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(54) Title: ANTIGEN BINDING PROTEINS THAT BIND DLL-4

Fig 1A. Binding of various anti-DLL4 antibodies to Cell Surface DLL4 Protein.

Fig 1B. Binding of selected anti-DLL4 antibodies to Cell Surface DLL4 Protein.

(57) Abstract: There is disclosed compositions and methods relating to or derived from anti- DLL-4 antibodies. More specifically, there is disclosed fully human antibodies that bind DLL-4, DLL-4-binding fragments and derivatives of such antibodies, and DLL-4 -binding polypeptides comprising such fragments. Further still, there is disclosed nucleic acids encoding such antibodies, antibody fragments and derivatives and polypeptides, cells comprising such polynucleotides, methods of making such antibodies, antibody fragments and derivatives and polypeptides, and methods of using such antibodies, antibody fragments and derivatives and polypeptides, including methods of treating or diagnosing subjects having DLL-4 related disorders or conditions, including various inflammatory disorders and various cancers.

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Antigen Binding Proteins that Bind DLL-4

Technical Field

The present disclosure provides compositions and methods relating to or derived from anti-DLL-4 antibodies. More specifically, the present disclosure provides human antibodies that bind DLL-4, DLL-4-binding fragments and derivatives of such antibodies, and DLL-4-binding polypeptides comprising such fragments. Further still, the present disclosure provides antibodies, antibody fragments and derivatives and polypeptides, and methods of using such antibodies, antibody fragments and derivatives and polypeptides, including methods of treating or diagnosing subjects having DLL-4-related disorders or conditions.

Background

Cell-to-cell communication is required for many biological processes such as differentiation, proliferation, and homeostasis. One system utilized by a wide range of eukaryotes is the Notch-signaling pathway. This pathway, especially the Notch receptor, is also critical for functional tumor angiogenesis. Thus, inhibition of Notch receptor function, blockage of the Notch receptor, and/or blockage of the Notch-signaling pathway are potential strategies for anti-cancer compositions and therapies. Small molecule inhibitors of the Notch receptor have proven to be toxic because they suppress wild type (normal) tissue expression of Notch receptors throughout the body. Thus, different members of the Notch-signaling pathway should be considered as potential targets for therapeutics.

A vasculature ligand for the Notch receptor is Delta 4 or Delta-like 4 (DLL-4). Largely expressed in the vasculature, DLL-4 is critical for vascular development (Yan et al., *Clin. Cancer Res.*, 13(24): 7243-7246 (2007); Shutter et al., *Genes Dev.*, 14(11): 1313-1318 (2000); Gale et al., *Proc. Natl. Acad. Sci. USA*, 101(45): 15949-15954 (2004); Krebs et al., *Genes Dev.*, 14(11): 1343-1352 (2000)). Mice heterozygous for DLL-4 are embryonically lethal due to major defects in vascular development (Gale et al., *Proc. Natl. Acad. Sci. USA*, 101(45): 15949-15954 (2004); Duarte et al., *Genes Dev.*, 18(20): 2474-2478 (2004); Krebs et al., *Genes Dev.*, 18(20): 2469-2473 (2004)). The expression of DLL-4 can be induced by VEGF (Liu et al., *Mol. Cell. Biol.*, 23(1): 14-25 (2003); Lobov et al., *Proc. Natl. Acad. Sci. USA*, 104(9): 3219-3224 (2007)). In sum, DLL-4 can negatively regulate VEGF signaling, in part through repressing VEGFR2 and inducing VEGFR1 (Harrington et al., *Microvasc. Res.*, 75(2): 144-154 (2008); Suchting et al., *Proc. Natl. Acad. Sci. USA*, 104(9): 3225-3230 (2007)). Exquisite coordination between DLL4 and VEGF is essential for functional angiogenesis.

In addition to its physiological role, DLL-4 is up-regulated in tumor blood vessels (Gale et al., *Proc. Natl. Acad. Sci. USA*, 101(45): 15949-15954 (2004); Mailhos et al., *Differentiation*, 69(2-3): 135-144 (2001); Patel et al., *Cancer Res.*, 65(19): 8690-8697 (2005); Patel et al., *Clin. Cancer Res.*, 12(16): 4836-4844 (2006); Noguera-Troise et al., *Nature*,

5   444(7122): 1032-1037 (2006)). Blockade of DLL-4 potently inhibited primary tumor growth in multiple models (Noguera-Troise et al., *Nature*, 444(7122): 1032-1037 (2006); Ridgway et al., *Nature*, 444(7122): 1083-1087 (2006); Scehnet et al., *Blood*, 109(11): 4753-4760 (2007)). The inhibition of DLL-4 was even effective against tumors that are resistant to anti-VEGF therapy. The combinatorial inhibition of both DLL-4 and VEGF provided an enhanced anti-

10   tumor activity. Interestingly, unlike VEGF inhibition that reduces tumor vessel formation, DLL-4 blockade leads to an increase in tumor vasculature density wherein the vessels are abnormal, cannot support efficient blood transport, and are effectively nonfunctional. Thus, DLL4 provides a potential target for cancer treatment.

Interactions between Notch receptors and their ligands represent an evolutionarily

15   conserved pathway important not only for cell fate decisions but also in regulating lineage decisions in hematopoiesis and in the developing thymus (Artavanis-Tsakonas et al. 1999, *Science* 284:770-776; Skokos et al. 2007; *J. Exp. Med.* 204:1525-1531; and Amsen et al. 2004, *Cell* 117:515-526). It has been recently shown that DLL-4-Notch1 inhibition leads to a complete block in T cell development accompanied by ectopic appearance of B cells and an

20   expansion of dendritic cells (DC) that can arise from Pro-T cell to DC fate conversion within the thymus (Hozumi et al. 2008, *J. Exp. Med.* 205(11):2507-2513; Koch et al. 2008, *J. Exp. Med.* 205(11):2515-2523; and Feyerabend et al. 2009, *Immunity* 30:1-13). Thus, there is accumulating evidence that Notch signaling is critical for the determination of cell fate decision from hematopoietic progenitor cells. Furthermore, a feedback control of regulatory

25   T cell (Treg) homeostasis by DCs *in vivo* has been shown (Darrasse-Jeze et al. 2009, *J. Exp. Med.* 206(9):1853-1862). However, the role of Notch signaling in controlling the origin and the development of DCs and consequently Treg homeostasis is still unknown. This is a question that is clinically important because identifying new methods of inducing Treg expansion could be used as a treatment for autoimmunity diseases and disorders.

30   Other DLL antagonists and their uses are disclosed in WO 2007/143689, WO 2007/070671, WO 2008/076379, WO 2008/042236, and WO/2008/019144. Therefore, there is a need in the art for therapeutic agents capable of targeting the DLL-4-Notch pathway and thereby inhibiting, or even preventing, tumor angiogenesis and growth.

Summary

The present disclosure provides a fully human antibody of an IgG class that binds to a DLL-4 epitope with a binding affinity of at least 10^{-6}M, which has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof. Preferably, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called C3 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called C5 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called C10 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called G6 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called F11 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called D2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called E10 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called F12 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called E5 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called H2 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called A1 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called A2 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called A11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called C12 herein), SEQ ID NO. 29/SEQ ID NO. 30 (called D9 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called E3 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called E6 herein), SEQ ID NO. 35/SEQ ID NO. 36 (called F1 herein), SEQ ID NO. 37/SEQ ID NO. 38 (called F6 herein), SEQ ID NO. 39/SEQ ID NO. 40 (called F10 herein), and combinations thereof.

The present disclosure provides a Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO.

37, SEQ ID NO. 39, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ

5   ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof. Preferably, the fully human antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID

10   NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO.

15   29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof.

The present disclosure provides a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a

20   peptide linker connection the heavy chain and light chain variable domain regions, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO.

25   27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ

30   ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof. Preferably, the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group

consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID

5   NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof.

The present disclosure further provides a method for treating a broad spectrum of

10   mammalian cancers, comprising administering an effective amount of an anti-DLL-4 polypeptide, wherein the anti-DLL-4 polypeptide is selected from the group consisting of a fully human antibody of an IgG class that binds to a DLL-4 epitope with a binding affinity of at least 10^{-6}M, a Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, a single chain human antibody,

15   having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, and combinations thereof;

wherein the fully human antibody has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ

20   ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid

25   sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof;

30   wherein the Fab fully human antibody fragment has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO.

31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ

5   ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof; and

        wherein the single chain human antibody has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group

10   consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to

15   the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof.

20   Preferably, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called C3 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called C5 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called C10 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called G6 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called F11 herein), SEQ ID NO.

25   11/SEQ ID NO. 12 (called D2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called E10 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called F12 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called E5 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called H2 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called A1 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called A2 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called A11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called C12 herein),

30   SEQ ID NO. 29/SEQ ID NO. 30 (called D9 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called E3 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called E6 herein), SEQ ID NO. 35/SEQ ID NO. 36 (called F1 herein), SEQ ID NO. 37/SEQ ID NO. 38 (called F6 herein), SEQ ID NO. 39/SEQ ID NO. 40 (called F10 herein), and combinations thereof. Preferably, the fully human antibody Fab fragment has both a heavy chain variable domain region and a light

chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called C3 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called C5 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called C10 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called G6 herein), SEQ ID NO. 9/SEQ

5   ID NO. 10 (called F11 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called D2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called E10 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called F12 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called E5 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called H2 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called A1 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called A2 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called A11 herein), SEQ ID

10   NO. 27/SEQ ID NO. 28 (called C12 herein), SEQ ID NO. 29/SEQ ID NO. 30 (called D9 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called E3 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called E6 herein), SEQ ID NO. 35/SEQ ID NO. 36 (called F1 herein), SEQ ID NO. 37/SEQ ID NO. 38 (called F6 herein), SEQ ID NO. 39/SEQ ID NO. 40 (called F10 herein), and combinations thereof. Preferably, the fully human single chain antibody has both a heavy

15   chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ

20   ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof.

25   Preferably, the broad spectrum of mammalian cancers to be treated is selected from the group consisting of ovarian, colon, breast, lung cancers, myelomas, neuroblastic-derived CNS tumors, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, and combinations thereof.

30   **Brief Description of the Figures**

          Figure 1A shows the initial screening binding of various anti-DLL-4 antibodies to cell surface expressed DLL-4. Cells stably expressing DLL-4 were incubated with 1 µg/ml of anti-DLL-4 antibodies as indicated. The binding was analyzed by flow cytometry (HTFC, Intellicyt).

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Figure 1B shows the binding of exemplary anti-DLL-4 antibodies to cell surface expressed DLL-4. Cells stably expressing DLL-4 were incubated with increasing amounts of anti-DLL-4 antibodies. The binding was analyzed by flow cytometry. Anti-DLL-4 antibodies showed binding characteristics (EC$_{50}$) comparable to or better than Oncomed OMP21M18.

5   Figure 2 shows a Biacore analysis of various anti-DLL-4 antibodies. The resulting binding kinetic parameters are indicated in the accompanying table.

Figure 3 shows epitope mapping of anti-DLL-4 antibodies in relation to Oncomed OMP21M18 antibody. Additional binding registered by Octet indicates that anti-DLL-4 antibodies C3, C5 or G6 binds to an epitope of DLL-4 that is not occupied by 21M18.

10   Figure 4A shows how anti-DLL-4 antibodies block rhDLL4 binding to immobilized rrNotch. The greater the binding the higher the absorbance value, therefore the best blocking antibodies are C10 and G6 which show the lowest values and are comparable to Oncomed OMP21M18 on the basis of *in vitro* binding activity.

Figure 4B shows that anti-DLL-4 antibodies block the binding of a soluble

15   recombinant Notch-1 (either rat or human) to cellular human DLL-4, as measured by flow cytometry. In this assay, Notch-1 is fluorescently labeled, therefore a high Mean Fluorescence Intensity (MFI) would reflect a strong interaction between Notch-1 and DLL-4. In the presence of anti-DLL-4 antibodies, little to no fluorescence was detected indicating blocking of the interaction.

20   Figure 4C shows the dose-dependent inhibition of the DLL-4- Notch-1 interaction for selected antibodies. IC$_{50}$ values reflect the concentration of antibody which causes 50 % inhibition of interaction between soluble Notch-1 and DLL-4. Anti-DLL-4 antibodies disclosed within show greater inhibitory activity than OMP21M18.

Figure 5 shows a RT-PCR-based bioassay that was developed to determine the ability

25   of selected antibodies to neutralize DLL-4-mediated cellular function *in vitro*. Data show that anti-DLL-4 antibody C3, an exemplary antibody of this disclosure, was able to efficiently inhibit DLL-4-mediated activation of Notch-1 dependent gene expression in a breast cancer cell Line (MCF7).

Figure 6 shows that anti-DLL-4 antibodies can prevent DLL-4-mediated HUVEC

30   proliferation inhibition. DLL4 is known to inhibit VEGF-mediated HUVEC proliferation. Thus, when cultured on DLL-4-coated plate, HUVEC growth is inhibited. Data show that anti-DLL-4 antibodies were able to block this inhibition and to partially restore HUVEC growth.

Detailed Description

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The present disclosure provides a fully human antibody of an IgG class that binds to a DLL-4 epitope with a binding affinity of 10^{-6}M or less, that has a heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof. Preferably, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2 (called C3 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called C5 herein), SEQ ID NO. 5/SEQ ID NO. 6 (called C10 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called G6 herein), SEQ ID NO. 9/SEQ ID NO. 10 (called F11 herein), SEQ ID NO. 11/SEQ ID NO. 12 (called D2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called E10 herein), SEQ ID NO. 15/SEQ ID NO. 16 (called F12 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called E5 herein), SEQ ID NO. 19/SEQ ID NO. 20 (called H2 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called A1 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called A2 herein), SEQ ID NO. 25/SEQ ID NO. 26 (called A11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called C12 herein), SEQ ID NO. 29/SEQ ID NO. 30 (called D9 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called E3 herein), SEQ ID NO. 33/SEQ ID NO. 34 (called E6 herein), SEQ ID NO. 35/SEQ ID NO. 36 (called F1 herein), SEQ ID NO. 37/SEQ ID NO. 38 (called F6 herein), SEQ ID NO. 39/SEQ ID NO. 40 (called F10 herein), and combinations thereof.

The present disclosure provides a Fab fully human antibody fragment, having a variable domain region from a heavy chain and a variable domain region from a light chain, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO.

37, SEQ ID NO. 39, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ

5   ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof. Preferably, the fully human antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID

10   NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO.

15   29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof.

The present disclosure provides a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a

20   peptide linker connection the heavy chain and light chain variable domain regions, wherein the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO.

25   27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has a light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ

30   ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof. Preferably, the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group

consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO.
5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID
NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16,
SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID

5   NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO.
27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ
ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO.
38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof.

      The present disclosure further provides a method for treating a broad spectrum of

10   mammalian cancers, comprising administering an effective amount of an anti- DLL-4
polypeptide, wherein the anti- DLL-4 polypeptide is selected from the group consisting of a
fully human antibody of an IgG class that binds to a DLL-4 epitope with a binding affinity of
at least 10^{-6}M, a Fab fully human antibody fragment, having a variable domain region from a
heavy chain and a variable domain region from a light chain, a single chain human antibody,

15   having a variable domain region from a heavy chain and a variable domain region from a
light chain and a peptide linker connection the heavy chain and light chain variable domain
regions, and combinations thereof;

      wherein the fully human antibody has a heavy chain variable domain sequence that is
at least 95% identical to the amino acid sequences selected from the group consisting of SEQ

20   ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11,
SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ
ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO.
33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has
a light chain variable domain sequence that is at least 95% identical to the amino acid

25   sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO.
6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ
ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO.
28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38,
SEQ ID NO. 40, and combinations thereof;

30   wherein the Fab fully human antibody fragment has the heavy chain variable domain
sequence that is at least 95% identical to the amino acid sequences selected from the group
consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9,
SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ
ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO.

31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ

5   ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof; and

wherein the single chain human antibody has the heavy chain variable domain sequence that is at least 95% identical to the amino acid sequences selected from the group

10   consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has the light chain variable domain sequence that is at least 95% identical to

15   the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof.

20   Preferably, the fully human antibody has both a heavy chain and a light chain wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16,

25   SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof. Preferably, the fully human

30   antibody Fab fragment has both a heavy chain variable domain region and a light chain variable domain region wherein the antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ

ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ

5   ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof. Preferably, the fully human single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain fully human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO.

10   5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ

15   ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof.

Preferably, the broad spectrum of mammalian cancers to be treated is selected from the group consisting of ovarian, colon, breast, lung cancers, myelomas, neuroblastic-derived CNS tumors, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-

20   cell derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, and combinations thereof.

An "antigen binding protein" is a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen.

25   Examples of antigen binding proteins include antibodies, antibody fragments (*e.g.*, an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the

30   three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et al., 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129; Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics

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("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold.

An antigen binding protein can have, for example, the structure of a naturally occurring immunoglobulin. An "immunoglobulin" is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa or lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

The variable regions of naturally occurring immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat et al. in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991. Other numbering systems for the amino acids in immunoglobulin chains include IMGT.RTM. (international ImMunoGeneTics information system; Lefranc et al, *Dev. Comp. Immunol.* 29:185-203; 2005) and AHo (Honegger and Pluckthun, *J. Mol. Biol.* 309(3):657-670; 2001).

Antibodies can be obtained from sources such as serum or plasma that contain immunoglobulins having varied antigenic specificity. If such antibodies are subjected to affinity purification, they can be enriched for a particular antigenic specificity. Such enriched preparations of antibodies usually are made of less than about 10% antibody having specific binding activity for the particular antigen. Subjecting these preparations to several rounds of affinity purification can increase the proportion of antibody having specific binding activity

for the antigen. Antibodies prepared in this manner are often referred to as "monospecific." Monospecfic antibody preparations can be made up of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 99.9% antibody having specific binding activity for the particular antigen.

