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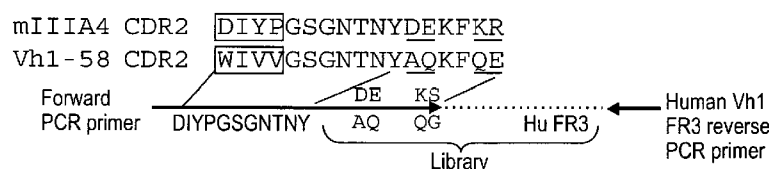
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(54) Title: ANTIBODIES TO EPHA3

FIG. 2



(57) Abstract: The current disclosure relates to high-affinity antibodies to EphA3 that have reduced immunogenicity when administered to a human to treat diseases and method of using such antibodies.

Antibodies to EphA3

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. provisional application no. 61/251,668, filed
5 October 14, 2009, which application is herein incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Eph receptor tyrosine kinases (Ephs) belong to a large group of receptor tyrosine
kinases (RTKs), kinases that phosphorylate proteins on tyrosine residues. Ephs and their
membrane bound ephrin ligands (ephrins) control cell positioning and tissue organization
10 (Poliakov, A., et al., *Dev Cell* 7:465-80 (2004)). In contrast to other receptor tyrosine
kinases, Eph receptor activation does not only require ligand binding and dimerization but
also involves preformed ligand oligomers. Thus, tyrosine phosphorylation of Eph receptors
requires presentation of ephrin ligands in their clustered or membrane-attached forms (Davis
et al., *Science* 266:816-819 (1994)). Functional and biochemical Eph responses occur at
15 higher ligand oligomerization states (Stein et al., *Genes Dev* 12:667-678 (1998)).

[0003] Among other patterning functions, various Ephs and ephrins have been shown to
play a role in vascular development. The de-regulated re-emergence of some ephrins and
their receptors in adults also has been observed to contribute to tumor invasion, metastasis
and neo-angiogenesis. For example, dominant-negative, soluble EphA2 or A3 proteins
20 exhibit effects on ephrin-induced endothelial cell function *in vitro*, and tumor angiogenesis
and progression *in vivo* (Nakamoto, et al., *Microsc Res Tech* 59:58-67 (2002); Brantley-
Sieders, et al., *Curr Pharm Des* 10:3431-42 (2004); Brantley, et al. *Oncogene* 21:7011-26
(2002); Cheng, et al. *Neoplasia* 5:445-56 (2003). Dobrzanski, et al. *Cancer Res* 64:910-9
(2004)). Furthermore, Eph family members have been found to be over-expressed on tumor
25 cells from a wide variety of human solid tumors (Brantley-Sieders, et al., *Curr Pharm Des*
10:3431-42 (2004); Marme, D. *Ann Hematol* 81 Suppl 2:S66 (2002); Booth, C. et al., *Nat*
Med 8:1360-1 (2002)).

[0004] EphA3 has also been reported to be activated and overexpressed on CD34⁺ cells in
chronic myeloid leukemia (CML) patients in the accelerated phase and blast crisis stage
30 (Cilloni et al., American Society of Hematology, Abstract 1092, 2008 (available on line
November 14, 2008)). Cilloni et al. reported that when primary CML cells or EphA3-

transfected normal cells were incubated with a specific monoclonal antibody, the antibody induced a significant reduction of proliferation in primary cells and transfected cells, reduced colony growth and induced changes to the adhesion properties. The antibody did not induce any significant changes in normal control cells or cells from CML patient in the chronic stage.

[0005] This invention is based, in part, on the discovery of new anti-EphA3 antibodies.

BRIEF SUMMARY OF THE INVENTION

[0006] The invention is directed to potent anti-EphA3 antibodies and methods of using such antibodies, *e.g.*, for the treatment of a disease involving EphA3. An antibody of the invention has the characteristics as described herein. Thus, in one aspect, an antibody of the invention comprises a V_H region that comprises a CDR3 comprising the amino acid sequence X₁GX₂YEX₃FDX₄, wherein X₁ is S or G, X₂ is Y or V, X₃ is E or D, and X₄ is S, V, or I, with the proviso that when the amino acid sequence is SGYYEDFDS the CDR1 is not SYWIN and when the amino acid sequence is SGYYEEFDS the CDR1 is not TYWIS. In some embodiments, the antibody has a CDR3 that comprises the amino acid sequence GGYEDFDS, SGYYEEFDS, SGVYEDFDS, SGYYEDFDV, or SGYYEDFDI. In some embodiments, the antibody has a J segment that comprises at least 80% identity, typically at 85%, or at least 90% identity, to a human germline J segment amino acid sequence; or that differs from a human germline J segment at no more than two positions; and a V-segment that comprises at least 80% identity, typically at least 85% identity, and preferably 90% identity, or greater, to a human germ line V-segment amino acid sequence. In some embodiment, the J segment comprises at least 90% identity to human JH6 amino acid sequence, and the V-segment comprises at least 90% identity to a human VH1 1-02 amino acid sequence. In some embodiments, the antibody has an FR4 that comprises WGQGTTVTVS, or an FR4 that differs no more than one amino acid from WGQGTTVTVS. In some embodiments, the antibody comprises a V_H CDR1, or a V_H CDR2, or both a V_H CDR1 and a V_H CDR2, as shown in a V_H region set forth in Figure 1. For example, an antibody of the invention can have a V_H CDR1 that has the amino acid sequence GYWMN, TYWIS, or SYWIN and/or a CDR2 that has the amino acid sequence DIYPGSGNTNYDEKFQG, DIYPGSGNTNYAQKFQG, DIYPGSGNTNYAQEFRG, DIYPGSGNTNYAQKFLG, DIYPGSGNTNYDEKFEG, or DIYPGSGNTNYDEKFKR. In some embodiments, the antibody has a V_H CDR1 GYWMN and a CDR2 DIYPGSGNTNYDEKFQG. In some embodiments, the antibody has a V_H CDR1 TYWIS

and a V_H CDR2 DIYPGSGNTNYAQ(K/E)F(Q/R/L)G. In some embodiments, an antibody of the invention has the V_H CDR1, CDR2, and CDR3 from one of the V regions as shown in Figure 1. In some embodiments, the antibody has a V_H V-segment amino acid sequence of a V-segment sequence shown in Figure 1. In some embodiments, the V_H has the sequence of a V_H region set forth in Figure 1.

[0007] The invention also provides an antibody that has a V_L region that comprises a CDR3 binding specificity determinant having the sequence X₁X₂YX₃X₄YPYT, wherein X₁ is G, V, or A; X₂ is Q, R, or G; X₃ is A, S, or L; and X₄ is N or K. In some embodiments, the CDR3 comprises GQYANYPYT, VQYAKYPYT, AQYANYPYT, VQYSNYPYT, VQYANYPYT, VGYANYPYT, VRYANYPYT, or VQYLNYPYT. In some embodiments, when the CDR3 is VQYANYPYT, the CDR1 is not RASQEISGYLG, or RASQGIISYLA and/or the CDR2 is not AASTLDS or AASSLQS. In some embodiments, the V_L region comprises a J segment that comprises at least 80% identity, typically at least 85% or 90% identity, to a human germline J segment amino acid sequence, or that differs no more than two amino acids from a human germline segment; and a V-segment that comprises at least 80% identity, typically at least 90% identity, or greater, to a human germ line V-segment amino acid sequence. In some embodiments, the J segment has no more than two amino acid changes, often no more than one amino acid change, relative to the sequence FGQGKLEIK from the human germline Jκ2 amino acid sequence and the V-segment comprises at least 90% identity to human germline JκI L15 amino acid sequence. In some embodiments, the FR4 of the antibody has the amino acid sequence FGQGKLEIK, or has no more than one amino acid residue changed relative to the sequence FGQGKLEIK. In some embodiments, the V_L region comprises a CDR1, or a CDR2, or both a CDR1 and CDR2 of a sequence VL region shown in Figure 1. For example, a CDR1 can have the sequence RASQGIISYLA, QASQDISTYLN, RASQEISGYLG, or RASQSISSYLA; and/or a CDR2 can have the sequence AASSLQS, GASSLQS, AASSLQR, or AASTLDS. In some embodiments, the CDR1 has the sequence RASQGIISYLA and the CDR2 has the sequence GASSLQS. In some embodiments, the CDR1 has the sequence QASQDISTYLN and the CDR2 has the sequence AASSLQR or AASSLQS. In some embodiments, the CDR1 has the sequence RASQSISSYLA and the CDR2 has the sequence AASSLQR. In some embodiments, the V_L region comprises the CDR1, CDR2, and CDR3 of one of the V_L regions set forth in Figure 1. In some embodiments, the V_L region comprises a V-segment that has a V-segment sequence as shown in Figure 1. In some embodiments, the V_L region has the sequence of a V_L region set forth in

Figure 1. In typical embodiments, the V_H region of the antibody comprises any of the V_H regions described in the preceding paragraph.

[0008] In some embodiments, the invention provide an antibody that comprises a V_L region that has a CDR3 comprising GQYANYPYT, VQYAKYPYT, AQYANYPYT,

- 5 VQYSNYPYT, VGYANYPYT, VRYANYPYT, or VQYLNYPYT. In some embodiments, the antibody comprises a heavy chain CDR3 comprising the amino acid sequence X₁GX₂YEX₃FDX₄, wherein X₁ is S or G, X₂ is Y or V, X₃ is E or D, and X₄ is S, V, or I. In some embodiments. In some embodiments, the heavy chain CDR3 comprises the amino acid sequence GGYEEDFDS, SGYYEEFDS, SGVYEDFDS, SGYYEDFDV, or SGYYEDFDI.
- 10 In some embodiments, the antibody comprises a light chain CDR1 or CDR2 set forth in Figure 1, or a heavy chain CDR1 or CDR2 set forth in Figure 1. In some embodiments, the antibody comprises a light chain CDR1 and CDR2 as set forth in Figure 1 and/or a heavy chain CDR1 and CDR2 set forth in Figure 1. In some embodiments, the antibody comprises a V_L V-segment set forth in Figure 1.

- 15 **[0009]** The invention additionally provides an antibody that comprises a V_H region comprising a CDR3 having the sequence SGYYE(E/D)FDS and a V_L region CDR3 sequence set forth in the preceding paragraph, with the proviso that the V_L region CDR3 sequence is not VQYANYPYT or VQYMNYPYT. In some embodiments, the antibody comprises a heavy chain CDR1 or CDR2 set forth in Figure 1, or a light chain CDR1 or CDR2 set forth in
- 20 Figure 1. In some embodiments, the antibody comprises a heavy chain CDR1 and CDR2 as set forth in Figure 1 and/or a light chain CDR1 and CDR2 set forth in Figure 1.

[0010] In some embodiments, an anti-EphA3 antibody of the invention comprises the V_H CDR1, CDR2, and CDR3 from one of the V_H regions set forth in Figure 1 and the V_L CDR1, CDR2, and CDR3 from one of the V_L regions set forth in Figure 1.

- 25 **[0011]** An antibody of the invention can comprise a V_H region as set forth in Figure 1 or a V_L region as set forth in Figure 1. Often, the antibody comprises a V_H region as set forth in Figure 1 and a V_L region as set forth in Figure 1. In some embodiments, the antibody comprises a combinations of V_H and V_L regions that comprise: a) SEQ ID NO:1 and SEQ ID NO:20, b) SEQ ID NO:2 and SEQ ID NO:11, c) SEQ ID NO: 2 and SEQ ID NO:12, d) SEQ
- 30 ID NO:2 and SEQ ID NO:19, e) SEQ ID NO:2 and SEQ ID NO:21, f) SEQ ID NO:2 and SEQ ID NO:22, g) SEQ ID NO:2 and SEQ ID NO:23, h) SEQ ID NO:3 and SEQ ID NO:11, i) SEQ ID NO:3 and SEQ ID NO:12, j) SEQ ID NO:3 and SEQ ID NO:21, k) SEQ ID NO:3

and SEQ ID NO:22, l) SEQ ID NO:4 and SEQ ID NO:11, m) SEQ ID NO:4 and SEQ ID NO:13, n) SEQ ID NO:5 and SEQ ID NO:11, o) SEQ ID NO:5 and SEQ ID NO:13, p) SEQ ID NO:5 and SEQ ID NO:21, q) SEQ ID NO:6 and SEQ ID NO:14, r) SEQ ID NO:6 and SEQ ID NO:15, s) SEQ ID NO:7 and SEQ ID NO:14, t) SEQ ID NO:7 and SEQ ID NO:15, u) SEQ ID NO:8 and SEQ ID NO:14, v) SEQ ID NO:8 and SEQ ID NO:15, w) SEQ ID NO:9 and SEQ ID NO:16, x) SEQ ID NO:9 and SEQ ID NO:17, y) SEQ ID NO:9 and SEQ ID NO:19, z) SEQ ID NO:10 and SEQ ID NO:17, aa) SEQ ID NO:10 and SEQ ID NO:18, or bb) SEQ ID NO:10 and SEQ ID NO:20.

[0012] In some embodiments, an antibody of the invention, *e.g.*, that has a V_H region sequence selected from the V_H region sequences in Figure 1 and a V_L region selected from the V_L region sequences in Figure 1, has a monovalent affinity better than about 10 nM, and often better than about 5 nM or 1 nM as determined by surface plasmon resonance analysis performed at 37°C. Thus, in some embodiments, the antibodies of the invention have an affinity (as measured using surface plasmon resonance) of about 10 nM, about 5 nM, about 2.5 nM, about 1 nM, about 0.5 nM, about 0.25 nM, or about 0.1 nM, or better.

[0013] An antibody of the invention as described herein may have a V_H region and/or a V_L region that comprises a methionine at the N-terminus.

[0014] In some embodiments, the antibody is an IgG. In some embodiments, the antibody is an IgG1 or an IgG3. In some embodiments, the antibody is an IgG2 or an IgG4.

[0015] In some embodiments, the antibody comprises a heavy chain constant regions having the amino acid sequence set forth in SEQ ID NO:24 and/or a kappa light chain constant region having the amino acid sequence set forth in SEQ ID NO:25.

[0016] In some embodiments, the heavy chain constant region of an antibody is afucosylated. In some embodiments, an antibody preparation comprising an antibody of the invention is hypofucosylated or afucosylated.

[0017] In some embodiments, an anti-EphA3 antibody of the invention has a heavy chain amino acid sequence and a light chain amino acid sequence that comprises SEQ ID NO:26 and SEQ ID NO:27, SEQ ID NO:28 and SEQ ID NO:29, SEQ ID NO:30 and SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35, or SEQ ID NO:36 and SEQ ID NO:27, respectively; and the antibody is afucosylated.

[0018] In some embodiments, the antibody is a (Fab')₂.

[0019] In some embodiments the antibody is PEGylated.

[0020] In some embodiments, the antibody activates EphA3.

[0021] In some embodiments, the antibody does not compete with a natural ligand, *e.g.*, ephrin A5, for binding to EphA3.

5 [0022] In another aspect, the invention provides a method of treating a patient that has an EphA3-dependent disease, the method comprising administering an antibody of the invention as described herein to the patient in a therapeutically effective amount. The patient may, *e.g.*, have a cancer. In some embodiments, the antibody is administered to a patient that has a solid tumor that comprises tumor cells that express EphA3. In other embodiments, the
10 antibody is administered to a patient that has a tumor that does not have tumor cells that express EphA3.

[0023] In some embodiments, the antibody is administered to a patient that has a myeloproliferative disorder. In some embodiments, the antibody is administered to a patient that has acute myeloid leukemia or chronic myeloid leukemia. In some some embodiments,
15 the antibody is administered to a patient that has a lymphoma. In some embodiments, the antibody is administered to a patient that has myelodysplastic syndrome, polycythemia vera, essential thrombocythemia, or idiopathic myelofibrosis.

