

DLL3 BINDING PROTEINS AND METHODS OF USE

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application Nos. 62/736,368 filed on September 25, 2018; 62/736,358 filed on September 25, 2018; and 62/877,227 filed July 22, 2019 each of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 25, 2019, is named 47517-733_601_SL.txt and is 951,519 bytes in size.

BACKGROUND OF THE INVENTION

The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumor cells, while leaving healthy cells and tissues intact and undamaged. One such method is by inducing an immune response against the tumor, to make immune effector cells such as natural killer (NK) cells or cytotoxic T lymphocytes (CTLs) attack and destroy tumor cells.

SUMMARY ~~OF THE INVENTION~~

[0003] There is still a need for having available further options for the treatment of tumorous diseases related to the overexpression of DLL3, such as neuroendocrine tumors. The present disclosure provides, in certain embodiments, single domain proteins which specifically bind to DLL3 on the surface of tumor target cells and multispecific proteins, such as trispecific proteins containing DLL3 binding domain as described herein. In some embodiments, the present disclosure provides Delta Like Ligand 3 (DLL3) binding proteins, or multispecific proteins as mentioned above, which can be used for diagnosing and treating indications correlated to the expression of DLL3.

[0003a] In one aspect, the present disclosure provides a DLL3 targeting trispecific protein, wherein said protein comprises (a) a first domain (A) which is a single chain variable fragment that specifically binds to a human CD3; (b) a second domain (B) which is a single domain antibody that specifically binds to a human serum albumin protein; and (c) a third domain (C) which is a single domain antibody that specifically binds to a DLL3 protein.

wherein the third domain (C) comprises a CDR1 that has the amino acid sequence of SEQ ID No. 874, a CDR2 that has the amino acid sequence of SEQ ID No. 1316, and a CDR3 that has the amino acid sequence of SEQ ID No. 1758.

[0003b] In another aspect, the present disclosure provides a DLL3 targeting trispecific protein, wherein said protein comprises (a) a first domain (A) which is a single chain variable fragment that specifically binds to a human CD3; (b) a second domain (B) which is a single domain antibody that specifically binds to a human serum albumin protein; and (c) a third domain (C) which is a single domain antibody that specifically binds to DLL3, wherein the third domain (C) comprises a sequence selected from the group consisting of SEQ ID Nos. 408, 425, 432, 430, 431, and 1886.

[0003c] In another aspect, the present disclosure provides a DLL3 targeting trispecific protein, comprising the sequence of SEQ ID No. 1890 or SEQ ID No. 1891.

[0003d] In another aspect, the present disclosure provides a DLL3 single domain antibody comprising a CDR1 that has the amino acid sequence of SEQ ID No. 874, a CDR2 that has the amino acid sequence of SEQ ID No. 1316, and a CDR3 that has the amino acid sequence of SEQ ID No. 1758.

[0003e] In another aspect, the present disclosure provides a use of the DLL3 targeting trispecific protein disclosed herein in the manufacture of a medicament for treating a cancer overexpressing DLL3 protein in a subject.

[0003f] In another aspect, the present disclosure provides the DLL3 targeting trispecific protein disclosed herein for use in therapy.

[0004] One embodiment provides a DLL3 targeting trispecific protein, wherein said protein comprises (a) a first domain (A) which is a single chain variable fragment that specifically binds to a human CD3; (b) a second domain (B) which is a single domain antibody that specifically binds to a human serum albumin protein; and (c) a third domain (C) which is a single domain antibody that specifically binds to a DLL3 protein.

[0005] In some embodiments, the domains are linked in the order H2N-(A)-(B)-(C)-COOH, H2N-(A)-(C)-(B)-COOH, H2N-(B)-(A)-(C)-COOH, H2N-(B)-(C)-(A)-COOH, H2N-(C)-(B)-(A)-COOH, or H2N-(C)-(A)-(B)-COOH, or by linkers L1 and L2, in the order H2N-(A)-L1-(B)

CLAIMS

WHAT IS CLAIMED IS:

1. A DLL3 targeting trispecific protein, wherein said protein comprises
 - (a) a first domain (A) which is a single chain variable fragment that specifically binds to a human CD3;
 - (b) a second domain (B) which is a ~~half-life extension~~ single domain antibody that specifically binds to a human serum albumin protein; and
 - (c) a third domain (C) which is a single domain antibody that specifically binds to a DLL3 protein, wherein the third domain (C) comprises a CDR1 that has the amino acid sequence of SEQ ID No. 874, a CDR2 that has the amino acid sequence of SEQ ID No. 1316, and a CDR3 that has the amino acid sequence of SEQ ID No. 1758.
2. The DLL3 targeting trispecific protein of claim 1, wherein the domains are linked in the order H2N-(A)-(B)-(C)-COOH, H2N-(A)-(C)-(B)-COOH, H2N-(B)-(A)-(C)-COOH, H2N-(B)-(C)-(A)-COOH, H2N-(C)-(B)-(A)-COOH, or H2N-(C)-(A)-(B)-COOH, or by linkers L1 and L2, in the order H2N-(A)-L1-(B)-L2-(C)-COOH, H2N-(A)-L1-(C)-L2-(B)-COOH, H2N-(B)-L1-(A)-L2-(C)-COOH, H2N-(B)-L1-(C)-L2-(A)-COOH, H2N-(C)-L1-(B)-L2-(A)-COOH, or H2N-(C)-L1-(A)-L2-(B)-COOH.
3. The DLL3 targeting trispecific protein of claim 1 ~~or 2~~, wherein the ~~third domain domains are linked in the order H2N-(A)-(B)-(C)-COOH or by linkers L1 and L2 in the order of H2N-(A)-L1-(B)-L2-(C)-COOH~~ comprises an affinity matured binding molecule.
4. The DLL3 targeting trispecific protein of claim ~~3~~ 1, wherein the domains are linked in the order H2N-(C)-(B)-(A)-COOH or by linkers L1 and L2 in the order of H2N-(C)-L1-(B)-L2-(A)-COOH ~~affinity matured binding molecule is derived from a parental molecule that specifically binds to the DLL3 protein.~~
5. The DLL3 targeting trispecific protein of claim ~~3 or 4~~ 1, wherein the third domain (C) is an affinity matured binding molecule ~~is derived~~ obtained from ~~the a~~ parental molecule that specifically binds to the DLL3 protein, after a round of affinity maturation.
6. The DLL3 targeting trispecific protein of claim 5, wherein the ~~round of~~ affinity maturation ~~matured DLL3 binding molecule has a binding affinity toward the DLL3 protein that is about 2-fold to about 50-fold greater than the binding affinity of the parental molecule toward the DLL3 protein~~ comprises panning a phage display library against the DLL3 protein.
7. The DLL3 targeting trispecific protein of claim ~~6~~ 2, wherein the linkers L1 and L2 are each independently selected from the group consisting of (GS)_n (SEQ ID No.1809),

(GGS)_n (SEQ ID No.1810), (GGGS)_n (SEQ ID No.1811), (GGSG)_n (SEQ ID No.1812), (GGSGG)_n (SEQ ID No.1813), and (GGGGS)_n (SEQ ID No.1814), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10~~phage display library is generated by mutating one or more residues of the parental molecule.~~

8. The DLL3 targeting trispecific protein of claim ~~7~~2, wherein the linkers L1 and L2 independently comprises~~phage display library expresses one or more molecules derived from the sequence of GGGSGGGGS (SEQ ID No. 1808)~~parental molecule.

9. The DLL3 targeting trispecific protein of claim ~~8~~1, wherein the second domain (B) comprises an amino acid sequence selected from the group consisting of SEQ ID Nos. 1769-1778~~affinity matured binding molecule is selected from the one or more molecules derived from the parental molecule.~~

10. The DLL3 targeting trispecific protein of ~~any one of claims 3-9~~claim 1, wherein the first domain (A) comprises an amino acid sequence selected from the group consisting of SEQ ID Nos. 1793-1802 and 1897-1898~~affinity matured DLL3 binding molecule has a greater binding affinity toward the DLL3 protein than a binding affinity of the parental molecule toward the DLL3 protein.~~

11. The DLL3 targeting trispecific protein of claim ~~10~~, comprising the sequence of SEQ ID No. 1890 or SEQ ID No. 1891~~wherein the affinity matured binding DLL3 binding molecule has a binding affinity toward the DLL3 protein that is about 2-fold about 50-fold greater than the binding affinity of the parental molecule toward the DLL3 protein.~~