5   An "antibody" refers to an intact immunoglobulin or to an antigen binding portion thereof that competes with the intact antibody for specific binding, unless otherwise specified. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include, *inter alia*, Fab, Fab', F(ab')$_2$, Fv, domain antibodies (dAbs), and complementarity determining

10   region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

A Fab fragment is a monovalent fragment having the V_L, V_H, C_L and C_{HI} domains; a F(ab')$_2$ fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge

15   at the hinge region; a Fd fragment has the V_H and C_{HI} domains; an Fv fragment has the V_L and V_H domains of a single arm of an antibody; and a dAb fragment has a V_H domain, a V_L domain, or an antigen-binding fragment of a V_H or VL domain (U.S. Patents 6,846,634; 6,696,245, US App. Pub.20/0202512; 2004/0202995; 2004/0038291; 2004/0009507;20 03/0039958, and Ward et al., *Nature* 341:544-546, 1989).

20   A single-chain antibody (scFv) is an antibody in which a V_L and a V_H region are joined via a linker (*e.g.*, a synthetic sequence of amino acid residues) to form a continuous protein chain wherein the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (Bird et al., 1988, *Science* 242:423-26 and Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-83). Diabodies are bivalent

25   antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V_H and V_L domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (Holliger et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:6444-48, and Poljak et al., 1994, *Structure* 2:1121-23). If the two polypeptide chains of a diabody are

30   identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

15

Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using the system described by Kabat et al. *supra*; Lefranc et al., *supra* and/or Honegger and Pluckthun, *supra*. One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. An antigen

5   binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

An antigen binding protein may have one or more binding sites. If there is more than

10   one binding site, the binding sites may be identical to one another or may be different. For example, a naturally occurring human immunoglobulin typically has two identical binding sites, while a "bispecific" or "bifunctional" antibody has two different binding sites.

The term "human antibody" includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of

15   the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). These antibodies may be prepared in a variety of ways, examples of which are described below, including through the immunization with an antigen of interest of a mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes.

20   A humanized antibody has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino

25   acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely

30   immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of

how to make humanized antibodies may be found in U.S. Patents 6,054,297, 5,886,152 and
5,877,293.

The term "chimeric antibody" refers to an antibody that contains one or more regions
from one antibody and one or more regions from one or more other antibodies. In one
5   embodiment, one or more of the CDRs are derived from a human anti-DLL-4 antibody. In
another embodiment, all of the CDRs are derived from a human anti-DLL-4 antibody. In
another embodiment, the CDRs from more than one human anti-DLL-4 antibodies are mixed
and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1
from the light chain of a first human anti-PAR-2 antibody, a CDR2 and a CDR3 from the
10   light chain of a second human anti-DLL-4 antibody, and the CDRs from the heavy chain
from a third anti-DLL-4 antibody. Other combinations are possible.

Further, the framework regions may be derived from one of the same anti-DLL-4
antibodies, from one or more different antibodies, such as a human antibody, or from a
humanized antibody. In one example of a chimeric antibody, a portion of the heavy and/or
15   light chain is identical with, homologous to, or derived from an antibody from a particular
species or belonging to a particular antibody class or subclass, while the remainder of the
chain(s) is/are identical with, homologous to, or derived from an antibody (-ies) from another
species or belonging to another antibody class or subclass. Also included are fragments of
such antibodies that exhibit the desired biological activity (*i.e.*, the ability to specifically bind
20   DLL-4).

A "neutralizing antibody" or an "inhibitory antibody" is an antibody that inhibits the
proteolytic activation of DLL-4 when an excess of the anti-DLL-4 antibody reduces the
amount of activation by at least about 20% using an assay such as those described herein in
the Examples. In various embodiments, the antigen binding protein reduces the amount of
25   amount of proteolytic activation of DLL-4 by at least 30%, 40%, 50%, 60%, 70%, 75%, 80%,
85%, 90%, 95%, 97%, 99%, and 99.9%.

Fragments or analogs of antibodies can be readily prepared by those of ordinary skill
in the art following the teachings of this specification and using techniques known in the art.
Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of
30   functional domains. Structural and functional domains can be identified by comparison of the
nucleotide and/or amino acid sequence data to public or proprietary sequence databases.
Computerized comparison methods can be used to identify sequence motifs or predicted
protein conformation domains that occur in other proteins of known structure and/or function.

Methods to identify protein sequences that fold into a known three-dimensional structure are known. (Bowie et al., 1991, *Science* 253:164).

A "CDR grafted antibody" is an antibody comprising one or more CDRs derived from an antibody of a particular species or isotype and the framework of another antibody of the same or different species or isotype.

A "multi-specific antibody" is an antibody that recognizes more than one epitope on one or more antigens. A subclass of this type of antibody is a "bi-specific antibody" which recognizes two distinct epitopes on the same or different antigens.

An antigen binding protein "specifically binds" to an antigen (*e.g.*, human DLL-4) if it binds to the antigen with a dissociation constant of 1 nanomolar or less.

An "antigen binding domain," "antigen binding region," or "antigen binding site" is a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

An "epitope" is the portion of a molecule that is bound by an antigen binding protein (*e.g.*, by an antibody). An epitope can comprise non-contiguous portions of the molecule (*e.g.*, in a polypeptide, amino acid residues that are not contiguous in the polypeptide's primary sequence but that, in the context of the polypeptide's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein).

The "percent identity" of two polynucleotide or two polypeptide sequences is determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif.)) using its default parameters.

The terms "polynucleotide," "oligonucleotide" and "nucleic acid" are used interchangeably throughout and include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs (*e.g.*, peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids thereof. The nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the invention comprise a contiguous open reading frame encoding an antibody, or a fragment, derivative, mutein, or variant thereof.

Two single-stranded polynucleotides are "the complement" of each other if their sequences can be aligned in an anti-parallel orientation such that every nucleotide in one polynucleotide is opposite its complementary nucleotide in the other polynucleotide, without the introduction of gaps, and without unpaired nucleotides at the 5' or the 3' end of either

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sequence. A polynucleotide is "complementary" to another polynucleotide if the two

polynucleotides can hybridize to one another under moderately stringent conditions. Thus, a

polynucleotide can be complementary to another polynucleotide without being its

complement.

5   A "vector" is a nucleic acid that can be used to introduce another nucleic acid linked

to it into a cell. One type of vector is a "plasmid," which refers to a linear or circular double

stranded DNA molecule into which additional nucleic acid segments can be ligated. Another

type of vector is a viral vector (*e.g.*, replication defective retroviruses, adenoviruses and

adeno-associated viruses), wherein additional DNA segments can be introduced into the viral

10   genome. Certain vectors are capable of autonomous replication in a host cell into which they

are introduced (*e.g.*, bacterial vectors comprising a bacterial origin of replication and

episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are

integrated into the genome of a host cell upon introduction into the host cell, and thereby are

replicated along with the host genome. An "expression vector" is a type of vector that can

15   direct the expression of a chosen polynucleotide.

A nucleotide sequence is "operably linked" to a regulatory sequence if the regulatory

sequence affects the expression (*e.g.*, the level, timing, or location of expression) of the

nucleotide sequence. A "regulatory sequence" is a nucleic acid that affects the expression

(*e.g.*, the level, timing, or location of expression) of a nucleic acid to which it is operably

20   linked. The regulatory sequence can, for example, exert its effects directly on the regulated

nucleic acid, or through the action of one or more other molecules (*e.g.*, polypeptides that

bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences

include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation

signals). Further examples of regulatory sequences are described in, for example, Goeddel,

25   1990, Gene Expression Technology: *Methods in Enzymology* 185, Academic Press, San

Diego, Calif. and Baron et al., 1995, *Nucleic Acids Res.* 23:3605-06.

A "host cell" is a cell that can be used to express a nucleic acid, *e.g.*, a nucleic acid of

the invention. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote,

for example, a single-celled eukaryote (*e.g.*, a yeast or other fungus), a plant cell (*e.g.*, a

30   tobacco or tomato plant cell), an animal cell (*e.g.*, a human cell, a monkey cell, a hamster

cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include

the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., 1981, *Cell*

23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells

or their derivatives such as Veggie CHO and related cell lines which grow in serum-free

media (Rasmussen et al., 1998, *Cytotechnology* 28:31) or CHO strain DX-B11, which is deficient in DHFR (Urlaub et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-20), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (McMahan et al., 1991, *EMBO J.* 10:2821),

5   human embryonic kidney cells such as 293,293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The

10   phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but also to the

15   progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, *e.g.*, mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Preferably, the mammalian cancer to be treated is selected from the group consisting

20   of ovarian, colon, breast or hepatic carcinoma cell lines, myelomas, neuroblastic-derived CNS tumors, monocytic leukemias, B-cell derived leukemia's, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, and combinations thereof.

Polypeptides of the present disclosure can be produced using any standard methods

25   known in the art. In one example, the polypeptides are produced by recombinant DNA methods by inserting a nucleic acid sequence (*e.g.*, a cDNA) encoding the polypeptide into a recombinant expression vector and expressing the DNA sequence under conditions promoting expression. Examples of nucleic acid sequences encoding VK-8B polypeptide disclosed herein are:

30   Nucleic acids encoding any of the various polypeptides disclosed herein may be synthesized chemically. Codon usage may be selected so as to improve expression in a cell. Such codon usage will depend on the cell type selected. Specialized codon usage patterns have been developed for *E. coli* and other bacteria, as well as mammalian cells, plant cells, yeast cells and insect cells. (Mayfield et al., *Proc. Natl. Acad. Sci. USA.* 2003 100(2):438-42;

Sinclair et al. *Protein Expr. Purif.* 2002 (1):96-105; Connell, *Curr. Opin. Biotechnol.* 2001 12(5):446-9; Makrides et al. *Microbiol. Rev.* 1996 60(3):512-38; and Sharp et al. *Yeast.* 1991 7(7):657-78).

5   General techniques for nucleic acid manipulation are described for example in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Vols. 1-3, Cold Spring Harbor Laboratory Press, 2 ed., 1989, or Ausubel et al., *Current Protocols in Molecular Biology* (Green Publishing and Wiley-Interscience: New York, 1987) and periodic updates, herein incorporated by reference. The DNA encoding the polypeptide is operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, viral, or insect

10   genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences that control the termination of transcription and translation. Moreover, a host can replicate, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants.

15   The recombinant DNA can also include any type of protein tag sequence that may be useful for purifying the protein. Examples of protein tags include but are not limited to a histidine tag, a FLAG tag, a myc tag, an HA tag, or a GST tag. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts can be found in *Cloning Vectors: A Laboratory Manual*, (Elsevier, N.Y., 1985).

20   The expression construct is introduced into the host cell using a method appropriate to the host cell. A variety of methods for introducing nucleic acids into host cells are known in the art, including, but not limited to, electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is an infectious

25   agent). Suitable host cells include prokaryotes, yeast, mammalian cells, or bacterial cells.

Suitable bacteria include gram negative or gram positive organisms, for example, *E. coli* or *Bacillus spp.* Yeast, preferably from the *Saccharomyces* species, such as *S. cerevisiae*, may also be used for production of polypeptides. Various mammalian or insect cell culture systems can also be employed to express recombinant proteins. *Baculovirus* systems for

30   production of heterologous proteins in insect cells are reviewed by Luckow and Summers, (*Bio/Technology*, 6:47, 1988). Examples of suitable mammalian host cell lines include endothelial cells, COS-7 monkey kidney cells, CV-1, L cells, C127, 3T3, Chinese hamster ovary (CHO), human embryonic kidney cells, HeLa, 293, 293T, and BHK cell lines. Purified polypeptides are prepared by culturing suitable host/vector systems to express the

recombinant proteins. For many applications, the small size of many of the polypeptides
disclosed herein would make expression in *E. coli* as the preferred method for expression.
The protein is then purified from culture media or cell extracts.

Proteins disclosed herein can also be produced using cell-translation systems. For

5   such purposes the nucleic acids encoding the polypeptide must be modified to allow in vitro
transcription to produce mRNA and to allow cell-free translation of the mRNA in the
particular cell-free system being utilized (eukaryotic such as a mammalian or yeast cell-free
translation system or prokaryotic such as a bacterial cell-free translation system.

DLL-4-binding polypeptides can also be produced by chemical synthesis (*e.g.*, by the

10   methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984, The Pierce Chemical Co.,
Rockford, Ill.). Modifications to the protein can also be produced by chemical synthesis.

The polypeptides of the present disclosure can be purified by isolation/purification
methods for proteins generally known in the field of protein chemistry. Non-limiting
examples include extraction, recrystallization, salting out (*e.g.*, with ammonium sulfate or

15   sodium sulfate), centrifugation, dialysis, ultrafiltration, adsorption chromatography, ion
exchange chromatography, hydrophobic chromatography, normal phase chromatography,
reversed-phase chromatography, gel filtration, gel permeation chromatography, affinity
chromatography, electrophoresis, countercurrent distribution or any combinations of these.
After purification, polypeptides may be exchanged into different buffers and/or concentrated

20   by any of a variety of methods known to the art, including, but not limited to, filtration and
dialysis.

The purified polypeptide is preferably at least 85% pure, more preferably at least 95%
pure, and most preferably at least 98% pure. Regardless of the exact numerical value of the
purity, the polypeptide is sufficiently pure for use as a pharmaceutical product.

25   Post-Translational Modifications of Polypeptides

In certain embodiments, the binding polypeptides of the invention may further
comprise post-translational modifications. Exemplary post-translational protein modifications
include phosphorylation, acetylation, methylation, ADP-ribosylation, ubiquitination,
glycosylation, carbonylation, sumoylation, biotinylation or addition of a polypeptide side

30   chain or of a hydrophobic group. As a result, the modified soluble polypeptides may contain
non-amino acid elements, such as lipids, poly- or mono-saccharide, and phosphates. A
preferred form of glycosylation is sialylation, which conjugates one or more sialic acid
moieties to the polypeptide. Sialic acid moieties improve solubility and serum half-life while
also reducing the possible immunogeneticity of the protein. (Raju et al. *Biochemistry*. 2001

31; 40(30):8868-76). Effects of such non-amino acid elements on the functionality of a polypeptide may be tested for its antagonizing role in DLL-4 function, *e.g.*, its inhibitory effect on angiogenesis or on tumor growth.

5   In one specific embodiment, modified forms of the subject soluble polypeptides comprise linking the subject soluble polypeptides to nonproteinaceous polymers. In one specific embodiment, the polymer is polyethylene glycol ("PEG"), polypropylene glycol, or polyoxyalkylenes, in the manner as set forth in U.S. Patents 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

       PEG is a water soluble polymer that is commercially available or can be prepared by
10   ring-opening polymerization of ethylene glycol according to methods well known in the art (Sandler and Karo, Polymer Synthesis, Academic Press, New York, Vol. 3, pages 138-161). The term "PEG" is used broadly to encompass any polyethylene glycol molecule, without regard to size or to modification at an end of the PEG, and can be represented by the formula: $X\text{--}O(CH_2CH_2O)_n\text{-}1CH_2CH_2OH$ (1), where n is 20 to 2300 and X is H or a terminal
15   modification, *e.g.*, a $C_{1\text{-}4}$ alkyl. In one embodiment, the PEG of the invention terminates on one end with hydroxy or methoxy, *i.e.*, X is H or CH_3 ("methoxy PEG"). A PEG can contain further chemical groups which are necessary for binding reactions; which results from the chemical synthesis of the molecule; or which is a spacer for optimal distance of parts of the molecule. In addition, such a PEG can consist of one or more PEG side-chains which are
20   linked together. PEGs with more than one PEG chain are called multiarmed or branched PEGs. Branched PEGs can be prepared, for example, by the addition of polyethylene oxide to various polyols, including glycerol, pentaerythriol, and sorbitol. For example, a four-armed branched PEG can be prepared from pentaerythriol and ethylene oxide. Branched PEG are described in, for example, EP-A 0 473 084 and U.S. Patent. 5,932,462. One form of PEGs
25   includes two PEG side-chains (PEG2) linked via the primary amino groups of a lysine (Monfardini et al., *Bioconjugate Chem.* 6 (1995) 62-69).

       Although PEG is well-known, this is, to our knowledge, the first demonstration that a pegylated[10F]n3 polypeptide can be pegylated and retain ligand binding activity. In a preferred embodiment, the pegylated[10F]n3 polypeptide is produced by site-directed pegylation,
30   particularly by conjugation of PEG to a cysteine moiety at the N- or C-terminus. Accordingly, the present disclosure provides a target-binding [10F]n3 polypeptide with improved pharmacokinetic properties, the polypeptide comprising: a [10F]n3 domain having from about 80 to about 150 amino acids, wherein at least one of the loops of said [10F]n3 domain participate in target binding; and a covalently bound PEG moiety, wherein said [10F]n3

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polypeptide binds to the target with a K_D of less than 100 nM and has a clearance rate of less than 30 mL/hr/kg in a mammal. The PEG moiety may be attached to the ^{10F}n3 polypeptide by site directed pegylation, such as by attachment to a Cys residue, where the Cys residue may be positioned at the N-terminus of the ^{0F}n3 polypeptide or between the N-terminus and the

5   most N-terminal beta or beta-like strand or at the C-terminus of the ^{10F}n3 polypeptide or between the C-terminus and the most C-terminal beta or beta-like strand. A Cys residue may be situated at other positions as well, particularly any of the loops that do not participate in target binding. A PEG moiety may also be attached by other chemistry, including by conjugation to amines.