BRIEF DESCRIPTION OF THE DRAWINGS

20 [0024] Figure 1 provides exemplary V_H and V_L sequences of anti-EphA3 antibodies of the invention.

[0025] Figure 2 provides a schematic of a heavy chain CDR2/FR3 cassette construction.

[0026] Figure 3 shows results of an antigen binding ELISA with anti-EphA3 IgGs. ELISA plates coated with EphA3-Fc were treated with chimeric IIIA4 (circles), an exemplary
25 engineered antibody of the invention (squares) or a control IgG (triangles) and probed for binding with an anti-human kappa chain HRP conjugate.

[0027] Figure 4 shows the specificity of an engineered antibody of the invention for EphA3. Various Eph proteins were coated onto an ELISA plate, treated with engineered antibody and probed for binding with an anti-human kappa chain HRP conjugate.

[0028] Figure 5 shows data from flow cytometric analysis of three cell lines in an experiment evaluated the ability of an exemplary engineered anti-EphA3 antibody of the invention to bind to cell surface expressed EphA3. B16, SKmel28, and LnCAP cells were blocked with 2% BSA and rat IgG and probed with engineered anti-EphA3 IgG, chimeric IIIA4 IgG or an isotype control IgG. Bound antibody was detected by an anti-human IgG Phycoerythrin conjugate using a FACS Caliber flow cytometry (BD). Dead cells were excluded by propidium iodide staining. For each graph, the control antibody profile is the left-most curve shown in the graph.

[0029] Figure 6 provides data showing that an afucosylated anti-EphA3 antibody has enhanced ADCC activity against AML cells compared with the fucosylated anti-EphA3 antibody.

DETAILED DESCRIPTION OF THE INVENTION

[0030] As used herein "EphA3" refers to the Eph receptor A3. This receptor has also been referred to as "Human embryo kinase", "hek", "eph-like tyrosine kinase 1", "etk1" or "tyro4". EphA3 belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family. EPH and EPH-related receptors have been implicated in mediating developmental events. Receptors in the EPH subfamily typically have a single kinase domain and an extracellular region containing a Cys-rich domain and 2 fibronectin type III repeats. The ephrin receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. EphA3 binds ephrin-A ligands. EphA3 nucleic acid and protein sequences are known. An exemplary human EphA3 amino acid sequence is available under accession number (EAW68857).

[0031] In the present invention, "activation" of EphA3 causes phosphorylation of EphA3. An antibody that activates EphA3, *i.e.*, causes phosphorylation of EphA3, is therefore considered to be an agonist in the context of this invention. EphA3 can be activated by dimerization. Such activation can lead to phosphorylation and apoptosis, although not necessarily to cell rounding. Activation, *e.g.*, when clustering of EphA3 occurs, can additionally lead to morphological changes, typically rounding of the cell.

[0032] In the present invention, "EphA3 antibody" or "anti-EphA3 antibody" are used interchangeably to refer to an antibody that specifically binds to EphA3. In some embodiments, the antibody can dimerize EphA3. The term encompasses antibodies that bind

to EphA3 in the presence of ephrin ligand (*e.g.*, ephrin A5) binding, as well as antibodies that bind to the ligand binding site and compete with ligand binding to EphA3.

[0033] An "EphA3 antibody that binds to EphA3 in the presence of binding of an ephrin ligand" refers to an antibody that does not significantly prevent binding of an ephrin ligand, such as ephrin A5, to EphA3. The presence of such an antibody in a binding reaction comprising EphA3 and an ephrin ligand, *e.g.*, ephrin A5, reduces ephrin ligand binding to EphA3 by less than about 30%, typically less than 20% or 10%.

[0034] The term "mAb IIIA4" refers to monoclonal antibody IIIA4 that was originally raised against LK63 human acute pre-B leukemia cells to affinity isolate EphA3 (Boyd, *et al. J Biol Chem* 267:3262-3267, 1992). mAb IIIA4 binds to the native EphA3 globular ephrin-binding domain (*e.g.*, Smith, *et al., J. Biol. Chem* 279:9522-9531, 2004). It is deposited in the European Collection of Animal Cell Cultures under accession no. 91061920 (*see, e.g.*, EP patent no. EP0590030).

[0035] An "antibody having an active isotype" as used herein refers to an antibody that has a human Fc region that binds to an Fc receptor present on immune effector cells. "Active isotypes" include IgG1, IgG3, IgM, IgA, and IgE. The term encompasses antibodies that have a human Fc region that comprises modifications, such as mutations or changes to the sugar composition and/or level of glycosylation, that modulate Fc effector function.

[0036] An "Fc region" refers to the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus, Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains C γ 2 and C γ 3 and the hinge between C γ 1 and C γ . It is understood in the art that the boundaries of the Fc region may vary, however, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, using the numbering is according to the EU index as in Kabat *et al.* (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, Va.). The term "Fc region" may refer to this region in isolation or this region in the context of an antibody or antibody fragment. "Fc region " includes naturally occurring allelic variants of the Fc region as well as modifications that modulate effector function. Fc regions also include variants that don't result in alterations to biological function. For example, one or more amino acids can be

deleted from the N-terminus or C-terminus of the Fc region of an immunoglobulin without substantial loss of biological function. Such variants can be selected according to general rules known in the art so as to have minimal effect on activity (see, *e.g.*, Bowie, *et al.*, *Science* 247:306-1310, 1990).

- 5 **[0037]** The term “equilibrium dissociation constant” or “affinity” abbreviated (K_D), refers to the dissociation rate constant (k_d , time^{-1}) divided by the association rate constant (k_a , $\text{time}^{-1} \text{M}^{-1}$). Equilibrium dissociation constants can be measured using any known method in the art. The antibodies of the present invention are high affinity antibodies. Such antibodies have a monovalent affinity better (less) than about about 50 nM and often less than about 10
- 10 nM as determined by surface plasmon resonance analysis performed at 37°C. Thus, in some embodiments, the antibodies of the invention have an affinity (as measured using surface plasmon resonance) of less than about 50 nM, typically less than about 25 nM, or even less than 10 nM, *e.g.*, about 5 nM or about 1 nM. In the context of the invention, an affinity is “better” if it has a higher affinity, *e.g.*, as evidenced by a lower numerical K_D .
- 15 **[0038]** The phrase “specifically (or selectively) binds” to an antibody or “specifically (or selectively) immunoreactive with,” when referring to a protein or peptide, refers to a binding reaction where the antibody binds to the protein of interest. In the context of this invention, the antibody typically binds to EphA3 with an affinity that is at least 100-fold greater than its affinity for other antigens.
- 20 **[0039]** As used herein, an “antibody” refers to a protein functionally defined as a binding protein and structurally defined as comprising an amino acid sequence that is recognized by one of skill as being derived from the framework region of an immunoglobulin encoding gene of an animal producing antibodies. An antibody can consist of one or more
- 25 polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.
- 30 **[0040]** A typical immunoglobulin (antibody) structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kD) and one “heavy” chain (about 50-70 kD). The N-terminus

of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0041] The term "antibody" as used herein includes antibody fragments that retain binding specificity. For example, there are a number of well characterized antibody fragments. Thus, for example, pepsin digests an antibody C-terminal to the disulfide linkages in the hinge region to produce F(ab')₂, a dimer of Fab which itself is a light chain joined to V_H-CH1 by a disulfide bond. The F(ab')₂ may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the (Fab')₂ dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (see, Fundamental Immunology, W.E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that fragments can be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized using recombinant DNA methodologies.

[0042] Antibodies include V_H-V_L dimers, including single chain antibodies (antibodies that exist as a single polypeptide chain), such as single chain Fv antibodies (sFv or scFv) in which a variable heavy and a variable light region are joined together (directly or through a peptide linker) to form a continuous polypeptide. The single chain Fv antibody is a covalently linked V_H-V_L which may be expressed from a nucleic acid including V_H- and V_L- encoding sequences either joined directly or joined by a peptide-encoding linker (*e.g.*, Huston, *et al. Proc. Nat. Acad. Sci. USA*, 85:5879-5883, 1988). While the V_H and V_L are connected to each as a single polypeptide chain, the V_H and V_L domains associate non-covalently.

Alternatively, the antibody can be another fragment. Other fragments can also be generated, *e.g.*, using recombinant techniques, as soluble proteins or as fragments obtained from display methods. Antibodies can also include diantibodies and miniantibodies. Antibodies of the invention also include heavy chain dimers, such as antibodies from camelids.

[0043] As used herein, "V-region" refers to an antibody variable region domain comprising the segments of Framework 1, CDR1, Framework 2, CDR2, and Framework 3, including CDR3 and Framework 4, which segments are added to the V-segment as a consequence of rearrangement of the heavy chain and light chain V-region genes during B-cell

differentiation. A "V-segment" as used herein refers to the region of the V-region (heavy or light chain) that is encoded by a V gene. The V-segment of the heavy chain variable region encodes FR1-CDR1-FR2-CDR2 and FR3. For the purposes of this invention, the V-segment of the light chain variable region is defined as extending though FR3 up to CDR3.

5 [0044] As used herein, the term "J-segment" refers to a subsequence of the variable region encoded comprising a C-terminal portion of a CDR3 and the FR4. A germline J-segment is encoded by an immunoglobulin J-gene segment.

[0045] As used herein, "complementarity-determining region (CDR)" refers to the three hypervariable regions in each chain that interrupt the four "framework" regions established
10 by the light and heavy chain variable regions. The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found,
15 whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found.

[0046] The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and
20 align the CDRs in three dimensional space.

[0047] The amino acid sequences of the CDRs and framework regions can be determined using various well known definitions in the art, *e.g.*, Kabat, Chothia, international ImMunoGeneTics database (IMGT), and AbM (*see, e.g.*, Johnson *et al.*, *supra*; Chothia & Lesk, 1987, Canonical structures for the hypervariable regions of immunoglobulins. *J. Mol. Biol.* 196, 901-917; Chothia C. *et al.*, 1989, Conformations of immunoglobulin hypervariable regions. *Nature* 342, 877-883; Chothia C. *et al.*, 1992, structural repertoire of the human VH segments *J. Mol. Biol.* 227, 799-817; Al-Lazikani *et al.*, *J.Mol.Biol* 1997, 273(4)).
25 Definitions of antigen combining sites are also described in the following: Ruiz *et al.*, IMGT, the international ImMunoGeneTics database. *Nucleic Acids Res.*, 28, 219-221 (2000); and Lefranc, M.-P. IMGT, the international ImMunoGeneTics database. *Nucleic Acids Res.* Jan 1;29(1):207-9 (2001); MacCallum *et al.*, Antibody-antigen interactions: Contact analysis and binding site topography, *J. Mol. Biol.*, 262 (5), 732-745 (1996); and Martin *et al.*, *Proc. Natl*

Acad. Sci. USA, 86, 9268–9272 (1989); Martin, *et al*, *Methods Enzymol.*, 203, 121–153, (1991); Pedersen *et al*, *Immunomethods*, 1, 126, (1992); and Rees *et al*, In Sternberg M.J.E. (ed.), *Protein Structure Prediction*. Oxford University Press, Oxford, 141–172 (1996).

[0048] “Epitope” or “antigenic determinant” refers to a site on an antigen to which an antibody binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., *Epitope Mapping Protocols in Methods in Molecular Biology*, Vol. 66, Glenn E. Morris, Ed (1996).

[0049] As used herein, “chimeric antibody” refers to an immunoglobulin molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region, or portion thereof, having a different or altered antigen specificity; or with corresponding sequences from another species or from another antibody class or subclass.

[0050] As used herein, “humanized antibody” refers to an immunoglobulin molecule in which CDRs from a donor antibody are grafted onto human framework sequences. Humanized antibodies may also comprise residues of donor origin in the framework sequences. The humanized antibody can also comprise at least a portion of a human immunoglobulin constant region. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. Humanization can be performed using methods known in the art (e.g., Jones *et al.*, *Nature* 321:522-525; 1986; Riechmann *et al.*, *Nature* 332:323-327, 1988; Verhoeven *et al.*, *Science* 239:1534-1536, 1988); Presta, *Curr. Op. Struct. Biol.* 2:593-596, 1992; U.S. Patent No. 4,816,567), including techniques such as “superhumanizing” antibodies (Tan *et al.*, *J. Immunol.* 169:

1119, 2002) and "resurfacing" (e.g., Staelens *et al.*, *Mol. Immunol.* 43: 1243, 2006; and Roguska *et al.*, *Proc. Natl. Acad. Sci USA* 91: 969, 1994).

[0051] A "HumaneeredTM" antibody in the context of this invention refers to an engineered human antibody having a binding specificity of a reference antibody. A "HumaneeredTM" antibody for use in this invention has an immunoglobulin molecule that contains minimal sequence derived from a donor immunoglobulin. Typically, an antibody is "HumaneeredTM" by joining a DNA sequence encoding a binding specificity determinant (BSD) from the CDR3 region of the heavy chain of the reference antibody to human V_H segment sequence and a light chain CDR3 BSD from the reference antibody to a human V_L segment sequence. Methods for humaneering are provided in US patent application publication no. 20050255552 and US patent application publication no. 20060134098.

[0052] The term "binding specificity determinant" or "BSD" as used in the context of the current invention refers to the minimum contiguous or non-contiguous amino acid sequence within a CDR region necessary for determining the binding specificity of an antibody. In the current invention, the minimum binding specificity determinants reside within a portion or the full-length of the CDR3 sequences of the heavy and light chains of the antibody.

[0053] A "human" antibody as used herein encompasses humanized and HumaneeredTM antibodies, as well as human monoclonal antibodies that are obtained using known techniques.

[0054] A "hypofucosylated" antibody preparation refers to an antibody preparation in which less than 50% of the oligosaccharide chains contain α 1,6-fucose. Typically, less than about 40%, less than about 30%, less than about 20%, less than about 10%, or less than 5% or less than 1% of the oligosaccharide chains contain α 1,6-fucose in a "hypofucosylated" antibody preparation.

[0055] An "afucosylated" antibody lacks α 1,6-fucose in the carbohydrate attached to the CH2 domain of the IgG heavy chain.

[0056] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not normally found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences, e.g., from unrelated genes arranged to make a new functional nucleic acid. Similarly, a heterologous protein will often

refer to two or more subsequences that are not found in the same relationship to each other in nature.

[0057] The term “recombinant” when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, *e.g.*, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all. By the term “recombinant nucleic acid” herein is meant nucleic acid, originally formed in vitro, in general, by the manipulation of nucleic acid, *e.g.*, using polymerases and endonucleases, in a form not normally found in nature. In this manner, operably linkage of different sequences is achieved. Thus an isolated nucleic acid, in a linear form, or an expression vector formed in vitro by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, *i.e.*, using the in vivo cellular machinery of the host cell rather than in vitro manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention. Similarly, a “recombinant protein” is a protein made using recombinant techniques, *i.e.*, through the expression of a recombinant nucleic acid as depicted above.

[0058] An EphA3-dependent disease, as used herein, refers to a disease in which a cell that expresses EphA3 is a target for therapy to treat the disease.

[0059] The term “vasculogenic bone marrow precursor cells” refers to bone-marrow-derived endothelial precursors and/or circulating endothelial cell precursor cells.

[0060] The term “cancer cell” or “tumor cell” as used herein refers to a neoplastic cell. The term includes cancer cells that are benign as well as malignant. Neoplastic transformation is associated with phenotypic changes of the tumor cell relative to the cell type from which it is derived. The changes can include loss of contact inhibition, morphological changes, and aberrant growth. (see, Freshney, *Culture of Animal Cells a Manual of Basic Technique* (3rd edition, 1994).