~~12. The DLL3 targeting trispecific protein of claim 10, wherein the affinity matured binding DLL3 binding molecule has a binding affinity toward the DLL3 protein that is about 3-fold greater than the binding affinity of the parental molecule toward the DLL3 protein.~~

~~13. The DLL3 targeting trispecific protein of any one of claims 1-12, wherein the third domain (C) comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-442 and 1886.~~

~~14. The DLL3 targeting trispecific protein of any one of claims 1-13, wherein the third domain (C) comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-52.~~

~~15. The DLL3 targeting trispecific protein of any one of claims 1-14, wherein the third domain (C) comprises a CDR1, a CDR2, and a CDR3.~~

~~16. The DLL3 targeting trispecific protein of claim 15, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID Nos. 443-884 and 1887, or one or~~

~~more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 443-884 and 1887.~~

~~17. — The DLL3 targeting trispecific protein of claim 15 or 16, wherein the CDR2 comprises a sequence selected from the group consisting of SEQ ID Nos. 885-1326 and 1888, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 885-1326 and 1888.~~

~~18. — The DLL3 targeting trispecific protein of any one of claims 15, 16, and 17, wherein the CDR3 comprises a sequence selected from the group consisting of SEQ ID Nos. 1327-1768 and 1889, or one or more substitutions relative to a sequence selected from SEQ ID Nos. 1327-1768 and 1889.~~

~~19. — The DLL3 targeting trispecific protein of claim 15, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID Nos. 495-528, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 495-528.~~

~~20. — The DLL3 targeting trispecific protein of claim 15 or 19, wherein the CDR2 comprises a sequence selected from the group consisting of SEQ ID Nos. 937-970, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 937-970.~~

~~21. — The DLL3 targeting trispecific protein of any one of claims 15, 19, and 20, wherein the CDR3 comprises a sequence selected from the group consisting of SEQ ID Nos. 1379-1412, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 1379 to 1412.~~

~~22. — The DLL3 targeting trispecific protein of claim 15, wherein the third domain (C) comprises a sequence that is at least about 80% identical to a sequence selected from the group consisting of SEQ ID Nos. 53-86.~~

~~23. — The DLL3 targeting trispecific protein of claim 22, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID Nos. 529-809, or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 529-809.~~

~~24. — The DLL3 targeting trispecific protein of claim 22 or 23, wherein the CDR2 comprises a sequence that has one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 971 to 1251.~~

~~25. — The DLL3 targeting trispecific protein of claim 23 or 24, wherein the CDR3 comprises a sequence selected from the group consisting of SEQ ID Nos. 1379-1412, or one~~

~~or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 1379 to 1412.~~

~~26. — The DLL3 targeting trispecific protein of claim 15, wherein the third domain (C) comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 87-367.~~

~~27. — The DLL3 targeting trispecific protein of claim 26, wherein the CDR1 comprises a sequence selected from the group consisting of SEQ ID Nos. 810-884, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 810 to 884.~~

~~28. — The DLL3 targeting trispecific protein of claim 26 or 27, wherein the CDR2 comprises a sequence selected from the group consisting of SEQ ID Nos. 1252 to 1326, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 1252 to 1326.~~

~~29. — The DLL3 targeting trispecific protein of claim 26, 27, or 28, wherein the CDR3 comprises a sequence selected from the group consisting of SEQ ID Nos. 1692 to 1768, or one or more amino acid substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 1692 to 1768.~~

~~30. — The DLL3 targeting trispecific protein of claim 15, wherein the third domain (C) comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 368-442.~~

~~31.~~ 12. A DLL3 targeting trispecific protein, wherein said protein comprises

(a) a first domain (A) which ~~is a single chain variable fragment that~~ specifically binds to a human CD3;

(b) a second domain (B) which is a ~~single half life extension~~ domain ~~antibody that specifically binds to a human serum albumin protein~~; and

(c) a third domain (C) which ~~is a single domain antibody that~~ specifically binds to DLL3, wherein the third domain ~~(C)~~ comprises ~~the a~~ sequence ~~selected from the group consisting of~~ SEQ ID ~~Nos. 408, 425, 432, 430, 431, and 1886~~Nos. 408, 425, 432, 430, 431, and 1886~~No. 68 or is derived from SEQ ID No. 68.~~