10   PEG conjugation to peptides or proteins generally involves the activation of PEG and coupling of the activated PEG-intermediates directly to target proteins/peptides or to a linker, which is subsequently activated and coupled to target proteins/peptides (see Abuchowski et al., *J. Biol. Chem.*, 252, 3571 (1977) and *J. Biol. Chem.*, 252, 3582 (1977), Zalipsky, et al., and Harris et. al., in: *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical*

15   *Applications*; (J. M. Harris ed.) Plenum Press: New York, 1992; Chap.21 and 22). It is noted that a binding polypeptide containing a PEG molecule is also known as a conjugated protein, whereas the protein lacking an attached PEG molecule can be referred to as unconjugated.

A variety of molecular mass forms of PEG can be selected, *e.g.*, from about 1,000 Daltons (Da) to 100,000 Da (n is 20 to 2300), for conjugating to DLL-4-binding

20   polypeptides. The number of repeating units "n" in the PEG is approximated for the molecular mass described in Daltons. It is preferred that the combined molecular mass of PEG on an activated linker is suitable for pharmaceutical use. Thus, in one embodiment, the molecular mass of the PEG molecules does not exceed 100,000 Da. For example, if three PEG molecules are attached to a linker, where each PEG molecule has the same molecular

25   mass of 12,000 Da (each n is about 270), then the total molecular mass of PEG on the linker is about 36,000 Da (total n is about 820). The molecular masses of the PEG attached to the linker can also be different, *e.g.*, of three molecules on a linker two PEG molecules can be 5,000 Da each (each n is about 110) and one PEG molecule can be 12,000 Da (n is about 270).

30   In a specific embodiment a DLL-4 binding polypeptide is covalently linked to one poly(ethylene glycol) group of the formula: $--CO--(CH_2)_x--(OCH_2CH_2)_m--OR$, with the $--CO$ (*i.e.* carbonyl) of the poly(ethylene glycol) group forming an amide bond with one of the amino groups of the binding polypeptide; R being lower alkyl; x being 2 or 3; m being from about 450 to about 950; and n and m being chosen so that the molecular weight of the

conjugate minus the binding polypeptide is from about 10 to 40 kDa. In one embodiment, a binding polypeptide's 6-amino group of a lysine is the available (free) amino group.

The above conjugates may be more specifically presented by formula (II): P--NHCO--$(CH_2)_x$--$(OCH_2CH_2)_m$--OR (II) , wherein P is the group of a binding polypeptide as
5 described herein, (i.e. without the amino group or amino groups which form an amide linkage with the carbonyl shown in formula (II); and wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 950 and is chosen so that the molecular weight of the conjugate minus the binding polypeptide is from about 10 to about 40 kDa. As used herein, the given ranges of "m" have an orientational meaning. The ranges of "m" are determined in any case, and
10 exactly, by the molecular weight of the PEG group. One skilled in the art can select a suitable molecular mass for PEG, e.g., based on how the pegylated binding polypeptide will be used therapeutically, the desired dosage, circulation time, resistance to proteolysis, immunogenicity, and other considerations.

In one embodiment, PEG molecules may be activated to react with amino groups on a
15 binding polypeptide, such as with lysines (Bencham et al., *Anal. Biochem.*, 131, 25 (1983); Veronese et al., *Appl. Biochem.*, 11, 141 (1985).; Zalipsky et al., *Polymeric Drugs and Drug Delivery Systems*, adrs 9-110 ACS Symposium Series 469 (1999); Zalipsky et al., *Europ. Polym. J.*, 19, 1177-1183 (1983); Delgado et al., *Biotechnology and Applied Biochemistry*, 12, 119-128 (1990)).

20 In one specific embodiment, carbonate esters of PEG are used to form the PEG-binding polypeptide conjugates. N,N'-disuccinimidylcarbonate (DSC) may be used in the reaction with PEG to form active mixed PEG-succinimidyl carbonate that may be subsequently reacted with a nucleophilic group of a linker or an amino group of a binding polypeptide (U.S. Patents 5,281,698 and 5,932,462). In a similar type of reaction, 1,1'-
25 (dibenzotriazolyl)carbonate and di-(2-pyridyl)carbonate may be reacted with PEG to form PEG-benzotriazolyl and PEG-pyridyl mixed carbonate (U.S. Patent 5,382,657), respectively.

Pegylation of a [10F]n3 polypeptide can be performed according to the methods of the state of the art, for example by reaction of the binding polypeptide with electrophilically active PEGs (supplier: Shearwater Corp., USA, www.shearwatercorp.com). Preferred PEG
30 reagents of the present invention are, e.g., N-hydroxysuccinimidyl propionates (PEG-SPA), butanoates (PEG-SBA), PEG-succinimidyl propionate or branched N-hydroxysuccinimides such as mPEG2-NHS (Monfardini et al., *Bioconjugate Chem.* 6 (1995) 62-69). Such methods may be used to pegylate at an f-amino group of a binding polypeptide lysine or the N-terminal amino group of the binding polypeptide.

In another embodiment, PEG molecules may be coupled to sulfhydryl groups on a binding polypeptide (Sartore et al., *Appl. Biochem. Biotechnol.*, 27, 45 (1991); Morpurgo et al., *Biocon. Chem.*, 7, 363-368 (1996); Goodson et al., *Bio/Technology* (1990) 8, 343; U.S. Patent 5,766,897). U.S. Patents 6,610,281 and 5,766,897 describes exemplary reactive PEG

5   species that may be coupled to sulfhydryl groups.

In some embodiments where PEG molecules are conjugated to cysteine residues on a binding polypeptide, the cysteine residues are native to the binding polypeptide, whereas in other embodiments, one or more cysteine residues are engineered into the binding polypeptide. Mutations may be introduced into a binding polypeptide coding sequence to

10   generate cysteine residues. This might be achieved, for example, by mutating one or more amino acid residues to cysteine. Preferred amino acids for mutating to a cysteine residue include serine, threonine, alanine and other hydrophilic residues. Preferably, the residue to be mutated to cysteine is a surface-exposed residue. Algorithms are well-known in the art for predicting surface accessibility of residues based on primary sequence or a protein.

15   Alternatively, surface residues may be predicted by comparing the amino acid sequences of binding polypeptides, given that the crystal structure of the framework based on which binding polypeptides are designed and evolved has been solved (Himanen et al., *Nature.* (2001) 20-27; 414(6866):933-8) and thus the surface-exposed residues identified. In one embodiment, cysteine residues are introduced into binding polypeptides at or near the N-

20   and/or C-terminus, or within loop regions.

In some embodiments, the pegylated binding polypeptide comprises a PEG molecule covalently attached to the alpha amino group of the N-terminal amino acid. Site specific N-terminal reductive amination is described in Pepinsky et al., (2001) *JPET*, 297, 1059, and U.S. Patent 5,824,784. The use of a PEG-aldehyde for the reductive amination of a protein

25   utilizing other available nucleophilic amino groups is described in U.S. Patent 4,002,531, in Wieder et al., (1979) *J. Biol. Chem.* 254,12579, and in Chamow et al., (1994) *Bioconjugate Chem.* 5, 133.

The ratio of a binding polypeptide to activated PEG in the conjugation reaction can be from about 1:0.5 to 1:50, between from about 1:1 to 1:30, or from about 1:5 to 1:15. Various

30   aqueous buffers can be used in the present method to catalyze the covalent addition of PEG to the binding polypeptide. In one embodiment, the pH of a buffer used is from about 7.0 to 9.0. In another embodiment, the pH is in a slightly basic range, *e.g.*, from about 7.5 to 8.5. Buffers having a pKa close to neutral pH range may be used, *e.g.*, phosphate buffer.

Conventional separation and purification techniques known in the art can be used to purify PEGylated binding polypeptide, such as size exclusion (*e.g.* gel filtration) and ion exchange chromatography. Products may also be separated using SDS-PAGE. Products that may be separated include mono-, di-, tri- poly- and un-pegylated binding polypeptide, as well

5   as free PEG. The percentage of mono-PEG conjugates can be controlled by pooling broader fractions around the elution peak to increase the percentage of mono-PEG in the composition. About ninety percent mono-PEG conjugates represents a good balance of yield and activity. Compositions in which, for example, at least ninety-two percent or at least ninety-six percent of the conjugates are mono-PEG species may be desired. In an embodiment of this invention

10   the percentage of mono-PEG conjugates is from ninety percent to ninety-six percent.

In one embodiment, PEGylated binding polypeptide of the invention contain one, two or more PEG moieties. In one embodiment, the PEG moiety(ies) are bound to an amino acid residue which is on the surface of the protein and/or away from the surface that contacts the target ligand. In one embodiment, the combined or total molecular mass of PEG in PEG-

15   binding polypeptide is from about 3,000 Da to 60,000 Da, optionally from about 10,000 Da to 36,000 Da.

In one embodiment of the invention, the PEG in pegylated binding polypeptide is not hydrolyzed from the pegylated amino acid residue using a hydroxylamine assay, *e.g.*, 450 mM hydroxylamine (pH 6.5) over 8 to 16 hours at room temperature, and is thus stable. In

20   one embodiment, greater than 80% of the composition is stable mono-PEG-binding polypeptide, more preferably at least 90%, and most preferably at least 95%.

In another embodiment, the pegylated binding polypeptides of the invention will preferably retain at least 25%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or 100% of the biological activity associated with the unmodified protein. In one embodiment, biological

25   activity refers to its ability to bind to DLL-4, as assessed by KD, k_{on} or k_{off}. In one specific embodiment, the pegylated binding polypeptide protein shows an increase in binding to DLL-4 relative to unpegylated binding polypeptide.

The serum clearance rate of PEG-modified polypeptide may be decreased by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or even 90%, relative to the clearance rate of

30   the unmodified binding polypeptide. The PEG-modified polypeptide may have a half-life ($t_{1/2}$) which is enhanced relative to the half-life of the unmodified protein. The half-life of PEG-binding polypeptide may be enhanced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 400% or 500%, or even by 1000% relative to the half-life of the unmodified binding polypeptide. In some embodiments,

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the protein half-life is determined *in vitro*, such as in a buffered saline solution or in serum. In other embodiments, the protein half-life is an *in vivo* half-life, such as the half-life of the protein in the serum or other bodily fluid of an animal.

Therapeutic Formulations and Modes of Administration

5   The present disclosure features methods for treating conditions or preventing pre-conditions which respond to an inhibition of DLL-4 biological activity. Preferred examples are conditions that are characterized by inflammation or cellular hyperproliferation. Techniques and dosages for administration vary depending on the type of specific polypeptide and the specific condition being treated but can be readily determined by the

10   skilled artisan. In general, regulatory agencies require that a protein reagent to be used as a therapeutic is formulated so as to have acceptably low levels of pyrogens. Accordingly, therapeutic formulations will generally be distinguished from other formulations in that they are substantially pyrogen free, or at least contain no more than acceptable levels of pyrogen as determined by the appropriate regulatory agency (*e.g.*, FDA).

15   Therapeutic compositions of the present disclosure may be administered with a pharmaceutically acceptable diluent, carrier, or excipient, in unit dosage form. Administration may be parenteral (*e.g.*, intravenous, subcutaneous), oral, or topical, as non-limiting examples. In addition, any gene therapy technique, using nucleic acids encoding the polypeptides of the invention, may be employed, such as naked DNA delivery, recombinant

20   genes and vectors, cell-based delivery, including ex vivo manipulation of patients' cells, and the like.

The composition can be in the form of a pill, tablet, capsule, liquid, or sustained release tablet for oral administration; or a liquid for intravenous, subcutaneous or parenteral administration; gel, lotion, ointment, cream, or a polymer or other sustained release vehicle

25   for local administration.

Methods well known in the art for making formulations are found, for example, in "Remington: The Science and Practice of Pharmacy" (20th ed., ed. A. R. Gennaro A R., 2000, Lippincott Williams & Wilkins, Philadelphia, Pa.). Formulations for parenteral administration may, for example, contain excipients, sterile water, saline, polyalkylene

30   glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Nanoparticulate formulations (*e.g.*, biodegradable nanoparticles, solid lipid nanoparticles, liposomes) may be used to control the biodistribution of the compounds. Other

potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. The concentration of the compound in the formulation varies depending upon a number of factors, including the dosage of the drug to be administered, and the route of administration.

5   The polypeptide may be optionally administered as a pharmaceutically acceptable salt, such as non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids

10   such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. In one example, the polypeptide is formulated in the presence of sodium acetate to increase thermal stability.

      Formulations for oral use include tablets containing the active ingredient(s) in a

15   mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose and sorbitol), lubricating agents, glidants, and anti-adhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc).

      Formulations for oral use may also be provided as chewable tablets, or as hard gelatin

20   capsules wherein the active ingredient is mixed with an inert solid diluent, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium.

      A therapeutically effective dose refers to a dose that produces the therapeutic effects for which it is administered. The exact dose will depend on the disorder to be treated, and may be ascertained by one skilled in the art using known techniques. In general, the

25   polypeptide is administered at about 0.01 µg/kg to about 50 mg/kg per day, preferably 0.01 mg/kg to about 30 mg/kg per day, most preferably 0.1 mg/kg to about 20 mg/kg per day. The polypeptide may be given daily (e.g., once, twice, three times, or four times daily) or preferably less frequently (e.g., weekly, every two weeks, every three weeks, monthly, or quarterly). In addition, as is known in the art, adjustments for age as well as the body weight,

30   general health, sex, diet, time of administration, drug interaction, and the severity of the disease may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

Exemplary Uses

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The DLL-4 binding proteins described herein and their related variants are useful in a number of therapeutic and diagnostic applications. These include the inhibition of the biological activity of DLL-4 by competing for or blocking the binding to a DLL-4 as well as the delivery of cytotoxic or imaging moieties to cells, preferably cells expressing DLL-4. The

5 small size and stable structure of these molecules can be particularly valuable with respect to manufacturing of the drug, rapid clearance from the body for certain applications where rapid clearance is desired or formulation into novel delivery systems that are suitable or improved using a molecule with such characteristics.

On the basis of their efficacy as inhibitors of DLL-4 biological activity, the

10 polypeptides of this disclosure are effective against a number of cancer conditions as well as complications arising from cancer, such as pleural effusion and ascites. Preferably, the DLL-4-binding polypeptides of the disclosure can be used for the treatment of prevention of hyperproliferative diseases or cancer and the metastatic spread of cancers. Preferred indications for the disclosed anti- DLL-4 antibodies include colorectal cancers, head and neck

15 cancers, small cell lung cancer, non-small cell lung cancer (NSCLC) and pancreatic cancer. Non-limiting examples of cancers include bladder, blood, bone, brain, breast, cartilage, colon kidney, liver, lung, lymph node, nervous tissue, ovary, pancreatic, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, or vaginal cancer.

20 In addition, various inflammatory disorders can be treated with the disclosed anti-DLL-4 binding polypeptides disclosed herein. Such inflammatory disorders include, for example, intestinal mucosa inflammation wasting diseases associated with colitis, multiple sclerosis, rheumatoid arthritis, osteoarthritis, psoriasis, and Crohn's disease.

A DLL-4 binding polypeptide can be administered alone or in combination with one

25 or more additional therapies such as chemotherapy radiotherapy, immunotherapy, surgical intervention, or any combination of these. Long-term therapy is equally possible as is adjuvant therapy in the context of other treatment strategies, as described above.

In certain embodiments of such methods, one or more polypeptide therapeutic agents can be administered, together (simultaneously) or at different times (sequentially). In

30 addition, polypeptide therapeutic agents can be administered with another type of compounds for treating cancer or for inhibiting angiogenesis.

In certain embodiments, the subject anti- DLL-4 antibodies agents of the invention can be used alone. Alternatively, the subject agents may be used in combination with other conventional anti-cancer therapeutic approaches directed to treatment or prevention of

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proliferative disorders (*e.g.*, tumor). For example, such methods can be used in prophylactic cancer prevention, prevention of cancer recurrence and metastases after surgery, and as an adjuvant of other conventional cancer therapy. The present disclosure recognizes that the effectiveness of conventional cancer therapies (*e.g.*, chemotherapy, radiation therapy,

5   phototherapy, immunotherapy, and surgery) can be enhanced through the use of a subject polypeptide therapeutic agent.

A wide array of conventional compounds have been shown to have anti-neoplastic activities. These compounds have been used as pharmaceutical agents in chemotherapy to shrink solid tumors, prevent metastases and further growth, or decrease the number of

10   malignant cells in leukemic or bone marrow malignancies. Although chemotherapy has been effective in treating various types of malignancies, many anti-neoplastic compounds induce undesirable side effects. It has been shown that when two or more different treatments are combined, the treatments may work synergistically and allow reduction of dosage of each of the treatments, thereby reducing the detrimental side effects exerted by each compound at

15   higher dosages. In other instances, malignancies that are refractory to a treatment may respond to a combination therapy of two or more different treatments.

When a polypeptide therapeutic agent of the present invention is administered in combination with another conventional anti-neoplastic agent, either concomitantly or sequentially, such therapeutic agent may be found to enhance the therapeutic effect of the

20   anti-neoplastic agent or overcome cellular resistance to such anti-neoplastic agent. This allows decrease of dosage of an anti-neoplastic agent, thereby reducing the undesirable side effects, or restores the effectiveness of an anti-neoplastic agent in resistant cells.

Pharmaceutical compounds that may be used for combinatory anti-tumor therapy include, merely to illustrate: aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg,

25   bicalutamide, bleomycin, buserelin, busulfan, campothecin, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone,

30   flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ironotecan, letrozole, leucovorin, leuprolide, levamisole, lomustine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, plicamycin, porfimer, procarbazine,

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raltitrexed, rituximab, streptozocin, suramin, tamoxifen, temozolomide, teniposide, testosterone, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, and vinorelbine.