[0061] "Inhibiting growth of a cancer" in the context of the invention refers to slowing growth and/or reducing the cancer cell burden of a patient that has cancer. "Inhibiting growth of a cancer" thus includes killing cancer cells as well as slowing or arresting cancer cell growth.

- 5 [0062] As used herein, "therapeutic agent" refers to an agent that when administered to a patient suffering from a disease, in a therapeutically effective dose, will cure, or at least partially arrest the symptoms of the disease and complications associated with the disease.

[0063] The terms "identical" or percent "identity," in the context of two or more polypeptide (or nucleic acid) sequences, refer to two or more sequences or subsequences that
10 are the same or have a specified percentage of amino acid residues (or nucleotides) that are the same (i.e., about 60% identity, preferably 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default
15 parameters described below, or by manual alignment and visual inspection (*see, e.g.*, NCBI web site). Such sequences are then said to be "substantially identical." "Substantially identical" sequences also includes sequences that have deletions and/or additions, as well as those that have substitutions, as well as naturally occurring, *e.g.*, polymorphic or allelic variants, and man-made variants. As described below, the preferred algorithms can account
20 for gaps and the like. Preferably, protein sequence identity exists over a region that is at least about 25 amino acids in length, or more preferably over a region that is 50-100 amino acids = in length, or over the length of a protein.

[0064] A "comparison window", as used herein, includes reference to a segment of one of the number of contiguous positions selected from the group consisting typically of from 20 to
25 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981),
30 by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT,

FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology (Ausubel et al., eds. 1995 supplement)).

[0065] Preferred examples of algorithms that are suitable for determining percent sequence identity and sequence similarity include the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990). BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0066] An indication that two polypeptides are substantially identical is that the first polypeptide is immunologically cross-reactive with the antibodies raised against the second polypeptide. Thus, a polypeptide is typically substantially identical to a second polypeptide, e.g., where the two peptides differ only by conservative substitutions.

[0067] The terms “isolated,” “purified,” or “biologically pure” refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. The term “purified” in some embodiments denotes that a protein gives rise to essentially one band in an electrophoretic gel. Preferably, it means that the protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

[0068] The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers, those containing modified residues, and non-naturally occurring amino acid polymer.

[0069] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ -

5 carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, e.g., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs may have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic
10 chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions similarly to a naturally occurring amino acid.

[0070] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical
15 Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0071] “Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino
20 acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical or associated, e.g., naturally contiguous, sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode most proteins. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can
25 be altered to another of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes silent variations of the nucleic acid. One of skill will recognize that in certain contexts each codon in a nucleic acid (except AUG, which is ordinarily the
30 only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, often silent variations of a nucleic acid which encodes a polypeptide is implicit in a described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0072] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention. Typically conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

[0073] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

I. Introduction

[0074] The invention relates to antibodies that bind to EphA3 with high affinity and typically, activate EphA3. The antibodies comprise variable regions with a high degree of amino acid sequence identity to human germ-line V_H and V_L sequences. In preferred embodiments, the CDRH3 of an antibody of the invention comprises the amino acid sequence X₁GX₂YE(X₃)FDX₄, wherein X₁ is S or G, X₂ is Y or V, X₃ is E or D, and X₄ is S, V, or I, with the proviso that the amino acid sequence is not SGYYEDFDS. In some embodiments, CDRL3 of an antibody of the invention comprises the amino acid sequence X₁X₂YX₃X₄YPYT, wherein X₁ is V or A, X₂ is Q, R, or G, X₃ is A, S, or L, and X₄ is N or K.

[0075] Typically, the portion of the CDR3 excluding the BSD and the complete FR4 are comprised of human germ-line sequences. In some embodiments, the CDR3-FR4 sequence excluding the BSD differs from human germ-line sequences by not more than 2 amino acids on each chain. In some embodiments, the J-segment comprises a human germline J-segment. Human germline sequences can be determined, for example, through the publicly available international ImMunoGeneTics database (IMGT) and V-base (on the worldwide web at vbase.mrc-cpe.cam.ac.uk).

[0076] The human germline V-segment repertoire consists of 51 heavy chain V-regions, 40 κ light chain V-segments, and 31 λ light chain V-segments, making a total of 3,621

germline V-region pairs. In addition, there are stable allelic variants for most of these V-segments, but the contribution of these variants to the structural diversity of the germline repertoire is limited. The sequences of all human germ-line V-segment genes are known and can be accessed in the V-base database, provided by the MRC Centre for Protein

5 Engineering, Cambridge, United Kingdom (see, also Chothia et al., 1992, J Mol Biol 227:776-798; Tomlinson et al., 1995, EMBO J 14:4628-4638; and Williams et al., 1996, J Mol Biol 264:220-232).

[0077] Antibodies or antibodies fragments as described herein can be expressed in prokaryotic or eukaryotic microbial systems or in the cells of higher eukaryotes such as
10 mammalian cells.

[0078] An antibody that is employed in the invention can be in any format. For example, in some embodiments, the antibody can be an including an constant region, e.g., a human constant region, *e.g.*, an intact Ig, a Fab, Fab', F(ab')₂ or a fragment of an intact immunoglobulin, *e.g.*, an scFv or Fv.

15 II. Heavy Chains

[0079] A heavy chain of an anti-EphA3 antibody of the invention comprises a heavy-chain V-region that comprises the following elements:

1) human heavy-chain V-segment sequences comprising FR1-CDR1-FR2-CDR2-
FR3

20 2) a CDRH3 region comprising the amino acid sequence X₁GX₂YEX₃FDX₄, wherein X₁ is S or G, X₂ is Y or V, X₃ is E or D, and X₄ is S, V, or I, with the proviso that the amino acid sequence is not SGYYEDFDS; and

3) a FR4 contributed by a human germ-line J-gene segment.

[0080] In some embodiment, the CDR3 comprises GGYIEDFDS, SGYYEEFDS,
25 SGVYEDFDS, SGYYEDFDV, or SGYYEDFDI.

[0081] The V-segment typically has at least 80% identity, or 85%, 90%, 95%, or greater identity to a human germline V-segment, *e.g.*, a human VH1 subclass. Thus, in some embodiments, the V-segment is a human V_{H1} 1-02 segment that at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or greater, identity to the germ-line segment VH1 1-02. In
30 some embodiments, the V-segment differs by not more than 15 residues from VH1 1-02 and preferably not more than 10 residues.

[0082] In some embodiments, an antibody of the invention comprises a V-segment that has at least 90% identity, or at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the germ-line segment VH 1-02; or to one of the V-segments of the V_H regions shown in Figure 1.

5 [0083] The FR4 sequence of the antibodies of the invention is provided by a human JH1, JH3, JH4, JH5 or JH6 gene germline segment, or a sequence that has a high degree of amino-acid sequence identity, *e.g.*, at least 90% or 95% identity, or differs at not more than 3, typically at not more than 2 amino acid residues in comparison to a human germline JH segment. In some embodiments, the J segment is from a human germline JH6 sequence and
10 the FR4 has the sequence WGQGTTVTVSS.

[0084] In some embodiments, the V-segment of the V_H region has a CDR1 and/or CDR2 as shown in Figure 1. For example, an antibody of the invention may have a CDR1 that has the sequence GYWMN, TYWIS, or SYWIN. An antibody of the invention may have a CDR2 that has the sequence DIYPGSGNTNYDEKFQG, DIYPGSGNTNYAQKFQG,
15 DIYPGSGNTNYAQEFRG, DIYPGSGNTNYAQKFLG, or DIYPGSGNTNYDEKFKR. Thus, in some embodiments, an anti-EphA3 antibody of the invention may have a V_H region CDR3 that has the sequence GGYIEDFDS, SGYYEEFDS, SGVYEDFDS, SGYYEDFDV, or SGYYEDFDI, and a CDR1 sequence GYWMN, TYWIS, or SYWIN and a CDR2 DIYPGSGNTNYDEKFQG, DIYPGSGNTNYAQKFQG, DIYPGSGNTNYAQEFRG,
20 DIYPGSGNTNYAQKFLG, or DIYPGSGNTNYDEKFKR.

[0085] In some embodiment, an anti-EphA3 antibody of the invention may have a V_H region CDR3 that has the sequence SGYYEDFDS and a CDR1 and/or a CDR2 of a V_H region set forth in Figure 1.

[0086] In some embodiments, a V_H region V-segment of an antibody of the invention has a
25 V-segment sequence shown in Figure 1.

[0087] In typical embodiment, an antibody of the invention has a V_H region sequence set forth in Figure 1.

III. Light chains

[0088] A light chain of an anti-EphA3 antibody of the invention comprises at light-chain
30 V-region that comprises the following elements:

1) human light-chain V-segment sequences comprising FR1-CDR1-FR2-CDR2-FR3

2) a CDRL3 region that has the sequence CDR3 that comprises the sequence $X_1X_2YX_3X_4PYT$, wherein X_1 is V or A; X_2 is Q, R, or G; X_3 is A, S, or L; and X_4 is N or K;

and

3) a FR4 contributed by a human germ-line J-gene segment.

[0089] In some embodiment, the CDR3 has a sequence $X_1X_2YX_3X_4YPYT$, wherein X_1 is V, X_2 is Q, X_3 is A, and X_4 is N. In some embodiments the V_L CDR3 is GQYANYPYT, VQYAKYPYT, AQYANYPYT, VQYSNYPYT, VQYANYPYT, VGYANYPYT, VRYANYPYT, or VQYLNYPYT.

[0090] The V_L region comprises either a V_{λ} or a V_{κ} V-segment. An example of a V_{κ} sequence that supports binding in combination with a complementary V_H -region is provided in Figure 1.

[0091] The V_{κ} segments may be of any subclass, *e.g.* and is often of the $V_{\kappa I}$ sub-class.

In some embodiments, the segments have at least 80% sequence identity to a human germline $V_{\kappa I}$ *e.g.*, at least 80% identity to the human germ-line $V_{\kappa I}$ L15 sequence. In some embodiments, the V_{κ} segment may differ by not more than 5 residues from $V_{\kappa I}$ L15. In other embodiments, the V_L region V-segment of an antibody of the invention has at least 85% identity, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the human kappa V-segment sequence of a V_L region shown in Figure 1.

[0092] The FR4 sequence of the V_L region of an antibody of the invention is provided by a human germline J segment, *e.g.* or a sequence that has a high degree of amino-acid sequence identity to a human germline J segment. In some embodiments, the J segment is a human germline $J_{\kappa 2}$ sequence and the FR4 of the antibody has the sequence FGQGKLEIK.

[0093] In some embodiments, the V-segment of the V_L region has a CDR1 and/or CDR2 as shown in Figure 1. Thus, an antibody of the invention may have a CDR1 sequence of RASQGIISYLA, QASQDISTYLN, RASQEISGYLG, or RASQSISSYLA and/or a CDR2 sequence AASSLQS, GASSLQS, AASSLQR, or AASTLDS.

[0094] In particular embodiments, an anti-EphA3 antibody of the invention may have a V_L region CDR1 and a CDR2 in a combination as shown in one of the V-segments of the V_L regions set forth in Figure 1 and a V_L region CDR3 sequence that comprises GQYANYPYT,

VQYAKYPYT, AQYANYPYT, VQYSNYPYT, VQYANYPYT, VGYANYPYT, VRYANYPYT, or VQYLNYPYT. In some embodiments, such an anti-EphA3 antibody may comprise a V_L region FR4 region that is FGQGKLEIK. Thus, a V_L region of an anti-EphA3 antibody of the invention, can comprise, *e.g.*, a CDR3 GQYANYPYT, VQYAKYPYT, AQYANYPYT, VQYSNYPYT, VQYANYPYT, VGYANYPYT, VRYANYPYT, or VQYLNYPYT; a CDR1 sequence RASQGIISYLA, QASQDISTYLN, RASQEISGYLG, or RASQSISSYLA; and a CDR2 sequence AASSLQS, GASSLQS, AASSLQR, or AASTLDS.

[0095] In some embodiments, a V_L region V-segment of an antibody of the invention has a V-segment sequence shown in Figure 1.

[0096] In typical embodiment, an antibody of the invention has a V_L region sequence set forth in Figure 1.

[0097] In some embodiments, an antibody of the invention comprises any one of the V_L regions set forth in SEQ ID NOs:11-23 with any one of the V_H regions set forth in SEQ ID NOs:1-10.

IV. Preparation of EphA3 antibodies

[0098] The affinity of an antibody may be assessed using well known assays to determine binding activity and affinity. Such techniques include ELISA assays as well as binding determinations that employ surface plasmon resonance or interferometry. For example, affinities can be determined by biolayer interferometry using a ForteBio (Mountain View, CA) Octet biosensor.

[0099] Antibodies of the invention typically compete with mIIIA4 for binding to EphA3. The ability of an antibody described herein to block or compete with mIIIA4 for binding to EphA3 indicates that the antibody binds to the same epitope or to an epitope that is close to, *e.g.*, overlapping, with the epitope that is bound by mIIIA4 for binding to EphA3. In other embodiments an antibody described herein, *e.g.*, an antibody comprising a V_H and V_L region combination as shown in the table provided in Figure 1, can be used as a reference antibody for assessing whether another antibody competes for binding to EphA3. A test antibody is considered to competitively inhibit binding of a reference antibody, if binding of the reference antibody to the antigen is reduced by at least 30%, usually at least about 40%, 50%, 60% or 75%, and often by at least about 90%, in the presence of the test antibody. Many

assays can be employed to assess binding, including ELISA, as well as other assays, such as immunoblots.

[0100] In typical embodiments, the antibody is an activating antibody. An antibody may be tested to confirm that the antibody retains the activity of activating EphA3. The activity can be determined using any number of endpoints, including phosphorylation assays, or an indirect endpoint such as apoptosis.

[0101] Methods for the isolation of antibodies with V-region sequences close to human germ-line sequences have previously been described (US patent application publication nos. 20050255552 and 20060134098). Antibody libraries may be expressed in a suitable host cell including mammalian cells, yeast cells or prokaryotic cells. For expression in some cell systems, a signal peptide can be introduced at the N-terminus to direct secretion to the extracellular medium. Antibodies may be secreted from bacterial cells such as *E. coli* with or without a signal peptide. Methods for signal-less secretion of antibody fragments from *E. coli* are described in US patent application 20070020685.

[0102] To generate a EphA3-binding antibody, one of the V_H-regions of the invention, *e.g.*, shown in Figure 1, is combined with one of the V_L-regions of the invention, *e.g.*, shown in Figure 1, and expressed in any of a number of formats in a suitable expression system. Thus the antibody may be expressed as a scFv, Fab, Fab' (containing an immunoglobulin hinge sequence), F(ab')₂, (formed by di-sulfide bond formation between the hinge sequences of two Fab' molecules), whole immunoglobulin or truncated immunoglobulin or as a fusion protein in a prokaryotic or eukaryotic host cell, either inside the host cell or by secretion. A methionine residue may optionally be present at the N-terminus, for example, in polypeptides produced in signal-less expression systems. Each of the V_H-regions described herein may be paired with each of the V_L regions to generate an anti-EphA3 antibody.

[0103] Antibodies may be produced using any number of expression systems, including both prokaryotic and eukaryotic expression systems. In some embodiments, the expression system is a mammalian cell expression, such as a CHO cell expression system. Many such systems are widely available from commercial suppliers. In embodiments in which an antibody comprises both a V_H and V_L region, the V_H and V_L regions may be expressed using a single vector, *e.g.*, in a discistronic expression unit, or under the control of different promoters. In other embodiments, the V_H and V_L region may be expressed using separate

vectors. A V_H or V_L region as described herein may optionally comprise a methionine at the N-terminus.