~~32.~~ 13. ~~The A~~ DLL3 targeting trispecific protein, ~~comprising the sequence of SEQ ID No. 1890 or SEQ ID No. 1891 of claim 31, wherein the third domain (C) is derived from SEQ ID No. 68.~~

~~33.~~ 14. A DLL3 single domain antibody comprising a CDR1 that has the amino acid sequence of SEQ ID No. 874, a CDR2 that has the amino acid sequence of SEQ ID No. 1316, and a CDR3 that has the amino acid sequence of SEQ ID No. 1758~~The DLL3 targeting~~

~~trispecific protein of claim 32, wherein SEQ ID No.68 comprises a CDR1, a CDR2, and a CDR3.~~

~~34.15. The DLL3 targeting trispecific protein of claim 33, wherein the CDR1 comprises the following sequence: GX₁X₂X₃X₄X₅NX₆X₇X₈. The DLL3 single domain antibody of claim 14, wherein the DLL3 single domain antibody comprises a sequence that is at least 80% identical to the amino acid sequence of SEQ ID No. 432.~~

~~35.16. The DLL3 targeting trispecific protein of claim 33 or 34, wherein the CDR2 comprises the following sequence: GJ₁SI₂J₃J₄J₅J₆J₇J₈J₉J₁₀SI₁₁KJ₁₂ (SEQ ID No. 1895) Use of the DLL3 targeting trispecific protein of claim 1 in the manufacture of a medicament for treating a cancer overexpressing DLL3 protein in a subject.~~

17. The DLL3 targeting trispecific protein of claim 1 for use in therapy.

~~36. The DLL3 targeting trispecific protein of claim 33, 34, or 35, wherein the CDR3 comprises the following sequence: Z₁Z₂Z₃Z₄Z₅Z₆Z₇Z₈Z₉Z₁₀Z₁₁.~~

~~37. The DLL3 targeting trispecific protein of any one of claims 34-36, wherein:~~

~~X₁ is A, E, F, G, I, K, L, N, Q, R, S, T, V, or Y;~~

~~X₂ is A, G, I, K, P, R, S, T, or V;~~

~~X₃ is A, D, F, K, L, N, P, Q, R, S, T, or Y;~~

~~X₄ is A, D, F, H, I, K, L, M, N, P, R, S, T, V, or Y;~~

~~X₅ is F, I, K, L, M, N, R, S, T, or V;~~

~~X₆ is A, or G;~~

~~X₇ is F, I, L, M, T, V, or Y; and~~

~~X₈ is A, or G.~~

~~38. The DLL3 targeting trispecific protein of any one of claims 34-37, wherein:~~

~~J₁ is I, or V;~~

~~J₂ is A, K, P, R, or S;~~

~~J₃ is D, or N;~~

~~J₄ is D, E, G, K, N, Q, R, S, T, or Y;~~

~~J₅ is S, or T;~~

~~J₆ is A, E, F, H, I, K, L, N, Q, R, S, T, V, or Y;~~

~~J₇ is A, I, L, M, V, or Y;~~

~~J₈ is D, F, H, I, L, N, S, T, V, or Y;~~

~~J₉ is A, D, E, F, G, I, K, L, N, Q, R, S, T, V, or Y;~~

~~J₁₀ is A, D, E, G, K, Q, S, or V;~~

~~_____ J₁₁ is A, or V; and~~

~~_____ J₁₂ is G, or V.~~

~~39. _____ The DLL3 targeting trispecific protein of any one of claims 34-38, wherein:~~

~~_____ Z₁ is F, or Y;~~

~~_____ Z₂ is G, H, I, K, N, R, S, or T;~~

~~_____ Z₃ is A, F, H, I, K, L, M, N, P, Q, R, S, T, or Y;~~

~~_____ Z₄ is A, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, or Y;~~

~~_____ Z₅ is A, C, D, E, G, H, I, K, L, N, P, Q, R, S, T, W, or Y;~~

~~_____ Z₆ is G, K, L, R, or T;~~

~~_____ Z₇ is A, G, H, L, Q, R, S, T, V, or Y;~~

~~_____ Z₈ is A, D, E, G, H, P, Q, S, T, W, or Y;~~

~~_____ Z₉ is A, G, I, K, L, M, N, Q, R, S, T, V, or Y;~~

~~_____ Z₁₀ is A, G, K, P, R, S, T, or V; and~~

~~_____ Z₁₁ is A, F, S, or Y.~~

~~40. _____ A DLL3 targeting trispecific protein, wherein said protein comprises~~