Certain chemotherapeutic anti-tumor compounds may be categorized by their
5   mechanism of action into, for example, following groups: anti-metabolites/anti-cancer agents, such as pyrimidine analogs (5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine) and purine analogs, folate antagonists and related inhibitors (mercaptopurine, thioguanine, pentostatin and 2-chlorodeoxyadenosine (cladribine)); antiproliferative/antimitotic agents including natural products such as vinca alkaloids
10   (vinblastine, vincristine, and vinorelbine), microtubule disruptors such as taxane (paclitaxel, docetaxel), vincristin, vinblastin, nocodazole, epothilones and navelbine, epidipodophyllotoxins (etoposide, teniposide), DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, cytoxan, dactinomycin, daunorubicin, doxorubicin, epirubicin,
15   hexamethylmelamineoxaliplatin, iphosphamide, melphalan, merchlorehtamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, teniposide, triethylenethiophosphoramide and etoposide (VP16)); antibiotics such as dactinomycin (actinomycin D), daunorubicin, doxorubicin (adriamycin), idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin; enzymes (L-
20   asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates-busulfan,
25   nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes--dacarbazinine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate); platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, nilutamide) and aromatase inhibitors (letrozole,
30   anastrozole); anticoagulants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine, clopidogrel, abciximab; antimigratory agents; antisecretory agents (breveldin); immunosuppressives (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); anti-angiogenic compounds

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(TNP-470, genistein) and growth factor inhibitors (*e.g.*, VEGF inhibitors, fibroblast growth factor (FGF) inhibitors); angiotensin receptor blocker; nitric oxide donors; anti-sense oligonucleotides; antibodies (trastuzumab); cell cycle inhibitors and differentiation inducers (tretinoin); mTOR inhibitors, topoisomerase inhibitors (doxorubicin (adriamycin), amsacrine,

5   camptothecin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin and mitoxantrone, topotecan, irinotecan), corticosteroids (cortisone, dexamethasone, hydrocortisone, methylpednisolone, prednisone, and prenisolone); growth factor signal transduction kinase inhibitors; mitochondrial dysfunction inducers and caspase activators; and chromatin disruptors.

10   Depending on the nature of the combinatory therapy, administration of the polypeptide therapeutic agents may be continued while the other therapy is being administered and/or thereafter. Administration of the polypeptide therapeutic agents may be made in a single dose, or in multiple doses. In some instances, administration of the polypeptide therapeutic agents is commenced at least several days prior to the conventional

15   therapy, while in other instances, administration is begun either immediately before or at the time of the administration of the conventional therapy.

The DLL-4 binding proteins described herein can also be detectably labeled and used to contact cells expressing DLL-4 for imaging applications or diagnostic applications. For diagnostic purposes, the polypeptide of the invention is preferably immobilized on a solid

20   support. Preferred solid supports include columns (for example, affinity columns, such as agarose-based affinity columns), microchips, or beads.

In one example of a diagnostic application, a biological sample, such as serum or a tissue biopsy, from a patient suspected of having a condition characterized by inappropriate angiogenesis is contacted with a detectably labeled polypeptide of the disclosure to detect

25   levels of DLL-4. The levels of DLL-4 detected are then compared to levels of DLL-4 detected in a normal sample also contacted with the labeled polypeptide. An increase of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% in the levels of the DLL-4 may be considered a diagnostic indicator.

In certain embodiments, the DLL-4 binding polypeptides are further attached to a

30   label that is able to be detected (*e.g.*, the label can be a radioisotope, fluorescent compound, enzyme or enzyme co-factor). The active moiety may be a radioactive agent, such as: radioactive heavy metals such as iron chelates, radioactive chelates of gadolinium or manganese, positron emitters of oxygen, nitrogen, iron, carbon, or gallium, ^{43}K, ^{52}Fe, ^{57}Co, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{123}I, ^{125}I, ^{131}I, ^{132}I, or ^{99}Tc. A binding agent affixed to such a moiety may be

used as an imaging agent and is administered in an amount effective for diagnostic use in a
mammal such as a human and the localization and accumulation of the imaging agent is then
detected. The localization and accumulation of the imaging agent may be detected by
radioscintigraphy, nuclear magnetic resonance imaging, computed tomography or positron

5   emission tomography. Immunoscintigraphy using DLL-4 binding polypeptides directed at
DLL-4 may be used to detect and/or diagnose cancers and vasculature. For example, any of
the binding polypeptide against a DLL-4 marker labeled with ^{99}Technetium, ^{111}Indium, or
^{125}Iodine may be effectively used for such imaging. As will be evident to the skilled artisan,
the amount of radioisotope to be administered is dependent upon the radioisotope. Those

10   having ordinary skill in the art can readily formulate the amount of the imaging agent to be
administered based upon the specific activity and energy of a given radionuclide used as the
active moiety. Typically 0.1-100 millicuries per dose of imaging agent, preferably 1-10
millicuries, most often 2-5 millicuries are administered. Thus, compositions according to the
present invention useful as imaging agents comprising a targeting moiety conjugated to a

15   radioactive moiety comprise 0.1-100 millicuries, in some embodiments preferably 1-10
millicuries, in some embodiments preferably 2-5 millicuries, in some embodiments more
preferably 1-5 millicuries.

The DLL-4 binding polypeptides can also be used to deliver additional therapeutic
agents (including but not limited to drug compounds, chemotherapeutic compounds, and

20   radiotherapeutic compounds) to a cell or tissue expressing DLL-4. In one example, the DLL-
4 binding polypeptide is fused to a chemotherapeutic agent for targeted delivery of the
chemotherapeutic agent to a tumor cell or tissue expressing DLL-4.

The DLL-4 binding polypeptides are useful in a variety of applications, including
research, diagnostic and therapeutic applications. For instance, they can be used to isolate

25   and/or purify receptor or portions thereof, and to study receptor structure (e.g., conformation)
and function.

In certain aspects, the various binding polypeptides can be used to detect or measure
the expression of DLL-4, for example, on endothelial cells (e.g., venous endothelial cells), or
on cells transfected with a DLL-4 gene. Thus, they also have utility in applications such as

30   cell sorting and imaging (e.g., flow cytometry, and fluorescence activated cell sorting), for
diagnostic or research purposes.

In certain embodiments, the binding polypeptides of fragments thereof can be labeled
or unlabeled for diagnostic purposes. Typically, diagnostic assays entail detecting the
formation of a complex resulting from the binding of a binding polypeptide to DLL-4. The

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binding polypeptides or fragments can be directly labeled, similar to antibodies. A variety of labels can be employed, including, but not limited to, radionuclides, fluorescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors and ligands (*e.g.*, biotin, haptens). Numerous appropriate immunoassays are known to the skilled artisan (see, for example, U.S.

5   Patents. 3,817,827; 3,850,752; 3,901,654; and 4,098,876). When unlabeled, the binding polypeptides can be used in assays, such as agglutination assays. Unlabeled binding polypeptides can also be used in combination with another (one or more) suitable reagent which can be used to detect the binding polypeptide, such as a labeled antibody reactive with the binding polypeptide or other suitable reagent (*e.g.*, labeled protein A).

10   In one embodiment, the binding polypeptides of the present invention can be utilized in enzyme immunoassays, wherein the subject polypeptides are conjugated to an enzyme. When a biological sample comprising a DLL-4 protein is combined with the subject binding polypeptides, binding occurs between the binding polypeptides and the DLL-4 protein. In one embodiment, a sample containing cells expressing a DLL-4 protein (*e.g.*, endothelial cells) is

15   combined with the subject antibodies, and binding occurs between the binding polypeptides and cells bearing a DLL-4 protein recognized by the binding polypeptide. These bound cells can be separated from unbound reagents and the presence of the binding polypeptide-enzyme conjugate specifically bound to the cells can be determined, for example, by contacting the sample with a substrate of the enzyme which produces a color or other detectable change

20   when acted on by the enzyme. In another embodiment, the subject binding polypeptides can be unlabeled, and a second, labeled polypeptide (*e.g.*, an antibody) can be added which recognizes the subject binding polypeptide.

        In certain aspects, kits for use in detecting the presence of a DLL-4 protein in a biological sample can also be prepared. Such kits will include a DLL-4 binding polypeptide

25   which binds to a DLL-4 protein or portion of said receptor, as well as one or more ancillary reagents suitable for detecting the presence of a complex between the binding polypeptide and the receptor protein or portions thereof. The polypeptide compositions of the present invention can be provided in lyophilized form, either alone or in combination with additional antibodies specific for other epitopes. The binding polypeptides and/or antibodies, which can

30   be labeled or unlabeled, can be included in the kits with adjunct ingredients (*e.g.*, buffers, such as Tris, phosphate and carbonate, stabilizers, excipients, biocides and/or inert proteins, e.g., bovine serum albumin). For example, the binding polypeptides and/or antibodies can be provided as a lyophilized mixture with the adjunct ingredients, or the adjunct ingredients can be separately provided for combination by the user. Generally these adjunct materials will be

present in less than about 5% weight based on the amount of active binding polypeptide or antibody, and usually will be present in a total amount of at least about 0.001% weight based on polypeptide or antibody concentration. Where a second antibody capable of binding to the binding polypeptide is employed, such antibody can be provided in the kit, for instance in a

5   separate vial or container. The second antibody, if present, is typically labeled, and can be formulated in an analogous manner with the antibody formulations described above.

Similarly, the present disclosure also provides a method of detecting and/or quantitating expression of DLL-4, wherein a composition comprising a cell or fraction thereof (*e.g.*, membrane fraction) is contacted with a binding polypeptide which binds to a

10   DLL-4 or portion of the receptor under conditions appropriate for binding thereto, and the binding is monitored. Detection of the binding polypeptide, indicative of the formation of a complex between binding polypeptide and DLL-4 or a portion thereof, indicates the presence of the receptor. Binding of a polypeptide to the cell can be determined by standard methods, such as those described in the working examples. The method can be used to detect

15   expression of DLL-4 on cells from an individual. Optionally, a quantitative expression of DLL-4 on the surface of endothelial cells can be evaluated, for instance, by flow cytometry, and the staining intensity can be correlated with disease susceptibility, progression or risk.

The present disclosure also provides a method of detecting the susceptibility of a mammal to certain diseases. To illustrate, the method can be used to detect the susceptibility

20   of a mammal to diseases which progress based on the amount of DLL-4 present on cells and/or the number of DLL-4-positive cells in a mammal. In one embodiment, the invention relates to a method of detecting susceptibility of a mammal to a tumor. In this embodiment, a sample to be tested is contacted with a binding polypeptide which binds to a DLL-4 or portion thereof under conditions appropriate for binding thereto, wherein the sample

25   comprises cells which express DLL-4 in normal individuals. The binding and/or amount of binding is detected, which indicates the susceptibility of the individual to a tumor, wherein higher levels of receptor correlate with increased susceptibility of the individual to a tumor.

Polypeptide sequences are indicated using standard one- or three-letter abbreviations. Unless otherwise indicated, each polypeptide sequence has amino termini at the left and a

30   carboxy termini at the right; each single-stranded nucleic acid sequence, and the top strand of each double-stranded nucleic acid sequence, has a 5' termini at the left and a 3' termini at the right. A particular polypeptide sequence also can be described by explaining how it differs from a reference sequence.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

The terms "DLL-4 inhibitor" and "DLL-4 antagonist" are used interchangeably. Each is a molecule that detectably inhibits at least one function of DLL-4. Conversely, a "DLL-4

5   agonist" is a molecule that detectably increases at least one function of DLL-4. The inhibition caused by a DLL-4 inhibitor need not be complete so long as it is detectable using an assay. Any assay of a function of DLL-4 can be used, examples of which are provided herein. Examples of functions of DLL-4 that can be inhibited by a DLL-4 inhibitor, or increased by a DLL-4 agonist, include cancer cell growth or apoptosis (programmed cell death), and so on.

10   Examples of types of DLL-4 inhibitors and DLL-4 agonists include, but are not limited to, DLL-4 binding polypeptides such as antigen binding proteins (e.g., DLL-4 inhibiting antigen binding proteins), antibodies, antibody fragments, and antibody derivatives.

The terms "peptide," "polypeptide" and "protein" each refers to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These

15   terms encompass, e.g., native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, and fusion proteins) of a protein sequence as well as post-translationally, or otherwise covalently or non-covalently, modified proteins. A peptide, polypeptide, or protein may be monomeric or polymeric.

A "variant" of a polypeptide (for example, an antibody) comprises an amino acid

20   sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. Disclosed variants include, for example, fusion proteins.

A "derivative" of a polypeptide is a polypeptide (e.g., an antibody) that has been chemically modified, e.g., via conjugation to another chemical moiety (such as, for example,

25   polyethylene glycol or albumin, e.g., human serum albumin), phosphorylation, and glycosylation. Unless otherwise indicated, the term "antibody" includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below.

An "antigen binding protein" is a protein comprising a portion that binds to an antigen

30   and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen binding protein to the antigen. Examples of antigen binding proteins include antibodies, antibody fragments (e.g., an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial

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scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et

5   al., 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129; Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold.

An antigen binding protein can have, for example, the structure of a naturally

10   occurring immunoglobulin. An "immunoglobulin" is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal

15   portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa or lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Preferably, the anti-DLL-4 antibodies disclosed herein are characterized by their variable domain region sequences in the heavy V_H and light V_L

20   amino acid sequences. The preferred antibody is A6 which is a kappa IgG antibody. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). The variable regions of each light/heavy chain pair form the antibody

25   binding site such that an intact immunoglobulin has two binding sites.

A "multi-specific antibody" is an antibody that recognizes more than one epitope on one or more antigens. A subclass of this type of antibody is a "bi-specific antibody" which recognizes two distinct epitopes on the same or different antigens.

An antigen binding protein "specifically binds" to an antigen (*e.g.*, human DLL-4) if it

30   binds to the antigen with a dissociation constant of 1 nanomolar or less.

An "antigen binding domain, "antigen binding region," or "antigen binding site" is a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and

affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

An "epitope" is the portion of a molecule that is bound by an antigen binding protein (*e.g.*, by an antibody). An epitope can comprise non-contiguous portions of the molecule

5   (*e.g.*, in a polypeptide, amino acid residues that are not contiguous in the polypeptide's primary sequence but that, in the context of the polypeptide's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein).

The "percent homology" of two polynucleotide or two polypeptide sequences is determined by comparing the sequences using the GAP computer program (a part of the GCG

10   Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif.)) using its default parameters.

A "host cell" is a cell that can be used to express a nucleic acid. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (*e.g.*, a yeast or other fungus), a plant cell (*e.g.*, a tobacco or tomato plant cell), an animal cell (*e.g.*, a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an

15   insect cell) or a hybridoma. Examples of host cells include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., 1981, *Cell* 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (Rasmussen et al., 1998, *Cytotechnology* 28:31) or CHO strain DX-B11, which is deficient in DHFR (Urlaub et al.,

20   1980, *Proc. Natl. Acad. Sci. USA* 77:4216-20), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (McMahan et al., 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293,293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro

25   culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not

30   express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g.,

mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Antigen Binding Proteins

　　　　Antigen binding proteins (*e.g.*, antibodies, antibody fragments, antibody derivatives,

5   antibody muteins, and antibody variants) are polypeptides that bind to DLL-4, (preferably, human DLL-4). Antigen binding proteins include antigen binding proteins that inhibit a biological activity of DLL-4.

　　　　Oligomers that contain one or more antigen binding proteins may be employed as DLL-4 antagonists. Oligomers may be in the form of covalently-linked or non-covalently-

10   linked dimers, trimers, or higher oligomers. Oligomers comprising two or more antigen binding protein are contemplated for use, with one example being a homodimer. Other oligomers include heterodimers, homotrimers, heterotrimers, homotetramers, heterotetramers, etc.

　　　　One embodiment is directed to oligomers comprising multiple antigen binding

15   proteins joined via covalent or non-covalent interactions between peptide moieties fused to the antigen binding proteins. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of antigen binding proteins attached thereto, as described in more detail below.

20   　　　　In particular embodiments, the oligomers comprise from two to four antigen binding proteins. The antigen binding proteins of the oligomer may be in any form, such as any of the forms described above, *e.g.*, variants or fragments. Preferably, the oligomers comprise antigen binding proteins that have DLL-4 binding activity.

　　　　In one embodiment, an oligomer is prepared using polypeptides derived from

25   immunoglobulins. Preparation of Fusion Proteins Comprising Certain Heterologous Polypeptides Fused to Various Portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:10535; Byrn et al., 1990, *Nature* 344:677; and Hollenbaugh et al., 1992 "Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages

30   10.19.1-10.19.11.

　　　　One embodiment is directed to a dimer comprising two fusion proteins created by fusing a DLL-4 binding fragment of an anti- DLL-4 antibody to the Fc region of an antibody. The dimer can be made by, for example, inserting a gene fusion encoding the fusion protein into an appropriate expression vector, expressing the gene fusion in host cells transformed

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with the recombinant expression vector, and allowing the expressed fusion protein to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield the dimer.

The term "Fc polypeptide" includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization also are included. Fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

Another method for preparing oligomeric antigen binding proteins involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., 1988, *Science* 240:1759), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al., 1994, *FEBS Letters* 344:191. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al., 1994, *Semin. Immunol.* 6:267-78. In one approach, recombinant fusion proteins comprising an anti-DLL-4 antibody fragment or derivative fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric anti- DLL-4 antibody fragments or derivatives that form are recovered from the culture supernatant.

The present disclosure provides a DLL-4 binding antigen binding protein (for example, an anti- DLL-4 antibody), that has one or more of the following characteristics: binds to both human and murine DLL-4, inhibits the activation of human DLL-4, inhibits the activation of murine DLL-4, binds to or near the proteolytic cleavage site of DLL-4, causes relatively little down-regulation of cell-surface expressed DLL-4.