[0104] An antibody of the invention may be produced in any number of formats, including as a Fab, a Fab', a F(ab')₂, a scFv, or a dAB. An antibody of the invention can also include a human constant region. The constant region of the light chain may be a human kappa or lambda constant region. The heavy chain constant region is often a gamma chain constant region, for example, a gamma-1, gamma-2, gamma-3, or gamma-4 constant region. In other embodiments, the antibody may be an IgA or IgM.

[0105] In some embodiments of the invention, the antibody V_L region, *e.g.*, a V_L region set forth in Figure 1, is combined with a human kappa constant region (*e.g.*, SEQ ID NO:25) to form the complete light-chain.

[0106] In some embodiments of the invention, the V_H region is combined a human gamma-1 constant region. Any suitable gamma-1 allotype can be chosen. Thus, in some embodiments, the antibody is an IgG having a constant region, *e.g.*, SEQ ID NO:24, that has a V_H selected from a V_H region sequence set forth in Figure 1. In some embodiments, the antibody has a V_L selected from the V_L region sequences set forth in Figure 1. In particular embodiments, the antibody has a kappa constant region as set forth in SEQ ID NO:25, and a heavy chain constant region as set forth in SEQ ID NO:24, where the heavy and light chain variable regions comprise one of the following combinations from the sequences set forth in Figure 1.

[0107] In some embodiments, *e.g.*, where the antibody is a fragment, the antibody can be conjugated to another molecule, *e.g.*, polyethylene glycol (PEGylation) or serum albumin, to provide an extended half-life *in vivo*. Examples of PEGylation of antibody fragments are provided in Knight *et al. Platelets* 15:409, 2004 (for abciximab); Pedley *et al., Br. J. Cancer* 70:1126, 1994 (for an anti-CEA antibody); Chapman *et al., Nature Biotech.* 17:780, 1999; and Humphreys, *et al., Protein Eng. Des.* 20: 227,2007).

[0108] In some embodiments, the antibodies of the invention are in the form of a Fab' or a (Fab')₂.

[0109] A full-length light chain is generated by fusion of a V_L-region to human kappa or lambda constant region. Either constant region may be used for any light chain; however, in

typical embodiments, a kappa constant region is used in combination with a Vkappa variable region and a lambda constant region is used with a Vlamba variable region.

[0110] The heavy chain of the Fab' is a Fd' fragment generated by fusion of a V_H-region of the invention to human heavy chain constant region sequences, the first constant (CH1) domain and hinge region. The heavy chain constant region sequences can be from any of the immunoglobulin classes, but is often from an IgG, and may be from an IgG1, IgG2, IgG3 or IgG4. The Fab' antibodies of the invention may also be hybrid sequences, *e.g.*, a hinge sequence may be from one immunoglobulin sub-class and the CH1 domain may be from a different sub-class.

[0111] An antibody that is employed in the invention can be in numerous formats. In some embodiments, the antibody can include an Fc region, *e.g.*, a human Fc region. For example, such antibodies include IgG antibodies that bind EphA3 and that have an active isotype. In some embodiments, the antibody can be an active fragment (*e.g.*, it can dimerize EphA3) or derivative of an antibody such as an Fab, Fab', F(ab')₂, Fv, scFv, or a single domain antibody ("dAb"). For example, in some embodiments, the antibody may be a F(ab')₂. Other exemplary embodiments of antibodies that can be employed in the invention include activating nanobodies or activating camellid antibodies. Such antibodies may additionally be recombinantly engineered by methods well known to persons of skill in the art. As noted above, such antibodies can be produced using known techniques. As appreciated by one of skill in the art, in some embodiments when an antibody is in a format that can be monovalent, *e.g.*, an Fv or Fab format, the antibody may be employed as a multivalent antibody, such as a trivalent or tetravalent antibody. Methods of generating multivalent antibodies are known (*see, e.g., King et al., Cancer Res. 54:6176-6185, 1994*).

[0112] In many embodiments, an antibody for use in the invention has an Fc constant region that has an effector function, *e.g.*, binds to an Fc receptor present on immune effector cells. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor), and the like. Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.* an antibody variable domain) and can be assessed using known assays (*see, e.g., the references cited hereinbelow.*)

[0113] Anti-EphA3 antibodies that have an active isotype and are bound to Fc-receptors on effector cells, such as macrophages, monocytes, neutrophils and NK cells, can induce cell death by ADCC.

[0114] The Fc region can be from a naturally occurring IgG1, or other active isotypes, including IgG3, IgM, IgA, and IgE. "Active isotypes" include antibodies where the Fc region comprises modifications to increase binding to the Fc receptor or otherwise improve the potency of the antibody. Such an Fc constant region may comprise modifications, such as mutations, changes to the level of glycosylation and the like, that increase binding to the Fc receptor. There are many methods of modifying Fc regions that are known in the art. For example, U.S. Patent Application Publication No. 20060039904 describes variants of Fc receptors that have enhanced effector function, including modified binding affinity to one or more Fc ligands (e.g., FcγR, C1q). Additionally, such Fc variants have altered antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) activity. Other Fc variants include those disclosed by Ghetie *et al.*, *Nat Biotech.*

15:637-40, 1997; Duncan *et al.*, *Nature* 332:563-564, 1988; Lund *et al.*, *J. Immunol* 147:2657-2662, 1991; Lund *et al.*, *Mol Immunol* 29:53-59, 1992; Alegre *et al.*, *Transplantation* 57:1537-1543, 1994; Hutchins *et al.*, *Proc Natl. Acad Sci USA* 92:11980-11984, 1995; Jefferis *et al.*, *Immunol Lett.* 44:111-117, 1995; Lund *et al.*, *FASEB J* 9:115-119, 1995; Jefferis *et al.*, *Immunol Lett* 54:101-104, 1996; Lund *et al.*, *J Immunol* 157:4963-4969, 1996; Armour *et al.*, *Eur J Immunol* 29:2613-2624, 1999; Idusogie *et al.*, *J Immunol* 164:4178-4184, 2000; Reddy *et al.*, *J Immunol* 164:1925-1933, 2000; Xu *et al.*, *Cell Immunol* 200:16-26, 2000; Idusogie *et al.*, *J Immunol* 166:2571-2575, 2001; Shields *et al.*, *J Biol Chem* 276:6591-6604, 2001; Jefferis *et al.*, *Immunol Lett* 82:57-65, 2002; Presta *et al.*, *Biochem Soc Trans* 30:487-490, 2002; Lazar *et al.*, *Proc. Natl. Acad. Sci. USA* 103:4005-4010, 2006; U.S. Pat. Nos. 5,624,821; 5,885,573; 5,677,425; 6,165,745; 6,277,375; 5,869,046; 6,121,022; 5,624,821; 5,648,260; 6,194,551; 6,737,056; 6,821,505; 6,277,375; 7,335,742; and 7,317,091; and PCT Publications WO 94/2935; WO 99/58572; WO 00/42072; WO 02/060919, and WO 04/029207.

[0115] Glycosylation is a form of post-translational modification by which carbohydrates (sugars) are enzymatically linked to macromolecules to produce glycans. In the context of the present invention, carbohydrates are typically attached to antibody Fc region via one or more N-linkages (through a nitrogen of asparagine or arginine side chains); however, O-linkages (via hydroxy oxygen of serine, threonine, tyrosine, hydroxylysine, or hydroxyproline

side chains) are also possible. Generally, IgG antibodies have a conserved N-linked glycosylation site in the CH2 domain at residue Asn297, while some classes and subclasses also have O-linked sugars, often in the hinge region, e.g. IgD and IgA of some species. The sugars are typically complex, high-mannose, branched sugars; in the case of N-linkages, the sugar that attaches directly to the amino acid sidechain nitrogen is typically N-acetyl glucosamine.

[0116] In some embodiments, the glycosylation of Fc regions may be modified. For example, a modification may be aglycosylation, for example, by altering one or more sites of glycosylation within the antibody sequence. Such an approach is described in further detail in U.S. Pat. Nos. 5,714,350 and 6,350,861. An Fc region can also be made that has an altered type of glycosylation, such as a hypofucosylated Fc variant having reduced amounts of fucosyl residues, or an afucosylated Fc variant lacking fucosyl residues, or an Fc variant having increased bisecting GlcNAc structures. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery, including rat myeloma cells as well as yeast and plants, have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation.

Umana *et al*, *Nat. Biotechnol* 17:176-180, 1999, which describes bisected GlcNAc resulting in 10 times ADCC. Umana notes that such bisected molecules result in less fucosylation. Davies, *et al.*, *Biotechnol. Bioeng.* 74:288-294, 2001 describe CHO cells with inserted enzyme β 1-4-N-acetylglucosaminyltransferase III (GnTIII) (which causes the bisected GlcNAc structure) resulting in increased ADCC of anti-CD20 antibodies. U.S. Patent No. (Umana) 6,602,684 describes cells engineered to produce bisecting GlcNAc glycoproteins.

[0118] Examples of methods to reduce fucosylation of an antibody preparation are provided in Shields *et al*, *J Biol Chem* 277:26733-26740, 2002, which describes CHO cells (Lec13) deficient in fucosylation to produce IgG1 and further describes that binding of the fucose-deficient IgG1 to human Fc γ RIIIA was improved up to 50-fold and increased ADCC. In addition, Shinkawa *et al.*, *J Biol Chem* 278:3466-3473, 2003; compare IgG produced in YB2/0 and CHO cells. The YB2/0 cells have decreased fucosylation and increased bisecting GlcNAc content. Niwa *et al.*, *Clin. Cancer Res.* 1-:6248-6255, 2004 compare anti-CD20 antibodies with antibodies made in YB2/0 cells (low fucosylation) and

observed enhanced ADCC in the latter. Examples of techniques to produce afucosylated antibodies are provided, for example, in Kanda *et al*, *Glycobiology* 17:104-118, 2006. U.S. Patent Nos. 6,946,292 (Kanda) describes fucosyltransferase knock-out cells to produce afucosylated antibodies. U.S. Patent 7,214,775 and WO 00/61739 describe antibody preparations in which 100% of the antibodies are afucosylated.

[0119] Other techniques to modify glycosylation are also known. See, for example, U.S. Patent Application Publication Nos. 20070248600; 20070178551 (GlycoFi technology methods employing engineered lower eukaryotic cells (yeast) to produce "human" glycosylation structures); 20080060092 (Biolex technology methods employing engineered plants to produce "human" glycosylation structures); 20060253928 (which also described engineering of plants to produce "human" antibodies).

[0120] Additional techniques for reducing fucose include ProBioGen technology (von Horsten *et al.*, *Glycobiology*, (advance access publication July 23, 2010); Potelligent™ technology (Biowa, Inc. Princeton, N.J.); and GlycoMAb™ glycosylation engineering technology (GLYCART biotechnology AG, Zurich, Switzerland).

[0121] The N-linked oligosaccharide content of an antibody can be analyzed by methods known in the art. The following is an example of such a method: Antibodies are subjected to digestion with the enzyme N-glycosidase F (Roche; TaKaRa). Released carbohydrates are analyzed by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with positive ion mode (Papac *et al.*, *Glycobiol.* 8: 445-454, 1998). Monosaccharide composition is then characterized by modified high-performance anion exchange chromatography (HPAEC) (Shinkawa *et al.*, *J. Biol. Chem.* 278: 3466-3473, 2003).

[0122] In some embodiments of the invention, the antibody is additionally engineered to reduce immunogenicity, *e.g.*, so that the antibody is suitable for repeat administration.

Methods for generating antibodies with reduced immunogenicity include humanization and humanizing procedures and modification techniques such as de-immunization, in which an antibody is further engineered, *e.g.*, in one or more framework regions, to remove T cell epitopes.

[0123] In some embodiments, antibodies are employed in a form that can activate EphA3 present on the surface of EphA3-expressing cells, *e.g.*, vasculogenic bone marrow precursor cells, or that can kill such cells by ADCC. Thus, in some embodiments an antibody is

dimeric. In other embodiments, the antibody may be in a monomeric form that has an active isotype. In some embodiments the antibody is in a multivalent form, *e.g.*, a trivalent or tetravalent form, that can cross-link EphA3.

V. Administration of anti-EphA3 antibodies for the treatment of diseases in which EphA3 is a target.

[0124] The invention also provides methods of treating a patient that has a disease in which it is desirable to kill EphA3-expressing cells. In some embodiments, such a disease may be a neoplastic disease. Accordingly, in one aspect, the invention provides a method of treating a

neoplastic disease using an antibody of the invention where the method comprises administering an anti-EphA3 antibody of the invention to a patient (that has a tumor) to inhibit tumor growth. Tumors that can be treated include tumors of the breast, lung, colon, stomach, liver, kidney, ovary, esophagus, and prostate, and the others. A solid tumor treated with an antibody of the invention can therefore be a breast carcinoma, lung carcinoma, prostate carcinoma, gastric carcinoma, esophageal carcinoma, colorectal carcinoma, liver carcinoma, ovarian carcinoma, vulval carcinoma, kidney carcinoma, cervical carcinoma, endometrial carcinoma, choriocarcinoma, head and neck cancer, nasopharyngeal carcinoma, laryngeal carcinoma, hepatoblastoma, Kaposi's sarcoma, melanoma, skin carcinoma, hemangioma, cavernous hemangioma, hemangioblastoma, pancreatic carcinoma, retinoblastoma, astrocytoma, glioblastoma, Schwannoma, oligodendroglioma, medulloblastoma, neuroblastomas, sarcomas include fibrosarcomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcoma, urinary tract carcinoma, thyroid carcinoma, and Wilm's tumor. Abnormal vascular proliferation associated with phakomatoses, and edema (such as that associated with brain tumors) can also be treated with an antibody of the invention.

[0125] In some embodiments, the anti-EphA3 antibody is administered to a patient that expresses EphA3 on the surface of the tumor cells. In some embodiments, the anti-EphA3 is administered to a patient that expresses EphA3 on the endothelium of blood vessels in the tumor. In some embodiments, the patient may express EphA3 on both the tumor cell surface and the endothelium. In some embodiments, the antibody is in a format as described, *e.g.*, in WO/2008/112192.

[0126] In some embodiments, a non-neoplastic condition is treated using an antibody of the invention. The non-neoplastic condition is selected from the group consisting of undesired or aberrant hypertrophy, arthritis, rheumatoid arthritis (RA), psoriasis, psoriatic plaques, sarcoidosis, atherosclerosis, atherosclerotic plaques, edema from myocardial infarction, diabetic and other proliferative retinopathies, retrolental fibroplasia, neovascular glaucoma, age-related macular degeneration, diabetic macular edema, corneal neovascularization, corneal graft neovascularization, corneal graft rejection, retinal/choroidal neovascularization, neovascularization of the angle (rubeosis), ocular neovascular disease, vascular restenosis, arteriovenous malformations (AVM), meningioma, hemangioma, angiofibroma, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, chronic inflammation, lung inflammation, acute lung injury/ARDS, sepsis, primary pulmonary hypertension, malignant pulmonary effusions, cerebral edema (e.g., associated with acute stroke/ closed head injury/ trauma), synovial inflammation, pannus formation in RA, myositis ossificans, hypertrophic bone formation, osteoarthritis (OA), refractory ascites, polycystic ovarian disease, endometriosis, 3rd spacing of fluid diseases (pancreatitis, compartment syndrome, burns, bowel disease), uterine fibroids, premature labor, chronic inflammation such as IBD (Crohn's disease and ulcerative colitis), renal allograft rejection, inflammatory bowel disease, nephrotic syndrome, undesired or aberrant tissue mass growth (non-cancer), obesity, adipose tissue mass growth, hemophilic joints, hypertrophic scars, inhibition of hair growth, Osier- Weber syndrome, pyogenic granuloma retrolental fibroplasias, scleroderma, trachoma, vascular adhesions, synovitis, dermatitis, preeclampsia, ascites, pericardial effusion, and pleural effusion.