~~(a) a first domain (A) which specifically binds to human CD3;~~

~~(b) a second domain (B) which is a half life extension domain; and~~

~~(c) a third domain (C) which specifically binds to DLL3, wherein the third domain comprises the sequence of SEQ ID No.75 or is derived from SEQ ID No.75.~~

~~41. _____ The DLL3 targeting trispecific protein of claim 40, wherein SEQ ID No.75 comprises a CDR1, a CDR2, and a CDR3.~~

~~42. _____ The DLL3 targeting trispecific protein of claim 41, wherein the CDR1 comprises the following sequence: X₁X₂X₃X₄X₅X₆X₇SX₈A.~~

~~43. _____ The DLL3 targeting trispecific protein of claim 41 or 42, wherein the CDR2 comprises the following sequence: GJ₁SI₂J₃GJ₄J₅J₆YI₇J₈SVKG (SEQ ID NO: 1894).~~

~~44. _____ The DLL3 targeting trispecific protein of any one of claims 41, 42, and 43, wherein the CDR3 comprises the following sequence: Z₁Z₂Z₃Z₄Z₅Z₆Z₇Z₈Z₉.~~

~~45. _____ The DLL3 targeting trispecific protein of any one of claims 42-44, wherein:~~

~~X₁ is A, D, E, F, G, H, K, L, M, N, Q, R, S, V, W, or Y;~~

~~X₂ is D, E, H, K, M, P, R, S, T, or Y;~~

~~X₃ is A, D, G, H, K, N, P, Q, R, S, T, V, or Y;~~

~~X₄ is K, S, or V;~~

~~X₅ is A, F, G, H, K, L, M, N, Q, R, S, T, V, W, or Y;~~

~~X₆ is D, F, H, I, K, L, M, N, Q, R, S, V, or Y;~~

~~X₇ is L, or M; and~~

~~X₈ is I, L, M, S, T, or V.~~

~~46. The DLL3 targeting trispecific protein of any one of claims 42-45, wherein:~~

~~J₁ is I, or V;~~

~~J₂ is A, D, E, G, H, I, K, L, N, P, Q, R, S, T, V, or Y;~~

~~J₃ is A, D, E, G, H, N, R, or T;~~

~~J₄ is H, P, R, or S;~~

~~J₅ is A, H, I, K, M, N, Q, R, T, or V;~~

~~J₆ is A, D, G, H, I, L, M, N, S, T, V, or Y;~~

~~J₇ is A, F, I, L, M, R, S, T, V, or Y;~~

~~J₈ is A, D, E, G, H, K, L, N, R, S, or V.~~

~~47. The DLL3 targeting trispecific protein of any one of claims 42-46, wherein:~~

~~Z₁ is L, or Y;~~

~~Z₂ is D, E, G, H, K, N, Q, R, S, T, V, or Y;~~

~~Z₃ is Q, or W;~~

~~Z₄ is A, D, E, G, H, I, K, L, M, P, R, S, T, or V;~~

~~Z₅ is A, D, E, G, N, R, S, T, or Y;~~

~~Z₆ is A, P, R, or S;~~

~~Z₇ is A, D, F, G, H, L, M, N, Q, R, S, T, V, or Y;~~

~~Z₈ is A, G, I, K, P, Q, R, S, or T; and~~

~~Z₉ is F, H, or Y.~~

~~48. The DLL3 targeting trispecific protein of any one of claims 1-47, wherein the third domain (C) comprises a humanized antibody or an antigen binding fragment thereof.~~

~~49. The DLL3 targeting trispecific protein of any one of claims 1-48, wherein the third domain (C) comprises a single domain antibody, a VHH domain, a scFv, a VH domain, a VL domain, a Fab, a Fab', a non-Ig domain, a ligand, a knottin, or a small molecule entity that specifically binds to DLL3.~~

