, the invention provides~~ Also disclosed is 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.

[0041] ~~In another aspect, the invention provides~~Also disclosed is 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.

[0042] ~~In another aspect, the invention provides~~Also disclosed is 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.

[0042a] In another aspect, there is provided a use of the antibody as described herein in the manufacture of a medicament for inhibiting growth of a tumor.

[0042a] In another aspect, there is provided the antibody as described herein for use in inhibiting growth of a tumor.

[0043] ~~In another aspect, the invention provides~~Also disclosed is 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.

[0044] ~~In other aspects, the invention provides~~Also disclosed is 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.

[0044a] In another aspect, there is provided a use of the antibody as described herein in the manufacture of a medicament for treatment of cancer.

[0044b] In another aspect, there is provided the antibody as described herein for use in the treatment of cancer.

[0044c] In another aspect, there is provided a pharmaceutical composition comprising the antibody as disclosed herein and a pharmaceutically acceptable carrier.

[0045] 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.

[0045a] In yet another aspect, there is provided an in vitro method for the production of an antibody, comprising expression at least one polynucleotide of the present disclosure in a cell.

[0046] Where aspects or ~~embodiments~~examples of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention 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 invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0047] 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.

[0048] 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-).

[0049] 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 (-◊-).

What is claimed is:

1. A bispecific antibody comprising:

a) a first antigen-binding site that specifically binds human vascular endothelial growth factor (VEGF), and

b) a second antigen-binding site that specifically binds human delta-like 4 ligand (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 $YIX_1X_2YX_3X_4ATNYNQKFKG$ (SEQ ID NO:80), wherein X_1 is serine or alanine, X_2 is ~~serine,~~ asparagine, serine, or glycine, X_3 is asparagine or lysine, and X_4 is ~~glycine,~~ arginine, glycine, or aspartic acid , 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).

2. The bispecific antibody of claim 1, wherein 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), YIANYNRATNYNQKFKG (SEQ ID NO:14), YISSYNGATNYNQKFKG (SEQ ID NO:15), or YIAGYKDATNYNQKFKG (SEQ ID NO:59), ~~or YISNYNRATNYNQKFKG (SEQ ID NO:65),~~ and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16).

3. The bispecific antibody of claim 1 or claim 2, which 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: 64, SEQ ID NO:9, SEQ ID NO:10, or 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.

4. The bispecific antibody of any one of claims 1-3, which comprises a first CH3 domain and a second CH3 domain, each of which is modified to promote formation of heteromultimers.

5. The bispecific antibody of any one of claims 1-4, wherein the first and second CH3 domains are modified based upon electrostatic effects.

6. The bispecific antibody of any one of claims 1-5, which is a monoclonal antibody, a recombinant antibody, a chimeric antibody, a humanized antibody, a human antibody, an IgG1 antibody, or an IgG2 antibody.

7. The bispecific antibody of any one of claims 1-5, which comprises a first human IgG2 constant region with amino acid substitutions at positions corresponding to positions 249 and 288 of SEQ ID NO:42, wherein the amino acids are replaced with glutamate or aspartate, and a second human IgG2 constant region with amino acid substitutions at positions corresponding to positions 236 and 278 of SEQ ID NO:42, wherein the amino acids are replaced with lysine.

8. The bispecific antibody of any one of claims 1-7, which:
   (i) inhibits binding of VEGF to at least one VEGF receptor;
   (ii) inhibits binding of DLL4 to at least one Notch receptor;
   (iii) inhibits Notch signaling; and/or
   (iv) modulates angiogenesis.

9. A bispecific antibody that specifically binds human VEGF and human DLL4, comprising:
   (a) a heavy chain of SEQ ID NO:7;
   (b) a heavy chain of SEQ ID NO: 62, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:56 ~~or SEQ ID NO: 62~~; and
   (c) two light chains of SEQ ID NO:8.

10. A bispecific antibody selected from the group consisting of 219R45-MB-21R83, 219R45-MB-21M18, 219R45-MB-21R79, and 219R45-MB-21R75~~, and 219R45-MB-21R83~~.

11. An isolated antibody that specifically binds human VEGF, which 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).

12. The antibody of claim 11, which comprises:

(a) a heavy chain variable region having at least 90% sequence identity to SEQ ID NO:11; and

(b) a light chain variable region having at least 90% sequence identity to SEQ ID NO:12.

13. The antibody of claim 11, which comprises:

(a) a heavy chain comprising SEQ ID NO:49 or SEQ ID NO:7; and

(b) a light chain comprising SEQ ID NO:8.