[0127] In some embodiments, an antibody of the invention is administered to a patient suffering from a myeloproliferative disorder. In some embodiments, the myeloproliferative disorder is acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, polycythemia vera (PV), essential thrombocythemia (ET), or idiopathic myelofibrosis (IM). In some embodiments, an anti-EphA3 antibody of the invention can be used in combination with one or more additional therapeutic agents to treat a patient that has chronic myeloid leukemia where leukemic stem cells from the patient express EphA3. Such therapeutic agents include various chemotherapeutic agents and imatinib mesylate (GLEEVEC®).

[0128] The antibody may be administered alone, or in combination with other therapies to treat the disease of interest. In some embodiments, the anti-EphA3 antibody is administered in combination with a Bv8 antagonist, e.g., a Bv8 antibody antagonist. Bv8 antagonists are

known (*see, e.g.*, WO 2009039337 and the references relating to Bv8 antagonists cited therein). For example, Bv8 antagonists include, antibodies and antibody fragments specifically binding to a native sequence Bv8 polypeptide, or a native sequence Bv8 receptor (PKR-1 /EG-VEGFR1 or PKR-2/EG-VEGFR2) polypeptide. In some embodiments, a patient
5 may also be treated with additional therapeutic agents, including a VEGF antagonist, *e.g.*, an anti-VEGF antibody antagonist, as well as other therapeutic agents, examples of which are additionally described below.

[0129] In some embodiments, the anti-EphA3 antibody is administered to a patient that has previously been treated with a VEGF antagonist, *e.g.*, an anti-VEGF antibody antagonist. In
10 some embodiments, the tumor may be refractory to treatment with a VEGF antagonist. In some embodiments, the anti-EphA3 antibody is administered to a patient that has an early stage tumor, *e.g.*, a Stage I, Stage II, or Stage III tumor.

[0130] A patient that is considered to be refractory to treatment with a VEGF antagonist, *e.g.*, a VEGF antibody antagonist, or has a tumor that is refractory to treatment with a VEGF
15 antagonist as used herein refers to a patient who responds to therapy, but suffer from side effects, develops resistance, does not respond to the therapy, does not respond satisfactorily to the therapy, etc. Thus, in such patients, or the tumors from such patients, the number of tumor cells has not been significantly reduced, or has increased, or tumor size has not been significantly reduced, or has increased, or there is not further reduction in size or in number
20 of cancer cells. The determination that the patient is refractory to treatment can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells. Similarly, a patient who has a non-neoplastic condition that is refractory to treatment with a VEGF antagonist, *e.g.*, a VEGF antibody, in the context of this invention refers to a patient who does not respond satisfactorily to treatment with the VEGF
25 antagonist, for example, the patient suffers side effects, develops resistance, or does not exhibit reduction in therapeutic indicators for the condition.

[0131] The methods of the invention comprise administering an anti-EphA3 antibody as a pharmaceutical composition to a patient in a therapeutically effective amount using a dosing regimen suitable for treatment of the disease. The composition can be formulated for use in a
30 variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the compositions for proper formulation. Suitable

formulations for use in the present invention are found in *Remington: The Science and Practice of Pharmacy*, 21st Edition, Philadelphia, PA. Lippincott Williams & Wilkins, 2005.

[0132] The anti-EphA3 antibody is provided in a solution suitable for injection into the patient such as a sterile isotonic aqueous solution for injection. The antibody is dissolved or suspended at a suitable concentration in an acceptable carrier. In some embodiments the carrier is aqueous, e.g., water, saline, phosphate buffered saline, and the like. The compositions may contain auxiliary pharmaceutical substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, and the like.

[0133] The pharmaceutical compositions of the invention are administered to a patient, e.g., a patient that has a tumor or non-neoplastic condition, in an amount sufficient to cure or at least partially arrest the disease or symptoms of the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." A therapeutically effective dose is determined by monitoring a patient's response to therapy. Typical benchmarks indicative of a therapeutically effective dose include amelioration of symptoms of the disease in the patient. Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health, including other factors such as age, weight, gender, administration route, etc. Single or multiple administrations of the antibody may be administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the methods provide a sufficient quantity of anti-EphA3 antibody to effectively treat the patient.

[0134] The antibody can be administered by injection or infusion through any suitable route including but not limited to intravenous, sub-cutaneous, intramuscular or intraperitoneal routes. In some embodiments, the antibody may be administered by insufflation. In an exemplary embodiment, the antibody may be stored at 10 mg/ml in sterile isotonic aqueous saline solution for injection at 4°C and is diluted in either 100 ml or 200 ml 0.9% sodium chloride for injection prior to administration to the patient. The antibody is administered by intravenous infusion over the course of 1 hour at a dose of between 0.2 and 10 mg/kg. In other embodiments, the antibody is administered by intravenous infusion over a period of between 15 minutes and 2 hours. In still other embodiments, the administration procedure is via sub-cutaneous bolus injection.

[0135] The dose of antibody is chosen in order to provide effective therapy for the patient and is in the range of less than 0.1 mg/kg body weight to about 25 mg/kg body weight or in the range 1 mg – 2 g per patient. Preferably the dose is in the range 1 – 10 mg/kg or approximately 50 mg – 1000 mg / patient. The dose may be repeated at an appropriate frequency which may be in the range once per day to once every three months, depending on the pharmacokinetics of the antibody (*e.g.*, half-life of the antibody in the circulation) and the pharmacodynamic response (*e.g.*, the duration of the therapeutic effect of the antibody). In some embodiments, the *in vivo* half-life of between about 7 and about 25 days and antibody dosing is repeated between once per week and once every 3 months. In other embodiments, the antibody is administered approximately once per month.

[0136] A V_H region and/or V_L region of the invention may also be used for diagnostic purposes. For example, the V_H and/or V_L region may be used for clinical analysis, such as detection of EphA3 levels on cells from a patient. A V_H or V_L region of the invention may also be used, *e.g.*, to produce anti-Id antibodies.

EXAMPLES

Methodology

Sub-Cloning of Murine V-regions

[0137] The V-region DNA from murine monoclonal mIIIA4 were provided by Dr. Martin Lackmann (Department of Biochemistry and Molecular Biology, Monash University, Victoria, Australia). PCR was used to amplify the V-genes of the V-heavy and V-kappa regions and incorporate restriction enzyme sites suitable for cloning into the desired vectors. V-regions were cloned as Fab fragments and expressed in *E. coli*. This Fab was tested for EphA3-Fc antigen binding and is referred to as reference sequence FA4 in these examples.

Antibody Purification

[0138] Fab fragments were expressed by secretion from *E. coli*. Cells were grown in 2xYT medium to an OD₆₀₀ of 0.6. Expression was induced using IPTG for 3 hours at 33° C. Assembled Fabs were obtained from periplasmic fractions and purified by affinity chromatography using Streptococcal Protein G (HiTrap Protein G HP columns; GE Healthcare) according to standard methods. Fabs were eluted in pH 2.0 buffer, immediately

adjusted to pH 7.0 and dialyzed against PBS pH 7.4 (PBS is without calcium and magnesium).

ELISA

[0139] Typically 50 ng of antigen was bound to a 96 well microtiter plate by overnight incubation at 4° C. The plate was blocked with a solution of 5% milk in PBS for one hour at 33° C. Induced medium (50 µl) from *E.coli* expressing each Humaneered Fab or optimized reference Fab FA106 was added to each well. After a one hour incubation at 33° C, the plate was rinsed three times with PBS + 0.1% Tween 20 (PBST), 50 µl of anti-human-kappa chain HRP conjugate (Sigma; diluted to 0.1 ng/ml in PBST) was added to each well, and the plate was incubated for 40 min at 33° C. The plate was washed three times with PBST and once with PBS. The substrate 3,3',5,5' tetramethylbenzidine (TMB), 100 µl (Sigma), was added to each well and the plate was incubated for ~5 min at room temperature. To stop the reaction, 100 µl of 0.2 N H₂SO₄ was added to each well. The reactions were read at 450 nm by spectrophotometry.

[0140] For detection of binding to Eph family members, recombinant human Eph extracellular (ec) domains were obtained from R&D Systems Inc: EphA1-Fc fusion protein, EphA2 ec domain, EphA5 ec domain, EphB4 ec domain and EphB6-Fc fusion protein. ELISA wells were coated with 100 ng of Eph protein for 1 hr at 37° C. After washing once with PBST, wells were blocked with 5% milk in PBST at 37° C for one hour. Wells were washed once and a two fold dilution series of candidate humaneered antibody added to each set of coated Eph proteins. After one hour, the wells were washed three times in PBST and 50 µl of anti-human-kappa chain HRP conjugate (Sigma; diluted to 0.1 ng/ml in PBST) was added to each well. The plate was incubated for 45 minutes, washed 3 times with PBST, washed one with PBS and 100 µl of TMB added to each well. The reaction was stopped by addition of 0.2N H₂SO₄ and binding measured by absorbance at 450 nm.

Colony Lift Binding Assay (CLBA)

[0141] Screening of Humaneered libraries of Fab fragments was carried out as described (U.S patent Application Publication Nos. 20050255552 and 20060134098) using antigen coated nitrocellulose filters.

Affinity measurements

[0142] The binding kinetics of Fab fragments were analyzed using surface plasmon resonance analysis (spr; Biacore T100) at Biosensor Tools Inc. Affinities were calculated from the determined association and dissociation constants at three different Fab concentrations.

Construction of IgGs

[0143] Chimeric IIIA4 IgG was constructed to contain human IgG1 constant regions and variable regions from the original mouse IIIA4 antibody. PCR was used to amplify the heavy and light chains from mouse IIIA4 DNA provided by Martin Lackmann and also to incorporate restriction sites for cloning. The heavy chain variable region was cloned into a vector expressing the full IgG1 heavy chain expressed from a CMV promoter located downstream of a UCOE sequence. The plasmid contains the Neo gene for selection in mammalian cells and the Amp gene for plasmid production in *E.coli*. Similarly, the light chain variable region was cloned into a vector expressing the human kappa light chain constant region, expressed from a CMV promoter downstream of a UCOE sequence. The plasmid contains a gene for hygromycin selection in mammalian cells and the Amp gene for production in *E. coli*. Engineered antibody IgG heavy and light chain vectors were constructed similarly except the DNA for the variable regions was obtained from *E. coli* expression vectors described above and the heavy chain expression vector contained the puromycin resistance gene instead of neomycin. Humanized antibody IgG and chimeric IIIA4 IgG were expressed in a modified CHO cell line by cotransfection of the heavy and light chain constructs using Fugene 6 reagent (Promega).

Flow Cytometry

[0144] Typically, 3×10^6 cells of SKmel28, LnCAP and B16-F10 were collected by centrifugation at 3000 rpm for 3 minutes. Media were removed and the cells blocked with 2% BSA in PBS for 30 minutes at 4° C followed by a second block with 10 µg/ml rat IgG for 30 minutes at 4° C. Cells were pelleted, re-suspended in 1.5 ml PBS and divided into 500 µl aliquots. Each aliquot was probed separately with 5 µg/ml of control IgG, humanized IgG or chimeric IIIA4 IgG for 45 minutes at 4° C. The samples were washed once in PBS and anti-human IgG-phycoerythrin-conjugate was added. After 45 minutes, cells were washed

once, re-suspended in PBS and analyzed by flow cytometry using a FACS Caliber flow cytometry. Propidium iodide was added just prior to analysis to exclude dead cells.

Example 1. Identification of engineered human anti-EphA3 antibodies

Murine and Reference V-region Amino Acid Sequences

- 5 Murine (mIIIA4) and a reference (FA4) V-region sequences are shown below. CDR sequences are underlined.

mIIIA4 and FA4 Vh:

EVKLEESGAELVKPGSSVKLSCKASGYNFTSYWINWVRLRPGQGLEWIGDIYPGSGN
TNYDEKFKRKATLTVDTSSSTAYMQLSSLASEDSALYYCTRSGYYEDFDSWGQGT

10 LIVSS

mIIIA4 and FA4 Vk:

DIVLTQTPSSLSASLEERVSLTCRASQEISGYLGWLQQKPDGTIKRLIYAASLTDSGVP
KKFSGNRSGSEYSLTISSLESEDFADYYCVQYANYPYTFGGGGTKLEIK

- 15 **[0145]** The Fab FA4 has intact murine V-regions from mIIIA4 fused with human constant regions and was purified from *E. coli*. A dilution ELISA of Fab fragments binding to the antigen produced binding curves that were dependent on antibody concentration.

- [0146]** In addition to the reference Fab (FA4), an optimized reference Fab (FA106) was constructed. Several framework amino acid residues in FA4 were changed to human germ-
20 line in the optimized reference Fab FA106. The V-region sequences of the optimized reference Fab are provided below. The residues altered to human germ-line are shown as bold font. CDR sequences are underlined.

FA106 Vh:

EVKLEESGAELVKPGSSVKLSCKASGYNFTSYWINWVRLRPGQGLEWIGDIYPGSGN
25 TNYDEKFKRKATLTVDTSSSTAYMQLSSLASEDTAVYYCARSGYYEDFDSWGQGT

VTVSS

FA106 Vk:

DIVLTQTPSSLSASLEERVSLTCRASQEISGYLGWLQQKPDGTIKRLIYAASTLD SGVP
KKFSGNRSGSEYSLTISSLESEDFATYYCVQYANYPYTFGQGGTKLEIK

5 *Library Construction and V-region Cassettes*

[0147] Epitope-focused libraries were constructed from a human V-segment library sequences linked to the unique CDR3-FR4 region containing the BSD and human germ-line J-segment sequences. The “full-length” Vh (Vh1 and Vh5) and Vk (VkI) libraries were used as a base for construction of “cassette” libraries in which only part of the murine V-segment is initially replaced by a library of human sequences. Several types of cassettes “libraries” were constructed for both the Vh and Vk chains. Cassettes for the V-heavy and V-kappa chains were made by bridge PCR with overlapping common sequences within the framework 2 region. In this way “front-end” human cassette libraries were constructed for both human Vh1 and Vh5 subclasses and a “middle” human cassette library was constructed for human Vh1. “Front end” and “middle” cassette libraries were constructed for the Vk I subclass.

[0148] Additionally, a cassette library consisting of Vh CDR2-FR3 was constructed. A schematic is shown in Figure 2. The first four residues (boxed) of the reference CDR2 were encoded by the 5' PCR primer. Also, the underlined amino acid residues in the mIIIA4 CDR2 and the human germ-line Vh1-58 CDR2 were varied in pair-wise combination in the forward primer (shown by the amino acid residues above and below the line). The 3' PCR primer is complementary to human germ-line Vh1 FR3. The primers were used to amplify and append a Hu Vh1 FR3 library (derived from spleen mRNA) to the engineered CDR2 library. Consecutive bridge PCR reactions were used to attach “front end” and CDR3-FR4 cassettes to construct a functional V-region.

[0149] Human Vh or Vk cassettes that supported binding to the antigen were identified by colony-lift binding assay and a rank order was determined according to affinity by ELISA. V-heavy screening identified “front-end”, “middle” and CDR2/FR3 cassettes that supported EphA3-Fc recombinant fusion protein antigen binding. V-kappa screening identified “front-end” and “middle” cassettes that supported antigen binding. The functional cassettes for each chain were recombined to construct a fully engineered, high affinity Fab that bound antigen.