- ~~50. The DLL3 targeting trispecific protein of any one of claims 49, wherein the third domain (C) comprises the single domain antibody.~~
- ~~51. The DLL3 targeting trispecific protein of any one of claims 1-50, wherein the second domain (B) binds a bulk serum protein.~~
- ~~52. The DLL3 targeting trispecific protein of claim 51, wherein the second domain (B) comprises a single domain antibody, a VHH domain, a scFv, a VH domain, a VL domain, a Fab, a Fab', a non-Ig domain, a ligand, a knottin, or a small molecule entity that specifically binds to the bulk serum protein.~~
- ~~53. The DLL3 targeting trispecific protein of claim 52, wherein the second domain (B) comprises the single domain antibody that specifically binds to the bulk serum protein.~~
- ~~54. The DLL3 targeting trispecific protein of claim 53, wherein the bulk serum protein comprises albumin, transferrin, IgG1, IgG2, IgG4, IgG3, IgA monomer, Factor XIII, Fibrinogen, IgE, pentameric IgM, or Igκ free light chain.~~
- ~~55. The DLL3 targeting trispecific protein of claim 54, wherein the bulk serum protein comprises the albumin.~~
- ~~56. The DLL3 targeting trispecific protein of claim 55, wherein the second domain (B) comprises a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1769-1778.~~
- ~~57. The DLL3 targeting trispecific protein of claim 56, wherein the second domain (B) comprises a sequence that is at least about 75% identical to SEQ ID No. 1774.~~
- ~~58. The DLL3 targeting trispecific protein of any one of claims 1-57, wherein the first domain (A) comprises a single domain antibody, a VHH domain, a scFv, a VH domain, a VL domain, a Fab, a Fab', a non-Ig domain, a ligand, a knottin, or a small molecule entity that specifically binds to CD3.~~
- ~~59. The DLL3 targeting trispecific protein of claim 58, wherein the first domain (A) comprises a sequence selected from the group consisting of SEQ ID Nos. 1793-1807.~~
- ~~60. The DLL3 targeting trispecific protein of any one of claims 1-59, wherein the third domain (C) comprises the following formula:~~

$$\text{f1-r1-f2-r2-f3-r3-f4}$$

~~wherein, r1 is identical to or comprises one or more amino acid residue substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 443-884 and 1887; r2 is identical to or comprises one or more amino acid residue substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 885-1326 and 1888; and r3 is identical to or comprises one or more amino acid residue substitutions relative to a sequence~~

selected from the group consisting of SEQ ID Nos. 1327-1768 and 1889; and wherein f1, f2, f3 and f4 are framework residues.

61. — The DLL3 targeting trispecific protein of claim 60, wherein the third domain (C) comprises a sequence that is at least 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-442 and 1886.

62. — The DLL3 targeting trispecific protein of any one of claims 1-61, wherein domains C and B are connected by linker L1 and domains B and A are connected by linker L2.

63. — The DLL3 targeting trispecific protein of claim 62, wherein linkers L1 and L2 are each independently selected from (GS)_n (SEQ ID No.1809), (GGS)_n (SEQ ID No.1810), (GGGS)_n (SEQ ID No.1811), (GGSG)_n (SEQ ID No.1812), (GGSGG)_n (SEQ ID No.1813), or (GGGGS)_n (SEQ ID No.1814), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or GGGGSGGGS (SEQ ID No. 1808).

64. — The DLL3 targeting trispecific protein of claim 63, wherein linkers L1 and L2 are each independently (GGGGS)₄ (SEQ ID No.1817), (GGGGS)₃ (SEQ ID No.1818), or GGGGSGGGS (SEQ ID No. 1808).

65. — The DLL3 targeting trispecific protein of any one claims 1-64, wherein the protein binds to DLL3 with a binding affinity (K_d) of about 0.1 nM to about 50 nM.

66. — The DLL3 targeting trispecific protein of any one of claims 1-65, wherein the protein binds to human DLL3, cynomolgus DLL3, or both human and cynomolgus DLL3.

67. — The DLL3 targeting trispecific protein of any one of claims 1-66, wherein the protein is less than about 80 kDa.

68. — The DLL3 targeting trispecific protein of any one of claims 1-67, wherein the protein is about 50 to about 75 kDa.

69. — The DLL3 targeting trispecific protein of any one of claims 1-68, wherein the protein is less than about 60 kDa.

70. — The DLL3 targeting trispecific protein of any one of claims 1-69, wherein the protein has an elimination half time of at least about 50 hours.