Antigen-binding fragments of antigen binding proteins of the invention may be produced by conventional techniques. Examples of such fragments include, but are not limited to, Fab and F(ab')$_2$ fragments.

The present disclosure provides monoclonal antibodies that bind to DLL-4. Monoclonal antibodies may be produced using any technique known in the art, *e.g.*, by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells can be immortalized using any technique known in

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the art, *e.g.*, by fusing them with myeloma cells to produce hybridomas. Myeloma cells for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells

5   (hybridomas). Examples of suitable cell lines for use in mouse fusions include Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; examples of cell lines used in rat fusions include R210.RCY3, Y3-Ag 1.2.3, IR983F and 48210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6.

10   Antigen binding proteins directed against DLL-4 can be used, for example, in assays to detect the presence of DLL-4 polypeptides, either *in vitro* or *in vivo*. The antigen binding proteins also may be employed in purifying DLL-4 proteins by immunoaffinity chromatography. Blocking antigen binding proteins can be used in the methods disclosed herein. Such antigen binding proteins that function as DLL-4 antagonists may be employed in

15   treating any DLL-4-induced condition, including but not limited to various cancers.

Antigen binding proteins may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit DLL-4-induced biological activity. Disorders caused or exacerbated (directly or indirectly) by the proteolytic activation of DLL-4, examples of which are provided herein, thus may be treated. In one embodiment, the present invention provides a

20   therapeutic method comprising *in vivo* administration of a DLL-4 blocking antigen binding protein to a mammal in need thereof in an amount effective for reducing an DLL-4-induced biological activity.

Antigen binding proteins include fully human monoclonal antibodies that inhibit a biological activity of DLL-4.

25   Antigen binding proteins may be prepared by any of a number of conventional techniques. For example, they may be purified from cells that naturally express them (*e.g.*, an antibody can be purified from a hybridoma that produces it), or produced in recombinant expression systems, using any technique known in the art. See, for example, Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Kennet et al. (eds.),

30   Plenum Press, New York (1980); and Antibodies: A Laboratory Manual, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988).

Any expression system known in the art can be used to make the recombinant polypeptides of the invention. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired polypeptide. Among the host cells

that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *bacilli*. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651)

5   (Gluzman et al., 1981, *Cell* 23:175), L cells, 293 cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, BHK (ATCC CRL 10) cell lines, and the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC: CCL 70) as described by McMahan et al., 1991, *EMBO J.* 10: 2821. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular

10   hosts are described by Pouwels et al. (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985).

The transformed cells can be cultured under conditions that promote expression of the polypeptide, and the polypeptide recovered by conventional protein purification procedures. One such purification procedure includes the use of affinity chromatography, *e.g.*, over a

15   matrix having all or a portion (*e.g.*, the extracellular domain) of DLL-4 bound thereto. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian anti- DLL-4 antibody polypeptides substantially free of contaminating endogenous materials.

Antigen binding proteins may be prepared, and screened for desired properties, by any

20   of a number of known techniques. Certain of the techniques involve isolating a nucleic acid encoding a polypeptide chain (or portion thereof) of an antigen binding protein of interest (*e.g.*, an anti- DLL-4 antibody), and manipulating the nucleic acid through recombinant DNA technology. The nucleic acid may be fused to another nucleic acid of interest, or altered (*e.g.*, by mutagenesis or other conventional techniques) to add, delete, or substitute one or more

25   amino acid residues, for example.

Single chain antibodies may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (V_L

30   and V_H). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (*e.g.*, dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt et al., 1997, *Prot. Eng.* 10:423; Kortt et al., 2001, *Biomol. Eng.* 18:95-108). By combining different V_L and V_H-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes

(Kriangkum et al., 2001, *Biomol. Eng.* 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Patent 4,946,778; Bird, 1988, *Science* 242:423; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879; Ward et al., 1989, *Nature* 334:544, de Graaf et al., 2002, *Methods Mol. Biol.* 178:379-87.

5   Techniques are known for deriving an antibody of a different subclass or isotype from an antibody of interest, *i.e.*, subclass switching. Thus, IgG antibodies may be derived from an IgM antibody, for example, and vice versa. Such techniques allow the preparation of new antibodies that possess the antigen-binding properties of a given antibody (the parent antibody), but also exhibit biological properties associated with an antibody isotype or
10   subclass different from that of the parent antibody. Recombinant DNA techniques may be employed. Cloned DNA encoding particular antibody polypeptides may be employed in such procedures, *e.g.*, DNA encoding the constant domain of an antibody of the desired isotype (Lantto et al., 2002, *Methods Mol. Biol.* 178:303-16). Moreover, if an IgG4 is desired, it may also be desired to introduce a point mutation (CPSCP->CPPCP) in the hinge region (Bloom
15   et al., 1997, *Protein Science* 6:407) to alleviate a tendency to form intra-H chain disulfide bonds that can lead to heterogeneity in the IgG4 antibodies.

In particular embodiments, antigen binding proteins of the present invention have a binding affinity (K_a) for DLL-4 of at least 10^6. In other embodiments, the antigen binding proteins exhibit a K_a of at least 10^7, at least 10^8, at least 10^9, or at least 10^{10}. In another
20   embodiment, the antigen binding protein exhibits a K_a substantially the same as that of an antibody described herein in the Examples.

In another embodiment, the present disclosure provides an antigen binding protein that has a low dissociation rate from DLL-4. In one embodiment, the antigen binding protein has a K_{off} of 1×10^{-4} to $^{-1}$ or lower. In another embodiment, the K_{off} is 5×10^{-5} to $^{-1}$ or lower.
25   In another embodiment, the K_{off} is substantially the same as an antibody described herein. In another embodiment, the antigen binding protein binds to DLL-4 with substantially the same K_{off} as an antibody described herein.

In another aspect, the present disclosure provides an antigen binding protein that inhibits an activity of DLL-4. In one embodiment, the antigen binding protein has an IC_{50} of
30   1000 nM or lower. In another embodiment, the IC_{50} is 100 nM or lower; in another embodiment, the IC_{50} is 10 nM or lower. In another embodiment, the IC_{50} is substantially the same as that of an antibody described herein in the Examples. In another embodiment, the antigen binding protein inhibits an activity of DLL-4 with substantially the same IC_{50} as an antibody described herein.

In another aspect, the present disclosure provides an antigen binding protein that binds to human DLL-4 expressed on the surface of a cell and, when so bound, inhibits DLL-4 signaling activity in the cell without causing a significant reduction in the amount of DLL-4 on the surface of the cell. Any method for determining or estimating the amount of DLL-4 on the surface and/or in the interior of the cell can be used. In other embodiments, binding of the antigen binding protein to the DLL-4-expressing cell causes less than about 75%, 50%, 40%, 30%, 20%, 15%, 10%, 5%, 1%, or 0.1% of the cell-surface DLL-4 to be internalized.

In another aspect, the present disclosure provides an antigen binding protein having a half-life of at least one day *in vitro* or *in vivo* (*e.g.*, when administered to a human subject). In one embodiment, the antigen binding protein has a half-life of at least three days. In another embodiment, the antigen binding protein has a half-life of four days or longer. In another embodiment, the antigen binding protein has a half-life of eight days or longer. In another embodiment, the antigen binding protein is derivatized or modified such that it has a longer half-life as compared to the underivatized or unmodified antigen binding protein. In another embodiment, the antigen binding protein contains one or more point mutations to increase serum half life, such as described in WO00/09560, incorporated by reference herein.

The present disclosure further provides multi-specific antigen binding proteins, for example, bispecific antigen binding protein, *e.g.*, antigen binding protein that bind to two different epitopes of DLL-4, or to an epitope of DLL-4 and an epitope of another molecule, via two different antigen binding sites or regions. Moreover, bispecific antigen binding protein as disclosed herein can comprise a DLL-4 binding site from one of the herein-described antibodies and a second DLL-4 binding region from another of the herein-described antibodies, including those described herein by reference to other publications. Alternatively, a bispecific antigen binding protein may comprise an antigen binding site from one of the herein described antibodies and a second antigen binding site from another DLL-4 antibody that is known in the art, or from an antibody that is prepared by known methods or the methods described herein.

Numerous methods of preparing bispecific antibodies are known in the art. Such methods include the use of hybrid-hybridomas as described by Milstein et al., 1983, *Nature* 305:537, and chemical coupling of antibody fragments (Brennan et al., 1985, *Science* 229:81; Glennie et al., 1987, *J. Immunol.* 139:2367; U.S. Patent 6,010,902). Moreover, bispecific antibodies can be produced via recombinant means, for example by using leucine zipper moieties (*i.e.*, from the *Fos* and *Jun* proteins, which preferentially form heterodimers; Kostelny et al., 1992, *J. Immunol.* 148:1547) or other lock and key interactive domain

structures as described in U.S. Patent 5,582,996. Additional useful techniques include those described in U.S. Patents 5,959,083; and 5,807,706.

In another aspect, the antigen binding protein comprises a derivative of an antibody. The derivatized antibody can comprise any molecule or substance that imparts a desired

5   property to the antibody, such as increased half-life in a particular use. The derivatized antibody can comprise, for example, a detectable (or labeling) moiety (*e.g.*, a radioactive, colorimetric, antigenic or enzymatic molecule, a detectable bead (such as a magnetic or electrodense (*e.g.*, gold) bead), or a molecule that binds to another molecule (*e.g.*, biotin or streptavidin), a therapeutic or diagnostic moiety (*e.g.*, a radioactive, cytotoxic, or

10   pharmaceutically active moiety), or a molecule that increases the suitability of the antibody for a particular use (*e.g.*, administration to a subject, such as a human subject, or other *in vivo* or *in vitro* uses). Examples of molecules that can be used to derivatize an antibody include albumin (*e.g.*, human serum albumin) and polyethylene glycol (PEG). Albumin-linked and PEGylated derivatives of antibodies can be prepared using techniques well known in the art.

15   In one embodiment, the antibody is conjugated or otherwise linked to transthyretin (TTR) or a TTR variant. The TTR or TTR variant can be chemically modified with, for example, a chemical selected from the group consisting of dextran, poly(n-vinyl pyurrolidone), polyethylene glycols, propropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols and polyvinyl alcohols.

20   Pharmaceutical compositions comprising the antibodies and fragments thereof of the disclosure are administered to a subject in a manner appropriate to the indication. Pharmaceutical compositions may be administered by any suitable technique, including but not limited to, parenterally, topically, or by inhalation. If injected, the pharmaceutical composition can be administered, for example, via intra-articular, intravenous, intramuscular,

25   intralesional, intraperitoneal or subcutaneous routes, by bolus injection, or continuous infusion. Localized administration, *e.g.* at a site of disease or injury is contemplated, as are transdermal delivery and sustained release from implants. Delivery by inhalation includes, for example, nasal or oral inhalation, use of a nebulizer, inhalation of the antagonist in aerosol form, and the like. Other alternatives include eyedrops; oral preparations including pills,

30   syrups, lozenges or chewing gum; and topical preparations such as lotions, gels, sprays, and ointments.

Use of antigen binding proteins in *ex vivo* procedures also is contemplated. For example, a patient's blood or other bodily fluid may be contacted with an antigen binding

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protein that binds DLL-4 *ex vivo*. The antigen binding protein may be bound to a suitable insoluble matrix or solid support material.

Advantageously, antigen binding proteins are administered in the form of a composition comprising one or more additional components such as a physiologically acceptable carrier, excipient or diluent. Optionally, the composition additionally comprises one or more physiologically active agents, for example, a second inflammation- or immune-inhibiting substance, an anti-angiogenic substance, an analgesic substance, etc., non-exclusive examples of which are provided herein. In various particular embodiments, the composition comprises one, two, three, four, five, or six physiologically active agents in addition to an DLL-4 binding antigen binding protein.

EXAMPLE 1

This example illustrates *in vitro* data for cell binding as part of an initial screen for several disclosed antibodies. CHO –K1 cells stably expressing DLL-4 (CHO-DLL-4 cells) were lifted in enzyme-free Dissociation Buffer and transferred to V-Bottom 96 well-plates (100,000 cells/ well). Cells were incubated with anti-DLL-4 Antibodies in FACS buffer (PBS+ 2% FBS) + NaN$_3$ on ice for 45 min. After 2 washes in FACS buffer, a 1:1000 dilution of Phycoerythrin conjugated anti-Human IgG (γ-chain specific) was added and incubated for 30 min. Following a final wash, fluorescence intensity was measured on an Intellicyt High Throughput Flow Cytometer (HTFC). Data were analyzed using Graphpad Prism software using non-linear regression fit. Data points are shown as the median fluorescence intensity (MFI) of positively labeled cells +/- Standard Error. EC$_{50}$ values are reported as the concentration of antibody to achieve 50% of maximal DLL-4 antibodies binding to DLL-4 expressing cells.

Results: This example shows that the disclosed anti-DLL-4 monoclonal antibodies were able to bind efficiently to DLL-4 expressed on the surface of cells. Several disclosed antibodies showed more potent binding characteristics compared to anti-DLL-4 antibody OMP21M18 (Oncomed), currently in clinical trial. EC$_{50}$ values were calculated for the disclosed anti- DLL4 antibodies C3, C10 and G6, and range from 0.1 to 0.3 nM, compared to 1.7 nM for OMP21M18 (Figure 2).

EXAMPLE 2

This example illustrates an antigen binding affinity determination for disclosed anti-DLL4 antibodies C3, C6 and G6. Affinity Determination Using BIACORE® Surface Plasmon Resonance Technology. Recombinant hDLL4 was immobilized on CM5 sensor chip

using standard NHS/EDC coupling methodology. All measurements were conducted in HBS-EP buffer with a flow rate of 30 μL/min. The antibody was diluted so as to obtain a series of concentrations. a 1:1 (Langmuir) binding model was used to fit the data.

     Epitope mapping of anti-DLL4 antibodies were done on Octet. AntiDLL-4 antibodies

5   C3, G6 and OMP21M18 were immobilized on AR2G sensor with amine-coupling kit, loaded rhDLL4, then ran different antibodies. Additional binding would indicate that the antibodies bind to different epitope.

     Results: This example illustrates an additional binding registered by Octet, indicating that C3, C5 and G6 antibodies bind to an epitope of DLL-4 that is not occupied by

10   OMP21M18.

EXAMPLE 3

     This example shows the blocking of DLL4 binding to Notch-1 by disclosed anti-DLL4- antibodies. Human DLL-4 is a type I transmembrane protein exclusively expressed in endothelial cells. DLL-4 is a ligand for the membrane bound Notch-1 receptor. Upon

15   binding to Notch-1, DLL-4 activates Notch signaling pathway, which plays a major role in angiogenesis and tumor vascular development. Blocking of this interaction would inhibit Notch-1 function in cells. The blocking was first evaluated in an ELISA-based immunoassay (Figure 4A). Briefly, rat recombinant Notch (5 μg/ml) was coated onto the wells of micro-titer plates at 4 °C overnight and the plate was then blocked with casein-PBS buffer. His-

20   tagged-rhDLL-4 (4μg/ml) was pre-incubated with the anti-DLL-4 antibodies for 30 min, then transferred to the Notch-coated plate and incubated for 0.5 hr with shaking. An HRP-conjugated anti-his-tag antibody was used to detect binding of rhDLL-4 to Notch-1.

     In parallel, the ability of anti-DLL-4 antibodies to block the binding of soluble recombinant Notch-1 protein to DLL-4-expressing CHO-K1 cells was assessed by HTFC.

25   CHO-DLL-4 cells were lifted with enzyme-free Cell Dissociation Buffer (GIBCO), and incubated in FACS buffer + Azide with 20 μg/ml FITC-rhNotch-1 in the presence or absence of anti-DLL-4 antibodies at fixed (Figure 4B) or increasing (Figure 4C) concentrations. Data were analyzed by flow cytometry.

     Results: Figure 4A illustrates that 3 of the disclosed anti-DLL-4 antibodies (C3, C10

30   and G6) were as efficient as OMP21M18 at blocking the binding of DLL-4 to Notch-1. This assay was repeated in a cellular context (Figure 4B), that confirms that C3, C10 and G6 are able to efficiently inhibit the interaction of human DLL-4 with both Human and Rat Notch-1. These three antibodies appear more efficient (IC_{50} values ranging from 2 to 6 nM) than OMP21M18 (IC50=1.72 nM) (Figure 4C).

EXAMPLE 4

This example shows the effect of anti-DLL-4 antibodies on Notch-1 pathway activation. Upon binding by DLL-4, the Notch-1 intracellular domain (NICD) is cleaved and translocates to the nucleus to activate the expression of specific target genes. Here we show

5   that anti-DLL-4 antibodies inhibit DLL-4-mediated Notch-1 pathway activation and modulate expression of Notch target genes in a breast cancer cell Line. MCF7 cells expressing Notch-1 were co-incubated for 24 hr with CHO cells either over-expressing Human DLL-4 (DLL-4) or not (No DLL-4). Cells were then harvested and total RNA was extracted with Trizol (Life Technology) according to manufacturer's recommendations. Gene

10   expression was analyzed by RT-PCR using Promega's Access RT-PCR System kit, according to the Company's instructions. The forward and reverse primers were as follows: Notch-1 Forward CACTGTGGGCGGGTCC SEQ ID NO. 41, Notch-1 Reverse: GTTGTATTGGTTCGGCACCAT SEQ ID NO. 42, HEY-1 Forward: GGAGAGGCGCCGCTGTAGTTA SEQ ID NO. 43, HEY-1 Reverse:

15   CAAGGGCGTGCGCGTCAAAGTA, SEQ ID NO. 44 GAPDH Forward: GGACCTGACCTGCCGTCTAGAA, SEQ ID NO. 45 GAPDH Reverse: GGTGTCGCTGTTGAAGTCAGAG SEQ ID NO. 46. PCR samples were run on a 3% agarose gel and bands intensities were quantified using Image Lab Software (BIORAD). Human GAPDH was used as a reference gene for normalization.