[0150] After the identification of a pool of high affinity, engineered Fabs, CDR3 affinity maturation libraries were built. The common CDR3 BSD sequences of a panel of engineered Fab clones were mutated using degenerate PCR primers to generate libraries. These mutagenic libraries were screened using colony lift binding and ELISA assays. The selected Fabs were ranked for affinity with ELISA. Mutations that supported similar or improved affinity for antigen when compared to the FA106 Fab were identified. The heavy chain and light chain CDR3 mutations that support or improve antigen binding help to define the BSD region for each CDR.

Reference and Humaneered Fab Sequence Alignment

[0151] Aligned V-segments of murine reference and two engineered Fabs amino acid sequences were compared with the closest single human germ-line V-gene, Vh1-02 or VkI L15. The FR4 for the humaneered Fab Vh-regions is from human germ-line JH6 and has the sequence WGQGTTVTVSS. The FR4 for the humaneered Fab Vk-regions is from human germ-line Jk2 and has the sequence FGQGKLEIK.

[0152] Each of the Vh-regions and Vk-regions of the engineered Fabs have a high homology to human germ-line amino acid sequence. Exemplary homologies are shown in Table 1.

Table 1: Percentage identity to human germ-line sequence for two engineered V-regions: all percentages represent identity to a single human germ-line sequence across the V-region and exclude the CDR3 BSD sequences

Humaneered Fab	Vh versus Vh1-02	Vk versus VkI L15
1	93%	95%
2	91%	95%

Example 2. Binding kinetics of engineered antibodies.

[0153] Engineered Fabs were isolated from colony-lift binding assays and affinity confirmed by antigen-binding ELISA. Engineered Fab clones with strong positive signals in antigen-binding ELISA were purified and further characterized by kinetic comparison with the optimized reference Fab FA106.

[0154] Binding kinetics of two engineered Fabs and reference Fab FA106 were analyzed using a Biacore. The three Fabs were diluted from the starting concentration of 500 nM to 100 nM and further tested in a three-fold dilution series using PBS, pH 7.4 with 0.005% Tween-20 and 0.1 mg/ml BSA. Each of the five concentrations was tested three times over the three different density surfaces. Assays were run at 25° C. The response data from each surface were fit to a 1:1 interaction model. Calculated association and dissociation constants are shown in Table 2. Kinetic analysis of engineered Fab clones 1 and 2 and the reference clone FA106 all showed low nanomolar affinities for the antigen.

Table 2 : Humaneered Fab Binding constants determined at 25 degrees C

	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
Low	1.83E6	0.00193	1.052
medium	1.01E6	0.001513	1.5
high	1.60E6	0.00186	1.16
Humaneered Fab 1 avg	1.4(4)E6	1.7(2)E-3	1.2(2)
low	4.10E6	0.0058	1.4
medium	6.11E5	0.00178	2.91
high	1.67E6	0.00345	2.06
FA106 avg	2(1)E6	3(2)E-3	2.1(8)
low	5.14E5	0.00332	6.45
medium	6.50E5	0.00399	6.16
high	3.52E5	0.00279	7.92
Humaneered Fab 2 avg	5(1)E5	3.3(6)E-3	6.8(9)

The number in parentheses represents the standard error in the last reported digit based on the data from the three different density surfaces. The terms “low”, “medium”, and “high” in the left column refer to chip densities.

[0155] Humaneered Fab1 was expressed as an IgG in mammalian cells and purified from culture supernatant. Fab fragment was generated by papain digestion for analysis of monovalant binding affinity. Binding of Fab to human and mouse EphA3-Fc was measured on a Biacore 3000 using biotinylated EphA3 variants captured on a Streptavidin chip. Fab was diluted in HBSP running buffer from 50nM to 0.62nM with 3x dilutions. Results were double blanked with an empty reference cell and multiple HBSP buffer injections. Global fit analysis of the Biacore data was carried out assuming a 1:1 interaction. The binding kinetics parameters are shown in Table 3. The data show that Humaneered Fab1 produced from mammalian cells binds with high affinity to both human and murine EphA3.

Table 3 Engineered Fab 1 Binds Human and Mouse EphA3 with Comparable Affinity

Analyte	k_a (1/Ms)	k_d (1/s)	K_D (pM)
Human EphA3-Fc	2.2×10^6	2.0×10^{-3}	930
Mouse EphA3-Fc	2.3×10^6	1.8×10^{-3}	800

Binding of Fab 1 to immobilized mouse and human EphA3-Fc fusion proteins was analyzed by surface plasmon resonance analysis using a Biacore 3000 instrument and global fit analysis.

k_a = association constant; k_d = dissociation constant; K_D = overall affinity.

Binding and Specificity

[0156] A full-length antibody (IgG1 κ) was constructed, expressed from CHO cells and purified by Protein A affinity chromatography. ELISA plates were coated with EphA3-Fc and binding of cIIIA4 IgG, an exemplary engineered IgG, and a control IgG1 were compared (Figure 3). The EC_{50} in this assay was approximately three-fold lower for the engineered IgG than for the original chimeric antibody.

[0157] EphA3 is a member of the Eph receptor family which binds Ephrin ligands. The most homologous members of the family are the EphA receptors whose extracellular domains vary between 39% and 62% sequence identity to EphA3.

[0158] To confirm the specificity of the exemplary engineered IgG, binding was assessed against three EphA proteins and two more distantly related EphB proteins (see Figure 4). EphA5 homology to EphA3 is 61% and represents one of the most homologous family members to EphA3. The engineered antibody bound EphA3 specifically; binding was not observed to EphA1, EphA2 or EphA5. There was also no detectable binding to either of two EphB receptors tested (EphB4 and EphB6).

[0159] The exemplary engineered antibody was also tested for binding to EphA3 expressed on the surface of live cells. Three tumor cell lines known to express EphA3 were used: a mouse melanoma (B16), a human melanoma (SKmel28), and a prostate cancer line (LnCAP). Live cells were probed with the engineered IgG, chimeric IIIA4 or a control antibody. Bound antibody was detected using flow cytometry by an anti-human IgG phycoerythrin conjugate (Figure 5). Strong binding was observed for both the engineered IgG and Chimeric IIIA4 to B16 and SKmel28 cells. Both IgGs also bound LnCAP.

Example 3. Evaluation of the ability of Fab and F(ab')₂ fragments to induce apoptosis

[0160] Fab fragments were generated by papain digestion of an exemplary engineered IgG1 antibody of the invention (Ab1) or control human IgG1 antibody. One mg of antibody was incubated with 0.01mg of papain (Roche # 10108014001) in 100mM Sodium Acetate pH 5.0

at 37°C for 18h. F(ab')₂ was produced by pepsin digestion. Five mg of IgG was incubated with 250 µl immobilized pepsin agarose slurry (Pierce # 20343) at 37°C for 18h in 100mM Sodium Acetate pH 4.0. Upon cleavage, both digests were incubated with protein A resin for 30 minutes to remove Fc fragments and the supernatants were collected. Fab and F(ab')₂ fragments were dialyzed into 50mM Na Succinate pH 6.0, 145mM NaCl and 0.05% Tween 80.

[0161] To evaluate the ability of Fab and F(ab')₂ fragments of Ab1 to induce apoptosis in primary cells isolated from leukemia patients, cells were seeded at 2×10^5 cells/ well in 96-well "U"-bottom plates in 0.1 ml culture medium (RPMI 1640 with 10% fetal bovine serum). Antibody or antibody fragment was added to final concentrations of 10 µg/ml and the plates were incubated at 37°C and 5% carbon dioxide in a tissue-culture incubator for 24 hours. As a positive control for apoptosis induction, separate cell samples were incubated with camptothecin (10 µM; Calbiochem). At the end of the incubation, cells were harvested and washed by centrifugation at 1000 rpm for 5 min followed by incubation in 0.1 ml buffer containing 10 µl FITC-conjugated Annexin V (BD Pharmingen) for 30 minutes on ice. Cells were washed once by centrifugation and resuspended in flow cytometry buffer containing propidium iodide (Sigma) diluted 1:1000. Annexin V-staining cells undergoing apoptosis were identified by flow cytometry.

Table 4: Induction of apoptosis by Ab1 antibody and antibody fragments

Treatment	Apoptosis (%) Experiment 1	Apoptosis (%) Experiment 2
Engineered Ab1 IgG	82.0	85
hIgG1 control	1.8	-
(Fab') ₂	16.3	23
(Fab') ₂ control	1.3	0.8
Engineered Ab1 Fab	1.1	1.1
Fab control	0	0
Camptothecin	91.0	97.0

[0162] The results in Table 4 show that an exemplary antibody (Ab1) F(ab')₂ fragment induced direct induction of apoptosis in primary leukemia cells expressing EphA3. Approximately 20% of the target cells were killed within 24 hours using 10 µg/ml Ab1 F(ab')₂. In contrast, the monovalent Fab fragment was not able to induce detectable levels of apoptosis, indicating that dimerization of EphA3 is necessary and sufficient to induce significant levels of apoptosis in these cells and Fc effector functions are not required for apoptosis induction.

Example 4. Evaluation of an afucosylated antibody

[0163] Antibody 1 was produced in two glycosylation forms by expression in different CHO cell lines. One form (fucosylated Antibody 1) has glycosylation patterns typical of an IgG1 κ (f-allotype) therapeutic antibody including α 1,6 fucose. The other form (designated afucosylated Antibody 1) is produced in a CHO cell line containing a homozygous deletion of the α -1,6 fucosyl transferase gene FUT8 to prevent the addition of α -1,6 fucose and is therefore afucosylated. These antibody preparations were compared in NK-cell mediated antibody-dependent cellular cytotoxicity (ADCC) assays.

[0164] Different concentrations of anti-EphA3 antibody preparations were incubated with leukemia primary target cells and normal human PBMC effector cells (Buffy coat from Stanford University Blood Bank). Both fucosylated Antibody 1 and afucosylated Antibody 1 at 0.0001 μ g/mL to 10.0 μ g/mL were tested with 1×10^4 target cells from a patient with acute myeloid leukemia (AML) and 1×10^6 normal PBMC effector cells (100:1 effector: target cell ratio). After 16 hours incubation at 37°C, 5% CO₂, LDH release was measured in comparison with LDH from detergent-lysed cells to determine the percent cytotoxicity (using a Promega Cytotox 96 kit).

[0165] Afucosylated Antibody 1 shows increased ADCC activity against EphA3-positive target cells compared with fucosylated Antibody 1 (Figure 6).

[0166] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[0167] All publications, accession numbers, patents, and patent applications cited in this specification are herein incorporated by reference as if each was specifically and individually indicated to be incorporated by reference.

Exemplary V_H region sequences of anti-EphA3 antibodies of the invention:**SEQ ID NO:1**

QVQLVQSGAEVKKPGTSLVKVSCKASGYTFTGYWMNWVRQASGQGLEWMGDIYPG
 SGNTNYDEKFKGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARSGVYEDFDSWGQ
 5 GTTVTVSS

SEQ ID NO:2

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYWMNWVRQAPGQGLEWMGDIYPG
 SGNTNYDEKFKGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARSGYEEFDSWGQ
 GTTVTVSS

10 SEQ ID NO:3

QVQLVQSGAEVKKPGTSLVKVSCKASGYTFTGYWMNWVRQASGQGLEWMGDIYPG
 SGNTNYDEKFKGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARSGYEEFDSWGQ
 GTTVTVSS

SEQ ID NO:4

15 QVQLVQSGAELKKPGASVKVSCKTSGYTFTGYWMNWVRQAPGQGLEWMGDIYPG
 SGNTNYDEKFKGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARSGYEEFDSWGQ
 GTTVTVSS

SEQ ID NO:5

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYWMNWVRQAPGQGLEWMGDIYPG
 20 SGNTNYDEKFKGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARGGYEDFDSWG
 QGTTTVTVSS

SEQ ID NO:6

EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWISWVRQMPGQGLEWMGDIYPGSG
 NTNYAQKFQGRVTITADKSTSTAYMGLSSLRSEDVAVYYCARSGYYEDFDSWGQGT
 25 TTVTVSS

SEQ ID NO:7

EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWISWVRQMPGQGLEWMGDIYPGSG
 NTNYAQEFRGRVTITADESTSTAYVELSSLRSEDVAVYYCARSGYYEDFDSWGQGT
 VTVSS

SEQ ID NO:8

EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWISWVRQMPGQGLEWMGDIYPGSG
NTNYAQKFLGRVTITADESTSTAYMELSSLRYDDTAVYYCARSGYYEDFDSWGQGT
TVTVSS

5 **SEQ ID NO:9**

EVKLEESGAELVKPGSSVKLSCKASGYNFTSYWINWVRLRPGQGLEWIGDIYPGSGN
TNYDEKFKRKATLTVDTSSSTAYMQLSSLASEDTAVYYCARSGYYEDFDVWGQGT
TVTVSS

SEQ ID NO:10

10 EVKLEESGAELVKPGSSVKLSCKASGYNFTSYWINWVRLRPGQGLEWIGDIYPGSGN
TNYDEKFKRKATLTVDTSSSTAYMQLSSLASEDTAVYYCARSGYYEDFDIWGQGT
TVTVSS

Exemplary V_L region sequences of anti-EphA3 antibodies of the invention:15 **SEQ ID NO:11**

DIQMTQSPSFLSASVGDRVITITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

SEQ ID NO:12

DIQMTQSPSFLSASVGDRVITITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
20 SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYSNYPYTFGQGTKLEIK

SEQ ID NO:13

DIQMTQSPSFLSASVGDRVITITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCGQYANYPYTFGQGTKLEIK

SEQ ID NO:14

25 DIQMTQSPSFLSASVGDRVITITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

SEQ ID NO:15

DIQLTQSPSFLSASVGDRVITITCRASQGIISYLAWEYQQKPEKAPKRLIYGASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

SEQ ID NO:16

DIQMTQSPSSLSASLGDRVTITCQASQDISTYLNWIQQKPGKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGGTKLEIK

SEQ ID NO:17

5 DIQMTQSPSSLSASLGDRVTITCQASQDISTYLNWIQQKPGKAPKRLIYAASSLQRGV
PSRFSGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGGTKLEIK

SEQ ID NO:18

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLAWEYQQKPGKAPKRLIYAASSLQRGV
PSRFSGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGGTKLEIK

10 **SEQ ID NO:19**

DIQMTQSPSFLSASVGDRVTITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCAQYANYPYTFGQGGTKLEIK

SEQ ID NO:20

DIQMTQSPSFLSASVGDRVTITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
15 SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYAKYPYTFGQGGTKLEIK

SEQ ID NO:21

DIQMTQSPSFLSASVGDRVTITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCVGYANYPYTFGQGGTKLEIK

SEQ ID NO:22

20 DIQMTQSPSFLSASVGDRVTITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCVRYANYPYTFGQGGTKLEIK

SEQ ID NO:23

DIQMTQSPSFLSASVGDRVTITCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYLNYPYTFGQGGTKLEIK

25

SEQ ID NO:24: Exemplary heavy chain constant region

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT
30 KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:25: Exemplary light chain constant region

5 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Exemplary antibodies of the invention (heavy chain and light chain sequences):

SEQ ID NO:26 (heavy chain, antibody comprising SEQ ID NO:26/27)

10 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYWMNWVRQAPGQGLEWMGDIYPG
SGNTNYDEKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSGYYEEFDSWGQ
GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDRVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
15 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:27 (light chain, antibody comprising SEQ ID NO:26/27)