14. The antibody of any one of claims 11-13, which inhibits binding of VEGF to at least one VEGF receptor.

15. An isolated antibody that specifically binds human DLL4, which comprises:

(a) a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13) or AYYIH (SEQ ID NO:79), a heavy chain CDR2 comprising $YIX_1X_2YX_3X_4ATNYNQKFKG$ (SEQ ID NO:80), wherein X_1 is serine or alanine, X_2 is ~~serine,~~ asparagine, serine, or glycine, X_3 is asparagine or lysine, and X_4 is ~~glycine,~~ arginine, glycine, or aspartic acid , 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).

16. The antibody of claim 15, which comprises a heavy chain CDR1 comprising TAYYIH (SEQ ID NO:13), a heavy chain CDR2 comprising, YISNYNRATNYNQKFKG (SEQ ID NO:65),YIANYNRATNYNQKFKG (SEQ ID NO:14), or YIAGYKDATNYNQKFKG (SEQ ID NO:59) ~~or YISNYNRATNYNQKFKG (SEQ ID NO:65),~~ and a heavy chain CDR3 comprising RDYDYDVGMDY (SEQ ID NO:16).

17. The antibody of claim 15 or claim 16, which comprises:

(a) a heavy chain variable region comprising SEQ ID NO: 64, SEQ ID NO:10, or SEQ ID NO:58~~, or SEQ ID NO: 64~~; and

(b) a light chain variable region comprising SEQ ID NO:12.

18. The antibody of any one of claims 11-17, which is a monoclonal antibody, a recombinant antibody, a chimeric antibody, a humanized antibody, a human antibody, a bispecific antibody, an IgG1 antibody, an IgG2 antibody, or an antibody fragment comprising an antigen-binding site.

19. A pharmaceutical composition comprising the antibody of any one of claims 1-18 and a pharmaceutically acceptable carrier.

20. A cell comprising or producing the antibody of any one of claims 1-18.

21. An isolated polynucleotide molecule comprising a polynucleotide that encodes the antibody of any one of claims 1-18.

22. A vector comprising the polynucleotide of claim 21.

23. A cell comprising the polynucleotide of claim 22.

24. A polypeptide comprising a sequence selected from the group consisting of: SEQ ID NO: 12, 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.

25. Use of the antibody of any one of claims 1-18 in the manufacture of a medicament for treatment of cancer.

26. The use of the antibody of claim 25, wherein the cancer is selected from the group consisting of colorectal cancer, colon cancer, ovarian cancer, pancreatic cancer, lung cancer, liver cancer, breast cancer, kidney cancer, prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, bladder cancer, glioblastoma, head and neck cancer, lymphoma and leukemia.

27. Use of the antibody of any one of claims 1-18 in the manufacture of a medicament for inhibiting growth of a tumor.

28. The use of the antibody of claim 27, wherein the tumor is selected from the group consisting of colorectal tumor, colon tumor, ovarian tumor, pancreatic tumor, lung tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor.

29. The use of the antibody of any one of claims 25-28, wherein the use comprises the antibody and at least one additional therapeutic agent.

30. The use of the antibody of claim 29, wherein the at least one additional therapeutic agent is a chemotherapeutic agent.

31. The use of the antibody of claim 29, wherein the at least one additional therapeutic agent is a second antibody.

32. The antibody of any one of claims 1-18 for use in the treatment of cancer.

33. The antibody of claim 32, wherein the cancer is selected from the group consisting of colorectal cancer, colon cancer, ovarian cancer, pancreatic cancer, lung cancer, liver cancer, breast cancer, kidney cancer, prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, bladder cancer, glioblastoma, head and neck cancer, lymphoma and leukemia.

34. The antibody of any one of claims 1-18 for use in inhibiting growth of a tumor.

35. The antibody of claim 34, wherein the tumor is selected from the group consisting of colorectal tumor, colon tumor, ovarian tumor, pancreatic tumor, lung tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor.

36. The antibody of any one of claims 32-35, wherein the treatment of cancer or the inhibiting growth of a tumor comprises the use of at least one additional therapeutic agent.

37. The antibody of claim 36, wherein the at least one additional therapeutic agent is a chemotherapeutic agent.

38. The antibody of claim 36, wherein the at least one additional therapeutic agent is a second antibody.

~~30.~~39. An *in* vitro method for the production of an antibody, comprising expressing at least one polynucleotide of claim 21 in a cell.