20   Results: Figure 5 shows that the presence of DLL-4 stimulated Hey-1 and Notch-1 expression, and that anti-DLL-4 antibody C3 efficiently inhibited DLL-4 stimulation of Notch-1 target gene regulation.

EXAMPLE 5

This example shows an *in vitro* cell proliferation assay illustrating the ability of the

25   disclosed anti-DLL-4 antibodies to antagonize DLL-4-mediated growth inhibition of Human Umbilical Vein Endothelial Cells (HUVEC). Angiogenesis is a complex finely regulated process. DLL-4 inhibits VEGF-mediated endothelial cell proliferation, and is induced by VEGF as a negative feedback regulator, thus preventing overly extensive angiogenic sprouting. It was previously shown that inhibition of DLL-4 rendered endothelial cells

30   hyperproliferative, causing defective cell fate specification or differentiation both *in vitro* and *in vivo*. Hence, blocking DLL-4 was shown to inhibit tumor growth in several tumor models. In this assay, low passage HUVEC cells were obtained from LONZA and cultured in EGM™-2 media (LONZA). One day prior to experiment, 96 well-plates were coated at 4 °C overnight with 0.1% Gelatin + 2 μg/ml rHuDLL-4 (SinoBiologics) or 0.1% Bovine Serum

Albumin (BSA, SIGMA). Then cells were lifted with StemPro Accutase (Life Technology) and added to the plates at 5,000 cells/ well in normal culture media, and returned for 3 days incubation at 37 °C. Results were analyzed using a standard MTT assay.

5   Results: Figure 6 shows that HUVEC proliferation was inhibited when cells were cultured on immobilized DLL-4, and that disclosed anti-DLL-4 antibodies (C3, C10 and G6) were capable of restoring HUVEC proliferation. This antagonism of DLL-4 function was as efficient, or more potent than Oncomed's OMP21M18 antibody.

Table 1

10   Human Anti-Dll-4 antibody sequences variable domain regions

Clones	Heavy chain	Light chain
C3	QVQLVQSGSELKKPGASVKVSCKAS GYTFTSYYMHWVRQVPGQGLEWMG IINPSGGSTSYAQKFQGRVTMTRDTS TSTVYMELSSLRSEDTAVYYCATDYY DSSGYVDFDYWGQGTLVTVSS SEQ ID No.1	QSVLTQPPSASGTPGQRVTISCSGSSSNIG SNTVNWYQQFPGTAPKLLIYRNNQRPSGV PDRFSGSKSGTSASLAISGLQSEDEADYY CAAWDDSPNGPVFGGGTKLTVL SEQ ID No.2
C5	QVQLQQSGPGLVKPSQTLSLTCAISG DSVSSNSAAWNWIRQSPSRGFEWL GRTEYRSKWYNDYAVSVESRITINPD TSKNHFSLQLNSVTPEDTAVYYCARD GNTFSFDYWGQGTLVTVSS SEQ ID NO. 3	QSVVTQPPSVSAAPGQKVTISCSGGSSNI GNNYVSWYQQIPGTAPKLLIYDNNNRPSGI PDRFSGSKSGASATLGISGLQTGDEANYY CGAWDSTLSGYVFGTGTKLTVL SEQ ID NO. 4
C10	QVQLVESGAEVKKPGASVKVSCKAS GYTFTSYYMHWVRQAPQGLEWMGII NPSGGSTSYAQKFQGRVTMTRDTST NTVYMELSSLRSEDTAVYYCARDVW GGYFDYWGQGTLVTVSS SEQ ID NO. 5	QSVLTQPASVSGSPGQSITISCTGTSSDVG GYNYVSWYQQHPGKAPKLMIYDVTNRPS GVSNRFSGSKSGNTASLTISGLQAEDEAD YYCSSYTSSSTVIFGGGTKLTVL SEQ ID NO. 6
G6	EVQLVESGGSLVQPGGSLRLSCAAS GFTFSNGWMTWLRQAPGKGLEWVA TIKPDGSDTAYVESVKGRFTISRDNA KNLLYLQMDSLRGDDTAVYFCARDL AYNAFDIWGQGTMVTVSS SEQ ID NO. 7	QSVLTQPPSASGTPGQRVTISCSGSSSNIG SNTVNWYQQLPGTAPKLLIYSNNQRPSGV PDRFSGSKSGTSASLAISGLQSEDEADYY CAAWDGSLNGYVFGTGTKLTVL SEQ ID NO. 8
F11	EVQLVESGAEVKKPGASVKVSCKAS GYTFTSYYMHWVRQAPGQGLEWMG IISPSGDSTSYAQKFQGRVTMTKDTS TSTVSMELSSLRSEDTAVYYCARDQ EGLRGSGYYGMDVWGQGTTVTVSS SEQ ID NO.9	SQPVLTQPASVSGSPGQSITISCTGTSSDI GDYNYVSWYQQHPGKAPKLMIYEVSKRPS GVPDRFSGSKSGNTASLTISGLQAEDEAD YYCYSYAGSYTYVFGTGTKLTV SEQ ID NO. 10
D2	QVQLVESGAEVKKPGASVKVSCKAS GYTFTTYFIHWVRQAPGQGLEWMGII NPSGGSTSYAQKFQGRVTMTRDTST STVYMELSSLRSEDTAVYYCAREFM ATGGFDYWGQGTLVTVSS SEQ ID NO. 11	QPVLTQPASVSGSPGQSITIPCTGTRSDVG GYNFVSWYQQHPGKAPKLLIYDNNKRPSG IPGRFSGSKSGTSATLGITGLQTGDEADYY CGTWDDSLNVWVFGGGTKVTVL SEQ ID NO. 12
E10	EVQLVQSGAEVKKPGASVKVSCKAS GYSFMNYDVTWVRQAAQGLEWMG	AIRMTQSPSFLSASVGDRITITCRASQDISA YLAWYQQKPGTAPKVLIYAASTLQSGVPS

	WMNPDSGNTGYADQFQGRITMTRD TSKSTAYMELTSLRSDDTAVYFCARA EVEVPGYYYKYGMDVWGQGTTVTV SS SEQ ID NO. 13	RFSGSGSGTEFTLTISSLQPEDFATYYCQQ LYDYLPITFGPGTKVDIK SEQ ID NO. 14
F12	QVQLVQSGAEVKKPGESVIISCKSSG FTFTRTAIHWMRQAPGQSFEWVGWI RGSNGDTSYSQKFRDKVTVTADTFS STSYLALNRLTSEDTAVYYCAREHPT SWDPDFWGQGTLVTVSS SEQ ID NO. 15	LPVLTQPRSVSGSPGQSVTISCTGTSSDV GGYNYVSWYQHHPGKAPKLMIYDVTKRPS GVPDRFSGSKSGNTASLTISGLQAEDEAD YYCSSYTSSSTPYVFGTGTKVTVL SEQ ID NO. 16
E5	QVQLQQSGPGLVQPSQTLSLTCVIS GDSVSNNNAAWTWIRQSPSRGLEW LGRTYYRSQWYSDYAVSVKSRMTIN PDTSKNQFSLQLNSLTPEDTAVYYCA REEVMDHDAFDIWGPGTMVTVSS SEQ ID NO. 17	LPVLTQSPSASGTPGQRVTISCSGSTSNIG SNTVNWYQQFPGTAPKLLIYYNDQRPSGV PDRFSGSKSGTSASLAISGLQSEDEADYY CAAWDDRLYGRLFGGGTKLIVL SEQ ID NO. 18
H2	EVQLLESGGGLVQPGGSLRLSCAAS GFNVSKNYMSWVRQAPGKGLEWVS SMSSSSSYKYYADSVKGRFTISRDNA KNSLYLQMNSLRAEDTAVYYCAREN DREAFDIWGQGTMVTVSS SEQ ID NO. 19	EIVMTQSPSSLSASVGDRVTITCRASQSISS YLNWYQQKPGKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQ SYSTPLTFGQGTRLEIK SEQ ID NO. 20
A1	QVQLQQSGPGLVKPSQTLSLTCAISG DSVSSNGVAWNWIRQSPSRGLEWL GRTYFNSKWYNDYAVSVESRITINPD TSKNQFSLQLNSVTPEDTAVYYCAR GRVSAFDYWGQGTPVTVSS SEQ ID NO. 21	QPVLTQPASVSGSPGQSITISCTGTSNDVG SYDLVSWYQQHPGKAPKLMTYDVSNRPS GVSNRFSGAKSGNTASLTISGLQAEDEAD YYCSSYTTSSTVVFGGGTKLTVL SEQ ID NO. 22
A2	EVQLVQSGAEVKKPGASVKVSCKAS GYTFTSDEINWVRQATGQGLEWLG WMNPHSGNTGYAQKFQGRVTMTRN TSISTADMELSSLTSDDTAVYYCARG HYYESSGYFFYGMDVWGQGTTVTV SS SEQ ID NO. 23	QPVLTQPASVSGSPGQSITISCTGTSSDVG GYNYVSWFQQHPGKAPKLMIYDVNNRPS GVSNRFSGSKSGNTASLTISGLQAEDGAD YYCSSYTSSSTYVFGTGTKVTVL SEQ ID NO. 24
A11	QVQLQQSGPGLVKPSQTLSLTCAISG DSVSSNSAAWNWIRQSPSRGLEWL GRTYYRSKWYNDYAVSVKSRITINPD TSKNQFSLQLNSVTPEDTAVYYCARE GDDYGDHFDYWGQGTLVTVSS SEQ ID NO. 25	QSVLTQPPSVSEAPRQGVTISCSGSRSNIG NNPVSWYQQVPGKPPKLLIYFDDLLPSGV SDRFSASKSGTSASLAISGLQSDDEADYFC AAWDDSLNGRVFGGGTKLTVL SEQ ID NO. 26
C12	EVQLLESGAEVKKPGASVKVSCKAS GYTFTSYAMHWVRQAPGQRLEWLG HINAANGNTKYSQKFQGRVTITRDTS ASTAYMELSSLRSEDTAVYYCARAR SGSYLVDYWGQGTLVTVSS SEQ ID NO. 27	YVLTQPPSASGTPGQRVTISCSGSNSNIGS NAVNWYQHLPGTAPKLLIYSNNQRPSGVP DRFSGSKSGTSASLAISGLQSEDESDYYCI AWDGSLSGYVFGTGTKVTVL SEQ ID NO. 28
D9	QVQLVESGAEVRKPGASVKVSCKAS GYTFTDYAIHWVRQAPGQRLEWMG WINAGNGNTKYSQKFQGRVTITRDT SASTAYMELSSLRSEDAAVYYCARG NGSGSYLVDYWGQGTLVTVSS SEQ ID NO. 29	QSVVTQPPSLSAAPGQRVSISCSGTSSNIG KNYVSWYQQVPGTAPRLLIYDNNKRASGI PARFSGSKSATSATLDIAGLQTGDEADYFC ETWDSSLRAEIFGGGTKLTVL SEQ ID NO. 30
E3	QVQLVESGAEVKKPGASVKVSCKAS	SYELMQPHSVSESPGKTVTISCTRSSGNIA

	GYTFTSYPMHWVRQAPGQRLEWMG WINAGNGDTKYSQKFQDRVTITSDTS ASTAYMELSSLRSEDTAVYYCAKDYY TSGTYQIDYWGQGTLVTVSS SEQ ID NO. 31	SNYVRWYQQRRDSAPTVVIFDDDQKPSG VADRFSGSIDTSSNSASLTISGLNTDDEAA YYCHSDESSTVIFGGRTKLTVL SEQ ID NO. 32
E6	EVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYTMHWVRQAPGQRLEWMG WINAGNGNTKYSQKFQGRVTITRDTF ASTAYMELSSLRSEDTAVYYCARSR YNSGGSLVDYWGQGTLVTVSS SEQ ID NO. 33	QSVVTQPPSVSAAPGQKVTISCSGSSSNIE NNYVSWYQQLPGTAPKLLIYDNNKRPSGIP DRFSGSKSGTSATLGITGLQTGDEADYYC GTWDSSLSAEVFGTGTKVTVL SEQ ID NO.34
F1	QVQLVQSGAEVKKPGTSVKVSCKTS GFPFTTYFFHWARQAPGQRPEWMG WIHGGNGNTKYSQKFQGRVTITRDT SASTAYMELSSLRSEDAAVYYCARG NGSGSYLVDYWGQGTLVTVSS SEQ ID NO.35	QAGLTQPPSASGSPGQPVTISCSGTSGDV GGYDYVSWYQQHPGKAPKLIIYDVNKRPS GVPDRFSGSKSGNTASLTVSGLQAEDEAD YHCSSYAGSNNVIFGGGTKLTVL SEQ ID NO.36
F6	QVQLVESGAEVKKPGASVKVSCKAS GYTFTNFFMHWVRQAPGQGLEWMG VINPSGPGTTYPQKFQDRVTMTRDT STSTVYMELSSLRSDDTAVYYCARDL INSGWSGAFDIWGQGTMVTVSS SEQ ID NO.37	QPVLTQPPSVSVAPGNTASITCGENNIGSK SVHWYQQKPGQAPVLVIYDSDRPSGIPE RFSGSNSGNTATLTISRVEAGDEADYYCQ VWDSSSDSWVFGGGTKVTVL SEQ ID NO.38
F10	QVQLVQSGAEVKEPGASVKVSCKTS GFTFTQIYVHWVRQAPGQGLEWMG LVRPSGSSRIYGQNFQGRVTLTRDTS TSTVYMDLSSLTSEDTAVYYCVTDVA GYGDGRVWGQGTLVTVSS SEQ ID NO.39	QSVLTQPPSVSAAPGQKVTISCSGSSSNIA NSYVSWYQQLPGTAPKLLIYDNNQRPSGIP DRFSGSKSGTSATLGITGLQTGDEADYYC GTWDSSLSAGVFGTGTKLTVL SEQ ID NO.40

We claim:

1.   A fully human antibody of an IgG class that binds to a DLL-4 epitope with a
binding affinity of at least 10^{-6}M, that has a heavy chain variable domain sequence that is at
least 95% identical to the amino acid sequences selected from the group consisting of SEQ
ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11,
SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ
ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO.
33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has
a light chain variable domain sequence that is at least 95% identical to the amino acid
sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO.
6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ
ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO.
28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38,
SEQ ID NO. 40, and combinations thereof.

2.   The fully human antibody of claim 1, wherein the antibody has a heavy
chain/light chain variable domain sequence selected from the group consisting of SEQ ID
NO. 1/SEQ ID NO. 2 (called C3 herein), SEQ ID NO. 3/SEQ ID NO. 4 (called C5 herein),
SEQ ID NO. 5/SEQ ID NO. 6 (called C10 herein), SEQ ID NO. 7/SEQ ID NO. 8 (called G6
herein), SEQ ID NO. 9/SEQ ID NO. 10 (called F11 herein), SEQ ID NO. 11/SEQ ID NO. 12
(called D2 herein), SEQ ID NO. 13/SEQ ID NO. 14 (called E10 herein), SEQ ID NO.
15/SEQ ID NO. 16 (called F12 herein), SEQ ID NO. 17/SEQ ID NO. 18 (called E5 herein),
SEQ ID NO. 19/SEQ ID NO. 20 (called H2 herein), SEQ ID NO. 21/SEQ ID NO. 22 (called
A1 herein), SEQ ID NO. 23/SEQ ID NO. 24 (called A2 herein), SEQ ID NO. 25/SEQ ID
NO. 26 (called A11 herein), SEQ ID NO. 27/SEQ ID NO. 28 (called C12 herein), SEQ ID
NO. 29/SEQ ID NO. 30 (called D9 herein), SEQ ID NO. 31/SEQ ID NO. 32 (called E3
herein), SEQ ID NO. 33/SEQ ID NO. 34 (called E6 herein), SEQ ID NO. 35/SEQ ID NO. 36
(called F1 herein), SEQ ID NO. 37/SEQ ID NO. 38 (called F6 herein), SEQ ID NO. 39/SEQ
ID NO. 40 (called F10 herein), and combinations thereof.

3.   A Fab fully human antibody fragment, having a variable domain region from a
heavy chain and a variable domain region from a light chain, wherein the heavy chain
variable domain sequence that is at least 95% identical to the amino acid sequences selected
from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7,
SEQ ID NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID
NO. 19, SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO.

29, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and

combinations thereof, and that has a light chain variable domain sequence that is at least 95%

identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2,

SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID

5   NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO.

24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34,

SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof.

4.   The fully human antibody Fab fragment of claim 3, wherein the antibody has a

heavy chain/light chain variable domain sequence selected from the group consisting of SEQ

10 ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ

ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12,

SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID

NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO.

23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ

15 ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO.

34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ

ID NO. 40, and combinations thereof.

5.   A single chain human antibody, having a variable domain region from a heavy

chain and a variable domain region from a light chain and a peptide linker connection the

20 heavy chain and light chain variable domain regions, wherein the heavy chain variable

domain sequence that is at least 95% identical to the amino acid sequences selected from the

group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID

NO. 9, SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19,

SEQ ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ

25 ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and

combinations thereof, and that has a light chain variable domain sequence that is at least 95%

identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 2,

SEQ ID NO. 4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID

NO. 14, SEQ ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO.

30 24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34,

SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof.

6.   The fully human single chain antibody of claim 5, wherein the single chain

fully human antibody has a heavy chain/light chain variable domain sequence selected from

the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ

ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10,

SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID

NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO.

21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ

5   ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO.

32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ

ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof.