DIQMTQSPSFLSASVGDRVITTCRASQGIISYLAWEYQQKPEKAPKRLIYAASSLQSGVP
20 SRFGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:28 (heavy chain, antibody comprising SEQ ID NO:28/29)

QVQLVQSGAEVKKPGTSVKVSCKASGYTFTGYWMNWVRQASGQGLEWMGDIYPG
25 SGNTNYDEKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSGVYEDFDSWGQ
GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDRVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK

AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
PVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:29 (light chain, antibody comprising SEQ ID NO:28/29)

DIQMTQSPSFLSASVGDRVITTCRASQGIISYLA WYQQKPEKAPKRLIYAASSLQSGVP
5 SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYAKYPYTFGQGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:30 (heavy chain, antibody comprising SEQ ID NO:30/31)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYWMNWVRQAPGQGLEWMGDIYPG
10 SGNTNYDEKFFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGGYYEDFDSWG
QGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
15 KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:31 (light chain, antibody comprising SEQ ID NO:30/31)

DIQMTQSPSFLSASVGDRVITTCRASQGIISYLA WYQQKPEKAPKRLIYAASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCGQYANYPYTFGQGTKLEIKRTVAAPSVFIF
20 PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:32 (heavy chain, antibody comprising SEQ ID NO:32/33)

QVQLVQSGAEVKKPGTSVKVSCKASGYTFTGYWMNWVRQASGQGLEWMGDIYPG
SGNTNYDEKFFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGSYEEFDSWGQ
25 GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
30 PVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:33 (light chain, antibody comprising SEQ ID NO:32/33)

DIQMTQSPSFLSASVGDRVITTCRASQGIISYLA WYQQKPEKAPKRLIYAASSLQSGVP
 SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIKRTVAAPSVFIF
 PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
 5 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:34 (heavy chain, antibody comprising SEQ ID NO:34/35)

QVQLVQSGAEVKKPGASVKV SCKASGYTFTGYWMNWVRQAPGQGLEWMGDIYPG
 SGNTNYDEKFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSGYEEFDSWGQ
 GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 10 HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT
 CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
 AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
 PVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

15 SEQ ID NO:35 (light chain, antibody comprising SEQ ID NO:34/35)

DIQMTQSPSFLSASVGDRVITTCRASQGIISYLA WYQQKPEKAPKRLIYAASSLQSGVP
 SRFSGSGSGTEFTLTISSLQPEDFATYYCAQYANYPYTFGQGTKLEIKRTVAAPSVFIF
 PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

20 SEQ ID NO:36 (heavy chain, antibody comprising SEQ ID NO:36/37)

EVQLVQSGAEVKKPGESLRISCKGSGYTFTTYWISWVRQMPGQGLEWMGDIYPGSG
 NTNYAQEFRGRVTITADESTSTAYVELSSLRSED TAVYYCARSGY YEDFDSWGQGT
 VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP
 25 CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
 NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
 QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
 DSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:37 (light chain, antibody comprising SEQ ID NO:36/37)

DIQLTQSPSFLSASVGDRVITICRASQGIISYLA WYQQKPEKAPKRLIYGASSLQSGVP
SRFSGSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS

5 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

WHAT IS CLAIMED IS:

- 1 1. An anti-EphA3 antibody, comprising:
2 a V_H region that has a CDR3 comprising the amino acid sequence
3 X₁GX₂YEX₃FDX₄, wherein X₁ is S or G, X₂ is Y or V, X₃ is E or D, and X₄ is S, V, or I,
4 with the proviso that when the amino acid sequence is SGYYEDFDS, the CDR1 is not
5 SYWIN and when the amino acid sequence is SGYYEEFDS, the CDR1 is not TYWIS;
6 a J segment that has no more than two amino acid changes relative to a human
7 germline J segment amino acid sequence; and
8 a V-segment that comprises at least 90% identity to a human germ line V-
9 segment amino acid sequence.
- 1 2. The antibody of claim 1, wherein the J segment has no more than two
2 amino acid changes relative to a human JH6 amino acid sequence, and the V-segment
3 comprises at least 90% identity to a human VH1 1-02 amino acid sequence
- 1 3. The antibody of claim 1 or claim 2, wherein FR4 comprises
2 WGQGTTVTVSS.
- 1 4. The antibody of any one of claims 1 to 3 , wherein the CDR3
2 comprises GGYIEDFDS, SGYYEEFDS, SGVYEDFDS, SGYYEDFDV, or SGYYEDFDI.
- 1 5. The antibody of any one of claims 1 to 4, wherein the antibody
2 comprises a V_H CDR1, or a V_H CDR2, or both a V_H CDR1 and a V_HCDR2, as shown in a V_H
3 region set forth in Figure 1.
- 1 6. The antibody of any one of claims 1 to 4, wherein the V_H region CDR1
2 has the sequence GYWMN, TYWIS, or SYWIN.
- 1 7. The antibody of any one of claims 1 to 4, wherein the V_H region CDR2
2 has the sequence DIYPGSGNTNYDEKFQG, DIYPGSGNTNYAQKFQG,
3 DIYPGSGNTNYAQEFRG, DIYPGSGNTNYAQKFLG, DIYPGSGNTNYDEKFEG, or
4 DIYPGSGNTNYDEKFKR.
- 1 8. The antibody of any one of claims 1 to 4, wherein the V_H region CDR1
2 has the sequence GYWMN, TYWIS, or SYWIN and the V_H region CDR2 has the sequence

DIYPGSGNTNYDEKFQG, DIYPGSGNTNYAQKFQG, DIYPGSGNTNYAQEFRG,
DIYPGSGNTNYAQKFLG, DIYPGSGNTNYDEKFEG, or DIYPGSGNTNYDEKFKR.

9. The antibody of any one of claims 1 to 4, wherein the V-segment sequence has a V_H V-segment sequence shown in Figure 1.

10. The antibody of claim 1, wherein the V_H has the sequence of a V_H region set forth in Figure 1.

11. The antibody of any one of the preceding claims, wherein the antibody has a V_L region that has a CDR3 comprising the sequence $X_1X_2YX_3X_4YPYT$, wherein X_1 is G, V, or A; X_2 is Q, R, or G; X_3 is A, S, or L; and X_4 is N or K;

a J segment that comprises at least 90% identity to a human germline J segment amino acid sequence; and

a V-segment that comprises at least 90% identity to a human germ line V-segment amino acid sequence.

12. The antibody of claim 11, wherein the V_L region J segment has no more than two amino acid changes in comparison to human germ-line J κ 2 amino acid sequence FGQGKLEIK and the V-segment comprises at least 90% identity to human germline J κ 1 L15 amino acid sequence.

13. The antibody of claim 11 or claim 12, wherein the FR4 comprises the amino acid sequence FGQGKLEIK.

14. The antibody of any one of claims 11, 12, or 13, wherein the V_L region CDR3 comprises GQYANYPYT, VQYAKYPYT, AQYANYPYT, VQYSNYPYT, VQYANYPYT, VGYANYPYT, VRYANYPYT, or VQYLNYPYT.

15. The antibody of any one of claims 11, 12, 13, or 14, where the V_L region comprises a CDR1, or a CDR2, or both a CDR1 and CDR2 of a sequence V_L region shown in Figure 1.

16. The antibody of any one of claims 11, 12, 13, or 14, wherein the V_L region CDR1 has the sequence RASQGIISYLA, QASQDISTYLN, RASQEISGYLG, or RASQSISSYLA.

1 17. The antibody of any one of claims 11, 12, 13, or 14, wherein the V_L
2 region CDR2 has the sequence AASSLQS, GASSLQS, AASSLQR, or AASTLDS.

1 18. The antibody of any one of claims 11, 12, 13, or 14, wherein the V_L
2 region CDR1 has the sequence RASQGIISYLA, QASQDISTYLN, RASQEISGYLG, or
3 RASQSISSYLA; and the V_L region CDR2 has the sequence AASSLQS, GASSLQS,
4 AASSLQR, or AASTLDS.

1 19. The antibody of any one of claims 11, 12, 13, or 14, wherein the V_L
2 region comprises a V-segment that has a V-segment sequence as shown in Figure 1.

1 20. The antibody of claim 11, wherein the V_L region has the sequence of a
2 V_L region set forth in Figure 1.

1 21. An antibody that comprises a V_L region that has a CDR3 comprising
2 GQYANYPYT, VQYAKYPYT, AQYANYPYT, VQYSNYPYT, VGYANYPYT,
3 VRYANYPYT, or VQYLNYPYT.

1 22. The antibody of claim 21, wherein the antibody comprises a heavy
2 chain CDR3 comprising the amino acid sequence X₁GX₂YEX₃FDX₄, wherein X₁ is S or G,
3 X₂ is Y or V, X₃ is E or D, and X₄ is S, V, or I.

1 23. The antibody of claim 22, wherein the heavy chain CDR3 comprises
2 the amino acid sequence GGYIEDFDS, SGYYEEFDS, SGVYEDFDS, SGYYEDFDV, or
3 SGYYEDFDI.

1 24. The antibody of any one of claims 21, 22, or 23, wherein the V_L region
2 comprises a CDR1, or a CDR2, or both a CDR1 and CDR2 of a sequence V_L region shown in
3 Figure 1.

1 25. The antibody of any one of claims 21, 22, or 23, wherein the V_L region
2 CDR1 has the sequence RASQGIISYLA, QASQDISTYLN, RASQEISGYLG, or
3 RASQSISSYLA.

1 26. The antibody of any one of claims 21, 22, or 23, wherein the V_L region
2 CDR2 has the sequence AASSLQS, GASSLQS, AASSLQR, or AASTLDS.

1 27. The antibody of any one of claims 21, 22, or 23, wherein the V_L region
2 CDR1 has the sequence RASQGIISYLA, QASQDISTYLN, RASQEISGYLG, or
3 RASQSISSYLA; and the V_L region CDR2 has the sequence AASSLQS, GASSLQS,
4 AASSLQR, or AASTLDS.

1 28. The antibody of any one of claims 21, 22, or 23, wherein the V_L region
2 comprises a V-segment that has a V-segment sequence as shown in Figure 1.

1 29. An antibody that comprises a V_H region as set forth in Figure 1 or a V_L
2 region as set forth in Figure 1.

1 30. An antibody that comprises a combination of a V_H region as set forth
2 in Figure 1 and a V_L region as set forth in Figure 1.

1 31. The antibody of claim 30, wherein the combination of V_H and V_L
2 regions comprises:

- 3 a) SEQ ID NO:1 and SEQ ID NO:20,
- 4 b) SEQ ID NO:2 and SEQ ID NO:11,
- 5 c) SEQ ID NO: 2 and SEQ ID NO:12,
- 6 d) SEQ ID NO:2 and SEQ ID NO:19,
- 7 e) SEQ ID NO:2 and SEQ ID NO:21,
- 8 f) SEQ ID NO:2 and SEQ ID NO:22,
- 9 g) SEQ ID NO:2 and SEQ ID NO:23,
- 10 h) SEQ ID NO:3 and SEQ ID NO:11,
- 11 i) SEQ ID NO:3 and SEQ ID NO:12,
- 12 j) SEQ ID NO:3 and SEQ ID NO:21,
- 13 k) SEQ ID NO:3 and SEQ ID NO:22,
- 14 l) SEQ ID NO:4 and SEQ ID NO:11,
- 15 m) SEQ ID NO:4 and SEQ ID NO:13,
- 16 n) SEQ ID NO:5 and SEQ ID NO:11,
- 17 o) SEQ ID NO:5 and SEQ ID NO:13,
- 18 p) SEQ ID NO:5 and SEQ ID NO:21,
- 19 q) SEQ ID NO:6 and SEQ ID NO:14,
- 20 r) SEQ ID NO:6 and SEQ ID NO:15,
- 21 s) SEQ ID NO:7 and SEQ ID NO:14,

- 22 t) SEQ ID NO:7 and SEQ ID NO:15,
23 u) SEQ ID NO:8 and SEQ ID NO:14,
24 v) SEQ ID NO:8 and SEQ ID NO:15,
25 w) SEQ ID NO:9 and SEQ ID NO:16,
26 x) SEQ ID NO:9 and SEQ ID NO:17,
27 y) SEQ ID NO:9 and SEQ ID NO:19,
28 z) SEQ ID NO:10 and SEQ ID NO:17,
29 aa) SEQ ID NO:10 and SEQ ID NO:18, or
30 bb) SEQ ID NO:10 and SEQ ID NO:20.

1 32. The antibody of any one of the preceding claims wherein the V_H region
2 or the V_L region, or both the V_H and V_L region amino acid sequences comprise a methionine
3 at the N-terminus.

1 33. The antibody of any one of the preceding claims wherein the antibody
2 is an IgG.

1 34. The antibody of claim 33, wherein the antibody is a IgG1 or IgG3.

1 35. The antibody of claim 33, wherein the antibody is a IgG2 or IgG4.

1 36. The antibody of claim 33, wherein the antibody comprises a heavy
2 chain constant region having the amino acid sequence set forth in SEQ ID NO:24.

1 37. The antibody of any one of the preceding claims wherein the antibody
2 comprises a kappa light chain constant region having the amino acid sequence set forth in
3 SEQ ID NO:25.

1 38. An antibody that has a heavy chain amino acid sequence and a and
2 light chain amino acid sequence that comprises SEQ ID NO:26 and SEQ ID NO:27, SEQ ID
3 NO:28 and SEQ ID NO:29, SEQ ID NO:30 and SEQ ID NO:31, SEQ ID NO:32 and SEQ ID
4 NO:33, SEQ ID NO:34 and SEQ ID NO:35, or SEQ ID NO:36 and SEQ ID NO:27.

1 39. An antibody preparation comprising an antibody of any one of claims
2 33 to 38, wherein the heavy chain constant region is hypofucosylated or afucosylated.

- 1 40. The antibody of any one of claims 1 to 32, wherein the antibody is a
2 (Fab')₂.
- 1 41. The antibody of any one of the preceding claims wherein the antibody
2 is PEGylated.
- 1 42. The antibody of any one of the preceding claims wherein the antibody .
2 activates EphA3.
- 1 43. The antibody of any one of the preceding claims, wherein the antibody
2 has an affinity of better than 10 nM.
- 1 44. The antibody of claim 43, wherein the antibody has an affinity of
2 better than 1 nM.
- 1 45. The antibody of any one of the preceding claims, wherein the antibody
2 does not compete with ephrin A5 for binding to EphA3.
- 1 46. An anti-EphA3 antibody that has a heavy chain amino acid sequence
2 and a light chain amino acid sequence that comprises: SEQ ID NO:26 and SEQ ID NO:27,
3 SEQ ID NO:28 and SEQ ID NO:29, SEQ ID NO:30 and SEQ ID NO:31, SEQ ID NO:32 and
4 SEQ ID NO:33, SEQ ID NO:34 and SEQ ID NO:35, or SEQ ID NO:36 and SEQ ID NO:27;
5 wherein the antibody is afucosylated.
- 1 47. An antibody preparation comprising the antibody of claim 46, wherein
2 the antibody preparation is hypofucosylated.
- 1 48. A method of treating a patient that has an EphA3-dependent disease,
2 the method comprising administering an antibody of any one of the preceding claims to the
3 patient in a therapeutically effective amount.
- 1 49. The method of claim 48, wherein the disease is a cancer.
- 1 50. The method of claim 49, wherein the patient has undergone treatment,
2 or is undergoing treatment, with a vascular endothelial growth factor (VEGF) antagonist.
- 1 51. The method of claim 50, wherein the VEGF antagonist is an anti-
2 VEGF antibody.

1 52. The method of claim 49, further comprising administering an anti-Bv8
2 antibody antagonist to the patient.