71. — The DLL3 targeting trispecific protein of any one of claims 1-70, wherein the protein has an elimination half time of at least about 100 hours.

72. — The DLL3 targeting trispecific protein of any one of claims 1-69, wherein the protein has an elimination half life of at least about 25 hours, at least about 50 hours, or at least about 100 hours when administered to a human subject.

73. — The DLL3 targeting trispecific protein of any one of claims 1-72, wherein the domains are linked in the order H2N-(A)-(B)-(C)-COOH.

~~74. A DLL3 binding protein comprising the following formula:~~

~~f1-r1-f2-r2-f3-r3-f4~~

~~wherein, r1 is a complementarity determining region 1 (CDR1) and is identical to or comprises one or more amino acid residue substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 443-884 and 1887; r2 is a CDR2 and is identical to or comprises one or more amino acid residue substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 885-1326 and 1888; and r3 is a CDR3 and identical to or comprises one or more amino acid residue substitutions relative to a sequence selected from the group consisting of SEQ ID Nos. 1327-1768 and 1889; and wherein f1, f2, f3 and f4 are framework residues.~~

~~75. The DLL3 binding protein of claim 74, comprising a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-442 and 1886.~~

~~76. The DLL3 binding protein of claim 74 or 75, comprising a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 1-52.~~

~~77. The DLL3 binding protein of claim 74 or 75, comprising a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 53-86.~~

~~78. The DLL3 binding protein of claim 74 or 75, comprising a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 87-367.~~

~~79. The DLL3 binding protein of claim 74 or 75, comprising a sequence that is at least about 75% identical to a sequence selected from the group consisting of SEQ ID Nos. 368-442.~~

~~80. A DLL3 binding protein that is derived from a parental DLL3 binding protein comprising the sequence of SEQ ID No. 68 or comprises the sequence of SEQ ID No. 68.~~

~~81. A DLL3 binding protein that is derived from a parental DLL3 binding protein comprising the sequence of SEQ ID No. 75 or comprises the sequence of SEQ ID No. 75.~~

~~82. A pharmaceutical composition comprising (i) the DLL3 targeting trispecific protein according to any one of claims 1-73 or the DLL3 binding protein according to any one of claims 74-81, and (ii) a pharmaceutically acceptable carrier.~~

~~83. A method for preparing a DLL3 targeting trispecific binding protein according to any one of claims 1-73, the method comprising: i) providing a DLL3 protein or a fragment thereof;~~

~~ii) exposing a recombinant library of DLL3 binding proteins to the DLL3 protein or a~~

fragment thereof; iii) selecting from the library a DLL3 binding protein which specifically binds to said oligomer or derivative thereof; and (iv) preparing the DLL3 targeting trispecific protein using the DLL3 binding protein identified in step (iii).

84. — The method of 83, wherein the recombinant library of DLL3 binding proteins is exposed to the DLL3 protein *in vitro* by screening the recombinant library with said DLL3 protein.

85. — The method of claim 83 or 84, wherein the recombinant library is expressed on the surface of a bacteriophage.

86. — The method of claim 83 or 84, wherein the recombinant library is expressed on the surface of yeast cells.

87. — The method of claim 83, wherein the recombinant library is expressed on the surface of bacterial cells.

88. — The method of claim 83 or 84, wherein the recombinant library is expressed as RNA-protein fusions.

89. — The method of any one of claims 83-88, wherein the recombinant library is an scFv library or an Fab library.

90. — The method of any one of claims 83-88, wherein the recombinant antibody library is a single domain library.

91. — A process for the production of a DLL3 targeting trispecific protein according to any one of claims 1-73, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding the DLL3 trispecific protein according to any one of claims 1-73 under conditions allowing the expression of the DLL3 targeting trispecific protein and recovering and purifying the produced protein from the culture.

92. — A method for preparing a DLL3 binding protein according to any one of claims 74-81, the method comprising: i) providing a DLL3 protein or a fragment thereof; ii) exposing a recombinant library of DLL3 binding proteins to the DLL3 protein or a fragment thereof; iii) selecting from the library a DLL3 binding protein which specifically binds to said oligomer or derivative thereof.

93. — The method of 92, wherein the recombinant library of DLL3 binding proteins is exposed to the DLL3 protein *in vitro* by screening the recombinant library with said DLL3 protein.