7.   The fully human single chain antibody of claim 5, wherein the fully human

single chain antibody has both a heavy chain variable domain region and a light chain

10   variable domain region, wherein the single chain fully human antibody has a heavy

chain/light chain variable domain sequence selected from the group consisting of SEQ ID

NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID

NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ

ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO.

15   18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ

ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO.

29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ

ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO.

40, and combinations thereof.

20   8.   A method for treating a broad spectrum of mammalian cancers, comprising

administering an effective amount of an anti-DLL-4 polypeptide, wherein the anti-DLL-4

polypeptide is selected from the group consisting of a fully human antibody of an IgG class

that binds to a DLL-4 epitope with a binding affinity of at least 10^{-6} M, a Fab fully human

antibody fragment, having a variable domain region from a heavy chain and a variable

25   domain region from a light chain, a single chain human antibody, having a variable domain

region from a heavy chain and a variable domain region from a light chain and a peptide

linker connection the heavy chain and light chain variable domain regions, and combinations

thereof;

wherein the fully human antibody has a heavy chain variable domain sequence that is

30   at least 95% identical to the amino acid sequences selected from the group consisting of SEQ

ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9, SEQ ID NO. 11,

SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ ID NO. 21, SEQ

ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO.

33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations thereof, and that has

a light chain variable domain sequence that is at least 95% identical to the amino acid

sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO.

6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16, SEQ

ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO. 26, SEQ ID NO.

5   28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36, SEQ ID NO. 38,

SEQ ID NO. 40, and combinations thereof;

wherein the Fab fully human antibody fragment has the heavy chain variable domain

sequence that is at least 95% identical to the amino acid sequences selected from the group

consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9,

10   SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ

ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO.

31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations

thereof, and that has the light chain variable domain sequence that is at least 95% identical to

the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO.

15   4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ

ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO.

26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36,

SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof; and

wherein the single chain human antibody has the heavy chain variable domain

20   sequence that is at least 95% identical to the amino acid sequences selected from the group

consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, SEQ ID NO. 9,

SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 17, SEQ ID NO. 19, SEQ

ID NO. 21, SEQ ID NO. 23, SEQ ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO.

31, SEQ ID NO. 33, SEQ ID NO. 35, SEQ ID NO. 37, SEQ ID NO. 39, and combinations

25   thereof, and that has the light chain variable domain sequence that is at least 95% identical to

the amino acid sequences selected from the group consisting of SEQ ID NO. 2, SEQ ID NO.

4, SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, SEQ ID NO. 12, SEQ ID NO. 14, SEQ

ID NO. 16, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 22, SEQ ID NO. 24, SEQ ID NO.

26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, SEQ ID NO. 36,

30   SEQ ID NO. 38, SEQ ID NO. 40, and combinations thereof.

9.   The method for treating a broad spectrum of mammalian cancers of claim 8,

wherein the fully human antibody has a heavy chain/light chain variable domain sequence

selected from the group consisting of SEQ ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID

NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID

NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO.

15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ

ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO.

26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ

5   ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO.

37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and combinations thereof.

       10.   The method for treating a broad spectrum of mammalian cancers of claim 8,

wherein the fully human antibody Fab fragment has both a heavy chain variable domain

region and a light chain variable domain region wherein the antibody has a heavy chain/light

10   chain variable domain sequence selected from the group consisting of SEQ ID NO. 1/SEQ ID

NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ ID NO. 7/SEQ ID

NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12, SEQ ID NO.

13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID NO. 18, SEQ

ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO. 23/SEQ ID NO.

15   24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ ID NO. 29/SEQ

ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO. 34, SEQ ID NO.

35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ ID NO. 40, and

combinations thereof.

       11.   The method for treating a broad spectrum of mammalian cancers of claim 8,

20   wherein the fully human single chain antibody has both a heavy chain variable domain region

and a light chain variable domain region, wherein the single chain fully human antibody has a

heavy chain/light chain variable domain sequence selected from the group consisting of SEQ

ID NO. 1/SEQ ID NO. 2, SEQ ID NO. 3/SEQ ID NO. 4, SEQ ID NO. 5/SEQ ID NO. 6, SEQ

ID NO. 7/SEQ ID NO. 8, SEQ ID NO. 9/SEQ ID NO. 10, SEQ ID NO. 11/SEQ ID NO. 12,

25   SEQ ID NO. 13/SEQ ID NO. 14, SEQ ID NO. 15/SEQ ID NO. 16, SEQ ID NO. 17/SEQ ID

NO. 18, SEQ ID NO. 19/SEQ ID NO. 20, SEQ ID NO. 21/SEQ ID NO. 22, SEQ ID NO.

23/SEQ ID NO. 24, SEQ ID NO. 25/SEQ ID NO. 26, SEQ ID NO. 27/SEQ ID NO. 28, SEQ

ID NO. 29/SEQ ID NO. 30, SEQ ID NO. 31/SEQ ID NO. 32, SEQ ID NO. 33/SEQ ID NO.

34, SEQ ID NO. 35/SEQ ID NO. 36, SEQ ID NO. 37/SEQ ID NO. 38, SEQ ID NO. 39/SEQ

30   ID NO. 40, and combinations thereof.

       12.   The method for treating a broad spectrum of mammalian cancers of claim 8,

wherein the broad spectrum of mammalian cancers to be treated is selected from the group

consisting of ovarian, colon, breast, lung cancers, myelomas, neuroblastic-derived CNS

tumors, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell

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derived lymphomas, T-cell derived lymphomas, mast cell derived tumors, and combinations thereof.
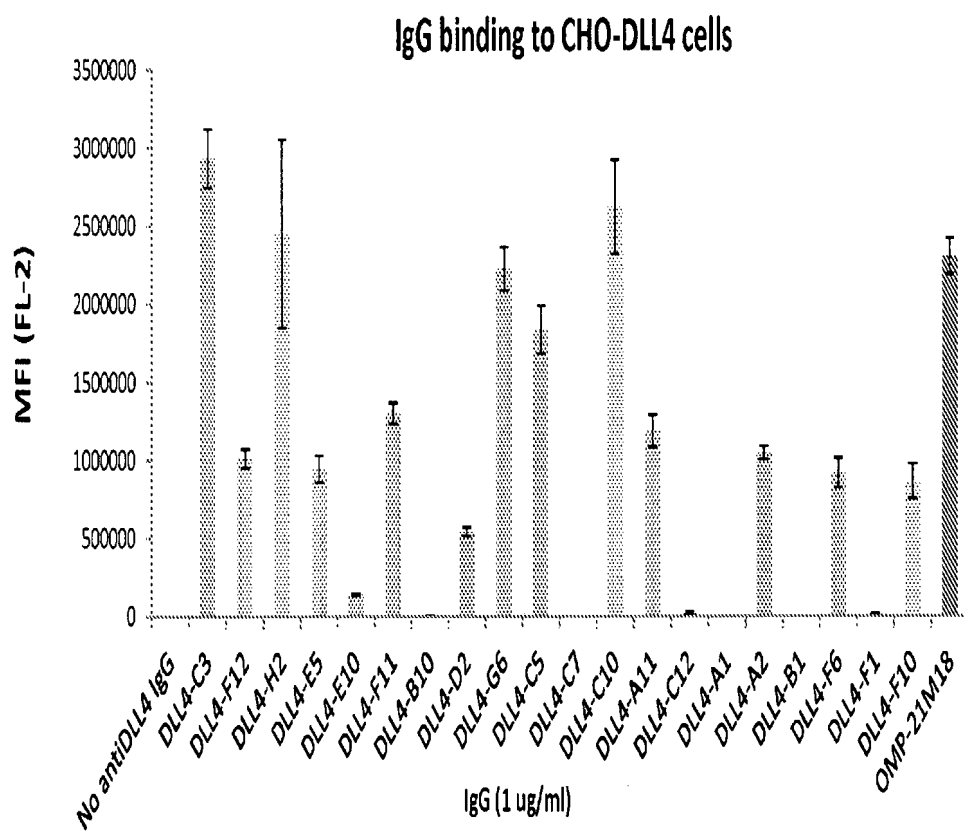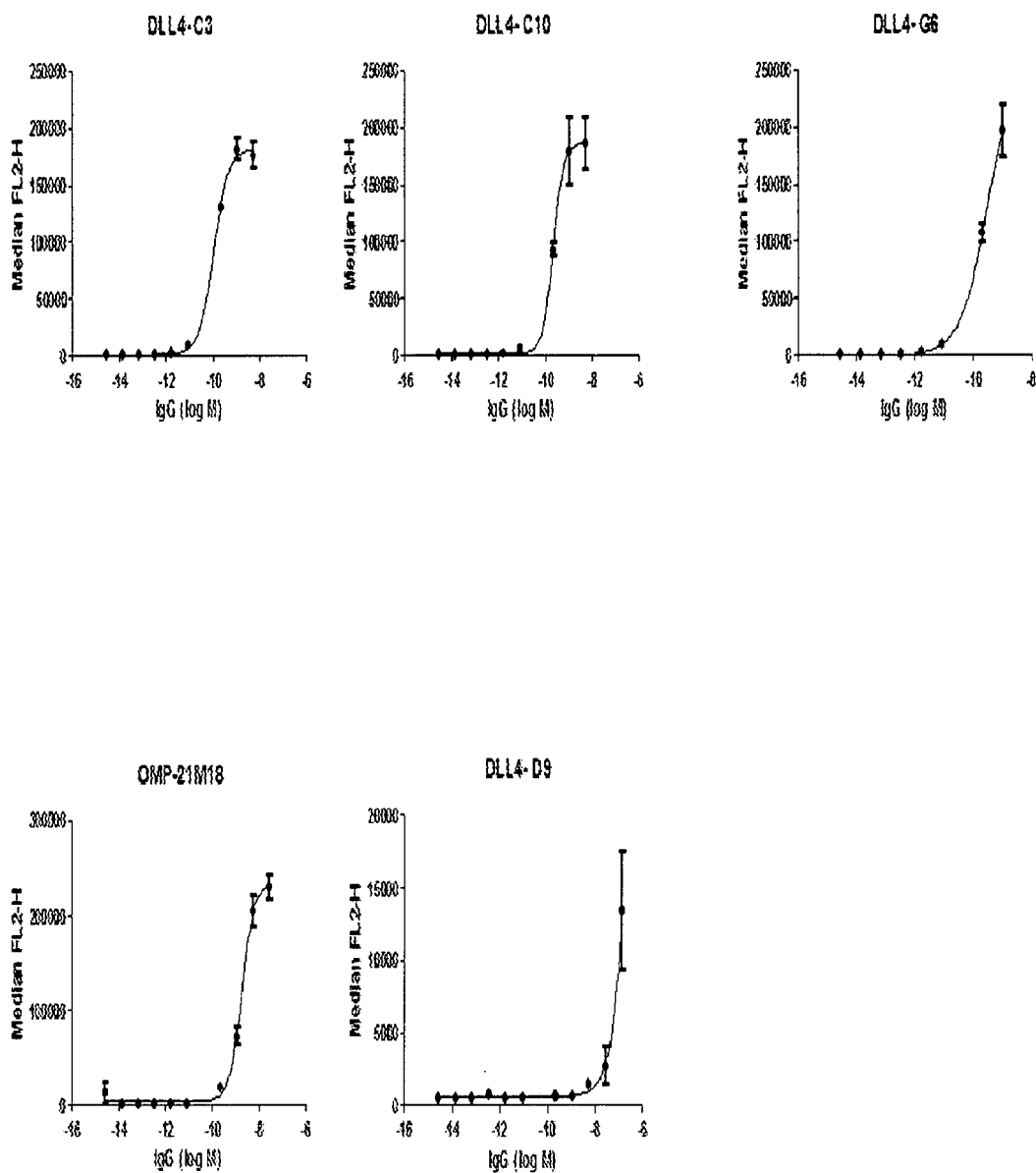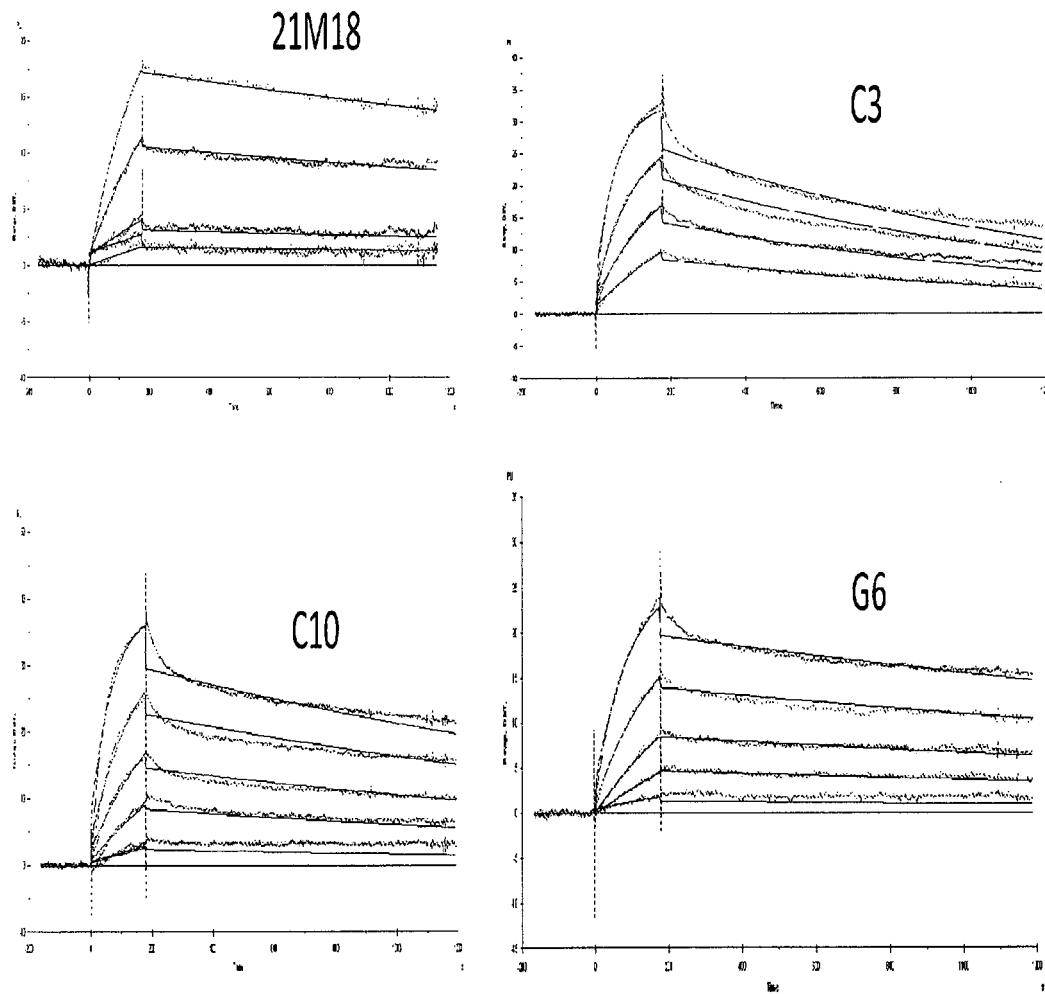
**Fig 1A.** Binding of various anti-DLL4 antibodies to Cell Surface DLL4 Protein.

Fig 1B. Binding of selected anti-DLL4 antibodies to Cell Surface DLL4 Protein.