1 53. The method of any one of claims 49 to 51, wherein the patient has a
2 solid tumor where the tumor cells do not express EphA3.

1 54. The method of any one of claims 49 to 51, wherein the patient has a
2 solid tumor where the tumor cells express EphA3.

1 55. The method of claim 49, wherein the EphA3-dependent disease is
2 acute myeloid leukemia or chronic myeloid leukemia

1 56. The method of claim 48, wherein the EphA3-dependent disease is
2 myelodysplastic syndrome, polycythemia vera, essential thrombocythemia, or idiopathic
3 myelofibrosis.

FIG. 1

Heavy chain V-regions

SEQ
ID:

```

1  QVQLVQSGAEVKKPGTSVKVSCKASGYTF1TCYWMN2WVRQASGQGLEWMGDI3YPGSGNTN4YDEK5FOGRVT6MT7RD8TS9ISTAYMEL10SRL11RSDD12TAVYYC13ARSGYVE14DFD15SWGQ16GT17TV18VSS
2  QVQLVQSGAEVKKPGASVKVSCKASGYTF1TCYWMN2WVRQAPGQGLEWMGDI3YPGSGNTN4YDEK5FOGRVT6MT7RD8TS9ISTAYMEL10SRL11RSDD12TAVYYC13ARSGYVEE14FFD15SWGQ16GT17TV18VSS
3  QVQLVQSGAEVKKPGTSVKVSCKASGYTF1TCYWMN2WVRQASGQGLEWMGDI3YPGSGNTN4YDEK5FOGRVT6MT7RD8TS9ISTAYMEL10SRL11RSDD12TAVYYC13ARSGYVEE14FFD15SWGQ16GT17TV18VSS
4  QVQLVQSGAELKKPGASVKVSCKTSGYTF1TCYWMN2WVRQAPGQGLEWMGDI3YPGSGNTN4YDEK5FOGRVT6MT7RD8TS9ISTAYMEL10SRL11RSDD12TAVYYC13ARSGYVEE14FFD15SWGQ16GT17TV18VSS
5  QVQLVQSGAEVKKPGASVKVSCKASGYTF1TCYWMN2WVRQAPGQGLEWMGDI3YPGSGNTN4YDEK5FOGRVT6MT7RD8TS9ISTAYMEL10SRL11RSDD12TAVYYC13ARGGYVE14DFD15SWGQ16GT17TV18VSS
6  EVQLVQSGAEVKKPGESLRISCKGSGYTF1TYWISW2VRQMPGQGLEWMGDI3YPGSGNTN4YAQK5FOGRVT6ITADK7ST8AYMGL9SSL10RSDD11TAVYYC12ARSGYVE13DFD14SWGQ15GT16TV17VSS
7  EVQLVQSGAEVKKPGESLRISCKGSGYTF1TYWISW2VRQMPGQGLEWMGDI3YPGSGNTN4YAQK5FOGRVT6ITADES7ST8AYVEL9SSL10RSDD11TAVYYC12ARSGYVE13DFD14SWGQ15GT16TV17VSS
8  EVQLVQSGAEVKKPGESLRISCKGSGYTF1TYWISW2VRQMPGQGLEWMGDI3YPGSGNTN4YAQK5FLGRVT6ITADES7ST8AYMEL9SSL10RYDD11TAVYYC12ARSGYVE13DFD14SWGQ15GT16TV17VSS
9  EVKLEESGAELV1PGSSV2KL3SKASGYNFT4SYW5INW6RLR7PPGQGLEWIGDI8YPGSGNTN9YDEK10FKR11KATL12TVDT13SS14STAYMQL15SSL16ASEDT17TAVYYC18ARSGYVE19DFD20W21WGQ22GT23TV24VSS
10 EVKLEESGAELV1PGSSV2KL3SKASGYNFT4SYW5INW6RLR7PPGQGLEWIGDI8YPGSGNTN9YDEK10FKR11KATL12TVDT13SS14STAYMQL15SSL16ASEDT17TAVYYC18ARSGYVE19DFD20W21WGQ22GT23TV24VSS

```

FIG. 1 (cont.)

Light chain V-regions

SEQ
ID:

11 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

12 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

13 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCGQYANYPYTFGQGTKLEIK

14 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

15 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

16 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

17 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

18 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

19 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCAQYANYPYTFGQGTKLEIK

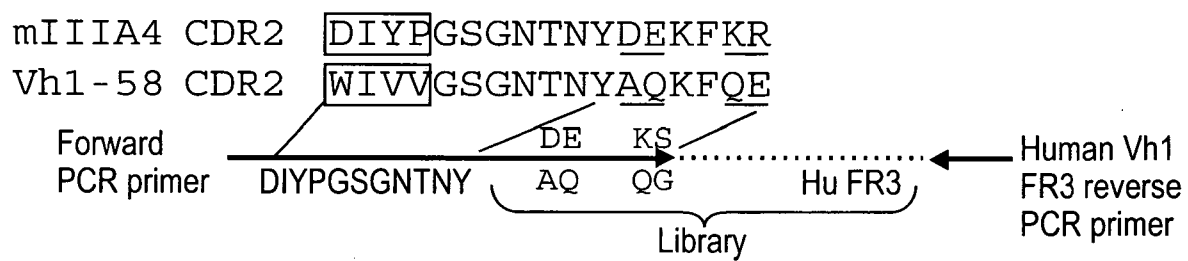
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21 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

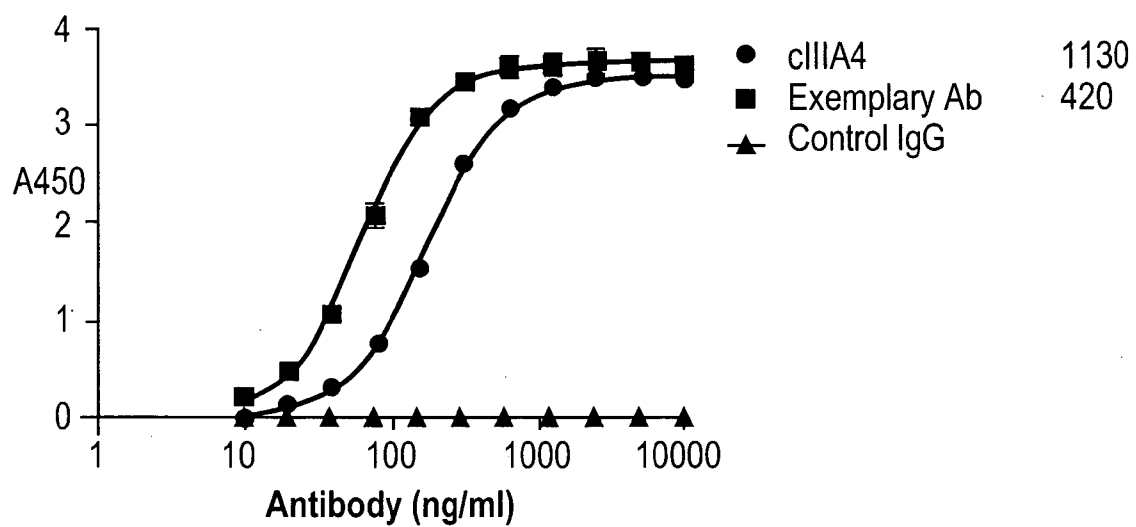
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23 DIQMTQSPSPFLSASVGDRVTITCRASQGIISYLAWYQQKPEKAPKRLIYAASSLQSGVPSRFSSGSGTEFTLTISSLQPEDFATYYCVQYANYPYTFGQGTKLEIK

3/7

FIG. 2

4/7

FIG. 3

5/7

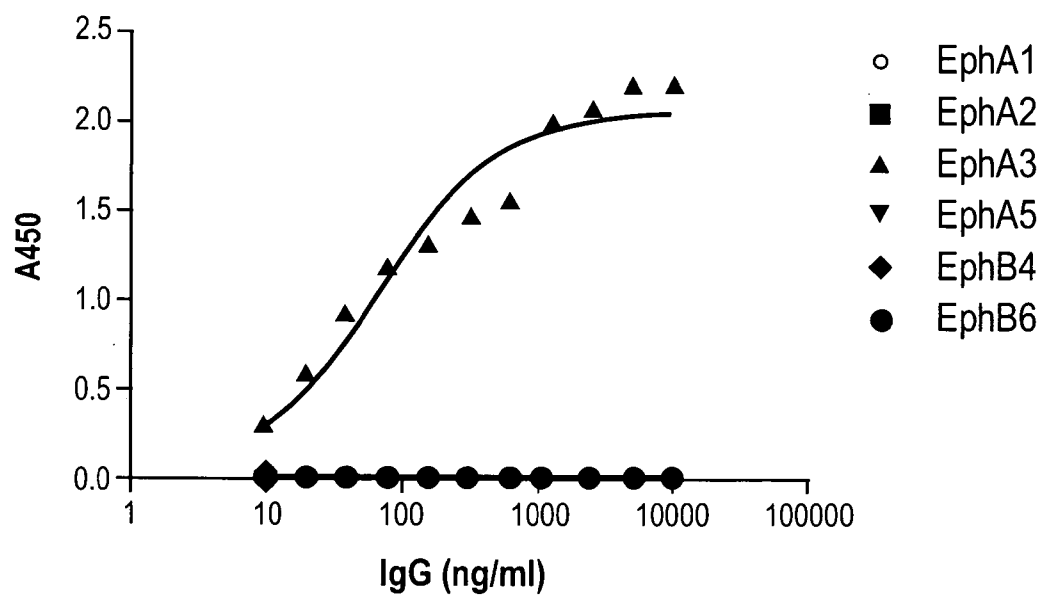
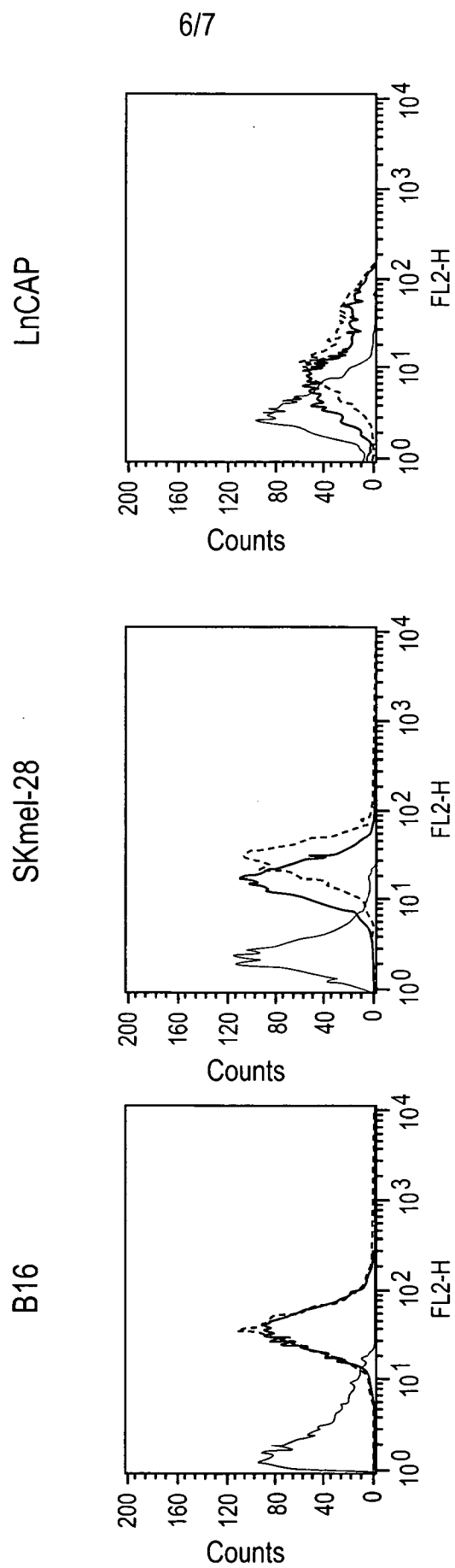
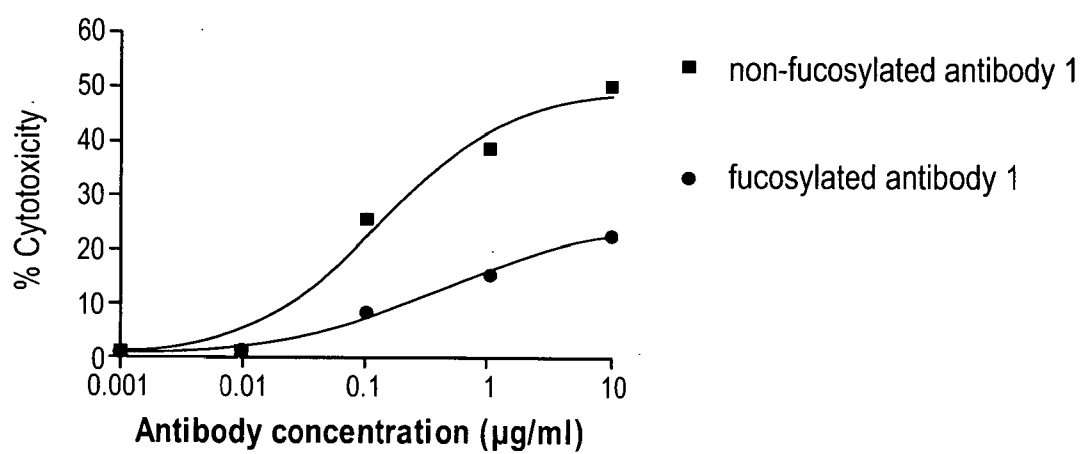
FIG. 4

FIG. 5



7/7

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/052725

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 C07K16/46
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/112192 A2 (KALOBIOUS PHARMACEUTICALS INC [US]; LACKMANN MARTIN [AU]; SCOTT ANDREW) 18 September 2008 (2008-09-18) cited in the application	1-54
Y	paragraph [0094]; figures 6-7; examples 7-8; table 1 ----- -/--	55,56



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

3 February 2011

Date of mailing of the international search report

15/02/2011

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Authorized officer

Luyten, Kattie

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/052725

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>ARRUGA FRANCESCA ET AL: "EphA3 is abnormally expressed in chronic myeloproliferative disorders and could represent a new molecular target", PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 50, 1 April 2009 (2009-04-01), page 692, XP009137278, ISSN: 0197-016X the whole document</p>	55,56
Y	<p>CILLONI DANIELA ET AL: "EphA3 Kinase Is Constitutively Activated in Chronic Myeloid Leukaemia during Progression to Accelerated and Blast Crisis and It Could Represent a New Molecular Target", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 112, no. 11, 1 November 2008 (2008-11-01), page 399, XP009137279, ISSN: 0006-4971 cited in the application the whole document</p>	55,56
Y	<p>LIST A: "Developmental therapeutics for MDS", LEUKEMIA RESEARCH, vol. 33, no. Suppl. 1, May 2009 (2009-05), pages S27-S28, XP002620034, & 10TH INTERNATIONAL SYMPOSIUM ON MYELODYSPLASTIC SYNDROMES; PATRAS, GREECE; MAY 06 -09, 2009 ISSN: 0145-2126 whole document, especially lines 29-30 on page S28</p>	55,56
A	<p>VEARING CHRISTOPHER ET AL: "Concurrent binding of anti-EphA3 antibody and ephrin-A5 amplifies EphA3 signaling and downstream responses: Potential as EphA3-specific tumor-targeting reagents", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 65, no. 15, 1 August 2005 (2005-08-01), pages 6745-6754, XP002493162, ISSN: 0008-5472, DOI: DOI:10.1158/0008-5472.CAN-05-0758 cited in the application Abstract; Discussion</p>	1-56
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/052725

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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