94. — The method of claim 92 or 93, wherein the recombinant library is expressed on the surface of a bacteriophage.

- ~~95.—The method of claim 92 or 93, wherein the recombinant library is expressed on the surface of yeast cells.~~
- ~~96.—The method of claim 93, wherein the recombinant library is expressed on the surface of bacterial cells.~~
- ~~97.—The method of claim 92 or 93, wherein the recombinant library is expressed as RNA-protein fusions.~~
- ~~98.—The method of any one of claims 92-97, wherein the recombinant library is an scFv library or an Fab library.~~
- ~~99.—The method of any one of claims 92-97, wherein the recombinant antibody library is a single domain library.~~
- ~~100.—A process for the production of a DLL3 binding protein according to any one of claims 74-81, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding the DLL3 binding protein according to any one of claims 74-81 under conditions allowing the expression of the DLL3 binding protein and recovering and purifying the produced protein from the culture.~~
- ~~101.—A method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of the DLL3 targeting trispecific protein according to any one of claims 1-73, or a DLL3 binding protein according to any one of claims 74-81, or a pharmaceutical composition according to claim 82, to a subject in need thereof.~~
- ~~102.—The method of claim 101, wherein the subject is human.~~
- ~~103.—The method of claim 102, wherein the method further comprises administration of an agent in combination with the DLL3 targeting trispecific protein according to any one of claims 1-73, the DLL3 binding protein according to any one of claims 74-81, or the pharmaceutical composition according to claim 82.~~
- ~~104.—The method of any one of claims 101-103, wherein the DLL3 targeting trispecific protein or the DLL3 binding protein selectively binds to tumor cells expressing DLL3.~~
- ~~105.—The method of claim 104, wherein the DLL3 targeting trispecific protein mediates T cell killing of tumor cells expressing DLL3.~~
- ~~106.—The method of any one of claims 101-105, wherein the tumorous disease comprises a solid tumor disease.~~
- ~~107.—The method of claim 106, wherein the solid tumor disease comprises lung cancer, gastric cancer, ovarian cancer, or triple negative breast cancer.~~
- ~~108.—The method of claim 107, wherein the solid tumor disease is metastatic.~~

~~109.—A method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising administration of a DLL3 targeting trispecific protein comprising a DLL3 binding domain comprising sequence selected from the group consisting of SEQ ID Nos. 1-442 and 1886, or a DLL3 binding protein comprising a sequence selected from the group consisting of SEQ ID Nos. 1-442 and 1886.~~

~~110.—The method of claim 109, wherein the DLL3 targeting trispecific protein or the DLL3 binding protein selectively binds to tumor cells expressing DLL3.~~

~~111.—The method of claim 110, wherein the DLL3 targeting trispecific protein directs T cell killing of tumor cells expressing DLL3.~~

~~112.—The method of any one of claims 109-111, wherein the tumorous disease comprises a solid tumor disease.~~

~~113.—The method of claim 112, wherein the solid tumor disease comprises lung cancer, gastric cancer, ovarian cancer, or triple negative breast cancer.~~

~~114.—The method of claim 113, wherein the solid tumor disease is metastatic.~~

~~115.—A method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising administration of a DLL3 targeting trispecific protein comprising a DLL3 binding domain comprising a sequence as set forth in SEQ ID No. 68 or 75 or a DLL3 binding protein comprising a sequence as set forth in SEQ ID No. 68 or 75.~~

~~116.—The method of any one of claims 101-115, comprising administering the DLL3 targeting trispecific protein or the DLL3 binding domain at a dose of up to 10 mg/kg.~~

~~117.—The method of any one of claims 101-116, wherein the protein is administered at least once a week.~~

~~118.—The method of any one of claims 101-117, wherein the protein is administered twice per week.~~

~~119.—The method of any one of claims 101-116, wherein the protein is administered every other week.~~

~~120.—The method of any one of claims 101-116, wherein the protein is administered every three weeks.~~

~~121.—A DLL3 binding protein comprising an amino acid sequence as set forth in SEQ ID No. 1890 or SEQ ID No. 1891.~~

~~122.—The DLL3 binding protein of any one of claims 1-81 and 104, wherein the protein is capable of binding a DLL3 sequence comprising an amino acid sequence as set forth in any one of SEQ ID Nos. 1892 and 1893.~~