	OMP21M18	C3	C10	G6	D9
EC50 (M)	1.74 E-09	1.069 E-10	2,156 E-10	3.050 E-10	~ 4,338 E-06

Fig 2. Kinetic Analysis of IgGs Against Recombinant Human DLL4 by Biacore

Name	ka (1/Ms)	kd (1/s)	Rmax (RU)	KA (1/M)	KD (M)	Chi2
21M18	1.32E+05	2.26E-04	28.8	5.86E+08	1.71E-09	0.0699
C3	8.91E+05	7.88E-04	27.9	1.13E+09	8.84E-10	0.425
C10	3.40E+05	4.01E-04	33.2	8.49E+08	1.18E-09	0.903
G6	2.52E+05	2.89E-04	24.2	8.72+E08	1.15E-09	0.211

Fig 3. Epitope mapping of STI Anti-DLL4 Antibodies compared to OMP21M18 using Octet
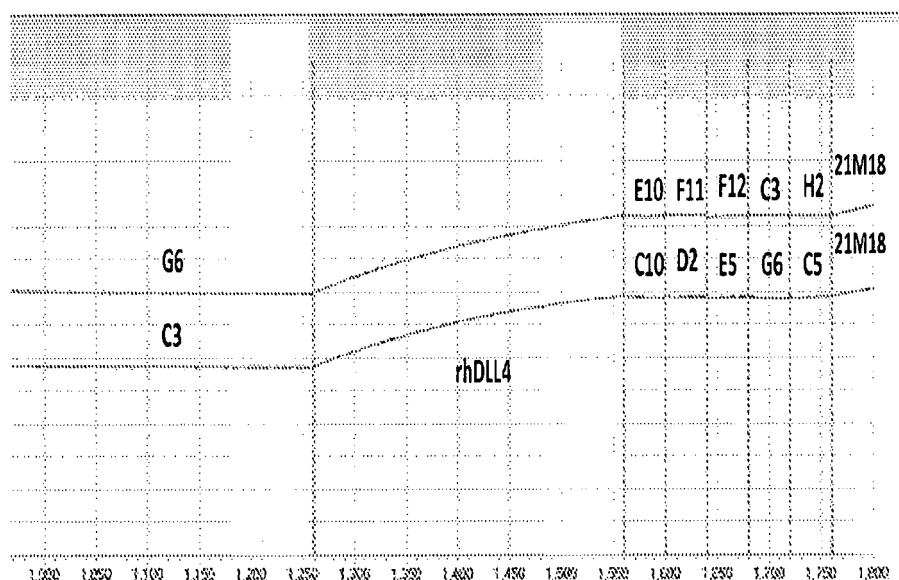
Sensor coated with IgG G6 or IgG C3, then captured rhDLL4 and run different antibodies
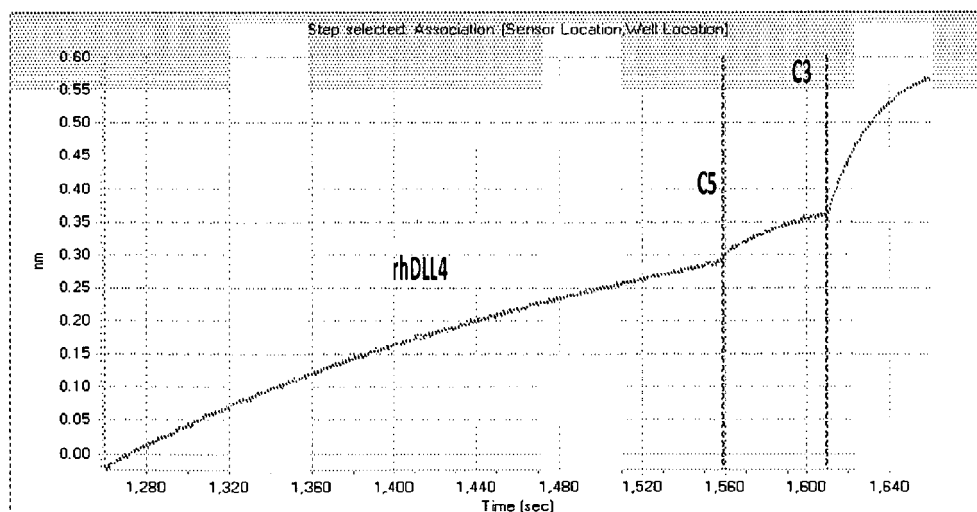
Sensor coated with OMP21M18, captured rhDLL4, then ran IgG C5 and C3

Fig 4A. Inhibition of human DLL4 Binding to rat recombinant Notch-1 Receptor
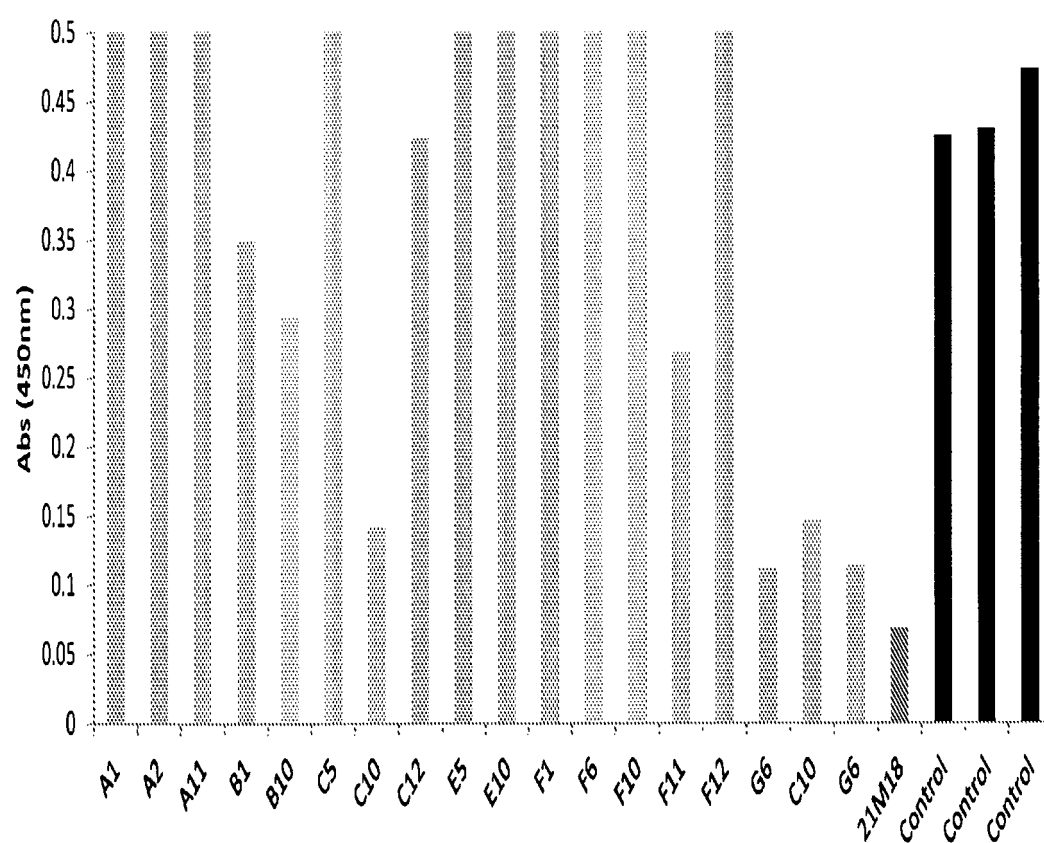
Fig 4B. Inhibition of human or rat soluble Notch-1 to Cellular DLL4
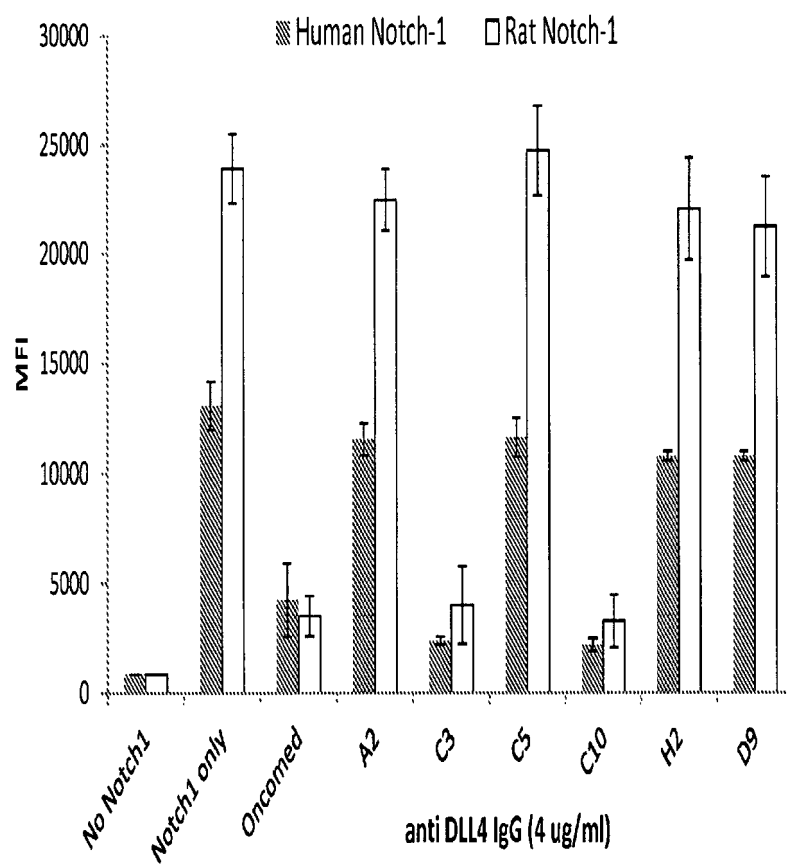
Fig 4C. Inhibition of human or rat soluble Notch-1 to Cellular DLL4
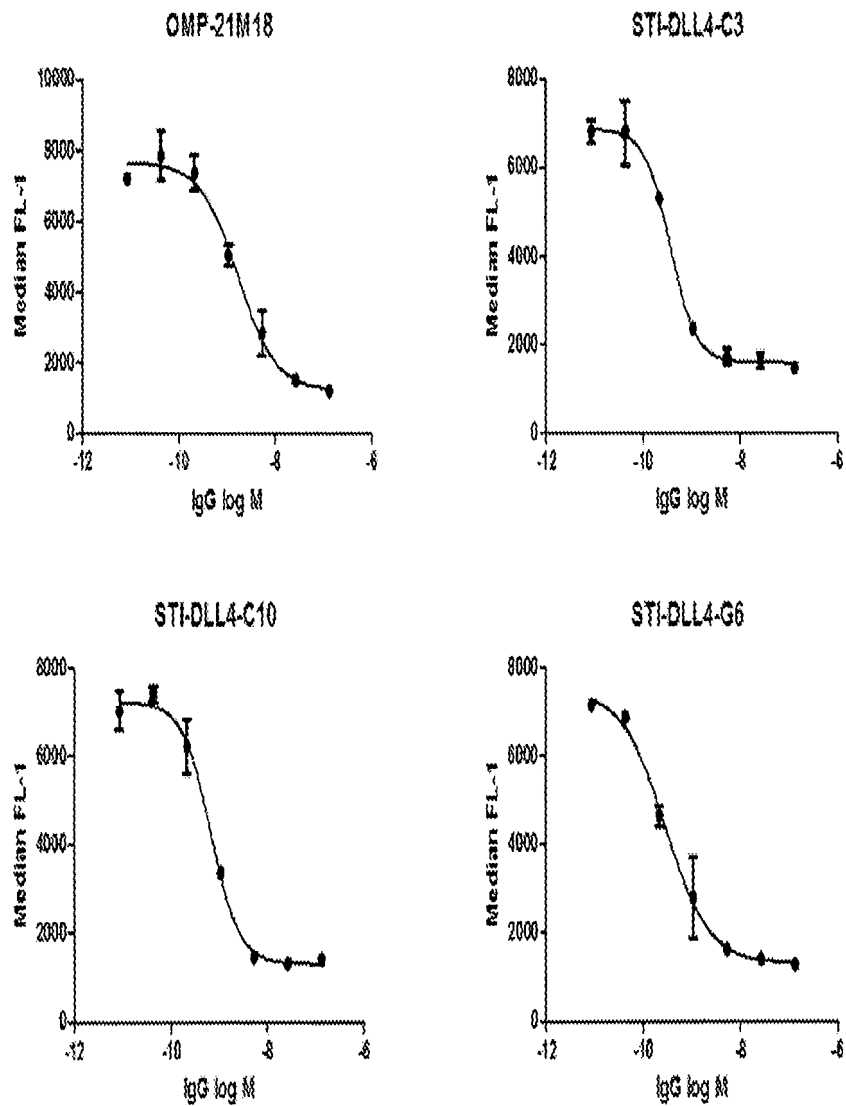
	OMP21M18	C3	C10	G6
IC50 (M)	1.72 E-09	3.64 E-10	6.76 E-10	2.86 E-10

Fig 5. Anti-DLL4 antibodies inhibit DLL4- mediated Notch-1 Pathway activation.

Notch-1 Pathway-dependent Gene Expression

☐ No DLL4   ■ DLL4 Cells   ▨ DLL4 Cells + anti-DLL4 IgG
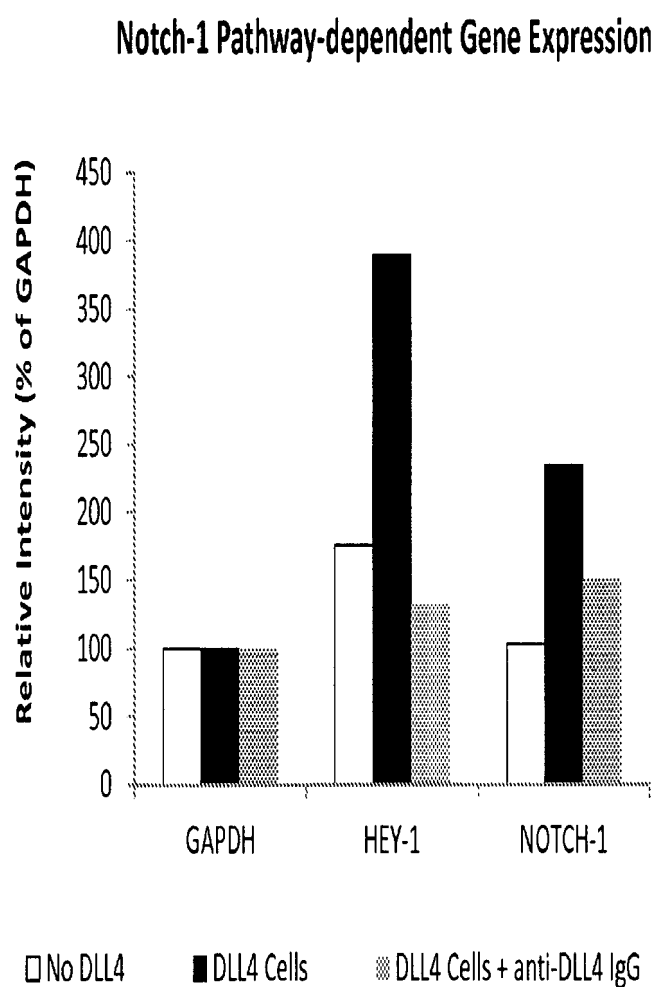
Fig 6. Anti-DLL4 antibodies block DLL4 mediated HUVEC Cell Growth Inhibition.

HUVEC Proliferation on Immobilized DLL4 (2ug/ml)

■ STI-DLL4-C3
▼ STI-DLL4-C10
◆ STI-DLL4-G6
▲ OMP-21M18

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A61K 39/395 (2006.01)

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

[Continued on next page]

(54) Title: ANTIGEN BINDING PROTEINS THAT BIND DLL-4

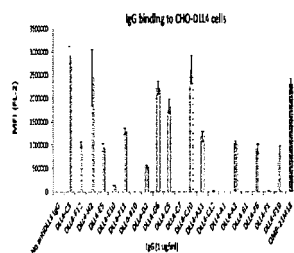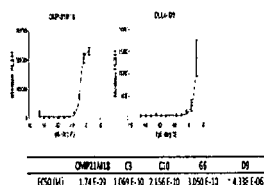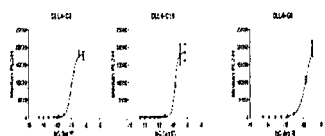Fig 1A. Binding of various anti-DLL4 antibodies to Cell Surface DLL4 Protein.

Fig 1B. Binding of selected anti-DLL4 antibodies to Cell Surface DLL4 Protein.

(57) **Abstract**: There is disclosed compositions and methods relating to or derived from anti- DLL-4 antibodies. More specifically, there is disclosed fully human antibodies that bind DLL-4, DLL-4-binding fragments and derivatives of such antibodies, and DLL-4 -binding polypeptides comprising such fragments. Further still, there is disclosed nucleic acids encoding such antibodies, antibody fragments and derivatives and polypeptides, cells comprising such polynucleotides, methods of making such antibodies, antibody fragments and derivatives and polypeptides, and methods of using such antibodies, antibody fragments and derivatives and polypeptides, including methods of treating or diagnosing subjects having DLL-4 related disorders or conditions, including various inflammatory disorders and various cancers.

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/22; A61K 39/395; C12P 21/08 (2014.01)

USPC - 424/135.1; 530/389.2, 388.8

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)

IPC(8): C07K 16/22, 16/00; A61K 39/395, 39/38, 39/00; C12P 21/08, 21/00 (2014.01)

USPC: 424/135.1, 158.1, 130.1, 139.1, 184.1, 133.1; 530/389.2, 387.3, 387.7, 387.9, 388.8, 388.75, 388.73;

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 practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); PubMed/PubMed Central/NCBI Blast; UNIPROT; ENA; Google; Google Scholar; 'DLL4,' 'anti-DLL4,' peptide, polypeptide, antibody, 'Fab,' 'antigen binding protein,' 'human antibody,' 'single chain antibody,' 'antibody fragment,' 'human antibody fragment,' 'human single chain antibody'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0292312 A1 (YAN, M et al.) November 18, 2010; paragraphs [0013], [0068], [0085],	1-12
A	US 2008/0025980 A1 (HARDY, B et al.) January 31, 2008; paragraphs [0031]-[0036], [0107], [0216]; Table 6	1-12
A	AKAHORI, Y et al. Construction And Characterization Of Antibody Libraries: Isolation Of Therapeutic Human Antibodies And Application To Functional Genomics. Genbank [database online]. National Center for Biotechnology Information. 02 July 2002 [retrieved on 30 June 2014]. Retrieved from the internet: <URL: http://www.ncbi.nlm.nih.gov/protein/?term=BAC01307 +OR+BAC01540+OR+BAC01602+OR+BAC01606+OR+BAC01631+OR+BAC02373>; entire document.	1-12
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☒ Further documents are listed in the continuation of Box C. ☐

*	Special categories of cited documents:	"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
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"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	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 July 2014 (02.07.2014)	3 0 JUL 2014

Name and mailing address of the ISA/US	Authorized officer:
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COMBRIATO, G et al. V-Lambda and J-Lambda-C-Lambda Gene Segments Of The Human Immunoglobulin Lambda Light Chain Locus Are Separated By 14 Kb And Rearrange By A Deletion Mechanism. European Journal of Immunology. June 1991, Vol. 21, No. 6; pages 1513-1522; abstract; Genbank Supplement; DOI: 10.1002/eji.1830210627.	1-12
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结合 DLL-4 的抗原结合蛋白

本发明公开了与抗-DLL-4 抗体有关或衍生自所述抗-DLL-4 抗体的组合物和方法。更具体地，本发明公开了结合 DLL-4 的完整的人抗体、这种抗体的 DLL-4-结合片段和衍生物、以及包括这种片段的 DLL-4-结合多肽。再而，本发明公开了编码这种抗体、抗体片段和衍生物以及多肽的核酸，包括这种多核苷酸的细胞，制备这种抗体、抗体片段和衍生物和多肽的方法，这种抗体、抗体片段和衍生物和多肽的使用方法，所述的使用方法包括治疗或诊断具有 DLL-4 相关病症或病况(包括各种炎性病症和各种癌在内)的受试者的方法。