

MULTIVALENT AND MULTISPECIFIC 41BB-BINDING FUSION PROTEINS

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/277,028, filed January 11, 2016; the contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] This invention relates generally to molecules that specifically engage 41BB, a member of the TNF receptor superfamily (TNFRSF). More specifically, this invention relates to multivalent and/or multispecific molecules that bind at least 41BB.

BACKGROUND OF THE INVENTION

[0003] The tumor necrosis factor receptor superfamily consists of several structurally related cell surface receptors. Activation by multimeric ligands is a common feature of many of these receptors. Many members of the TNFRSF have therapeutic utility in numerous pathologies, if activated properly. Agonism of this receptor family often requires higher order clustering, and conventional bivalent antibodies are not ideal for this purpose. Therefore, there exists a therapeutic need for more potent agonist molecules of the TNFRSF.

SUMMARY

[0004] The disclosure provides multivalent and multispecific TNF receptor superfamily (TNFRSF) binding fusion polypeptides that bind at least 41BB (also known as tumor necrosis factor receptor superfamily, member 4 (TNFRSF9) and/or CD137)). The use of the term “41BB” is intended to cover any variation thereof, such as, by way of non-limiting example, 41-BB and/or 4-1BB, and all variations are used herein interchangeably. These molecules that bind at least 41BB are referred to herein as “41BB-targeting molecules” or “41BB-targeting fusions” or “41BB-targeting proteins” or “41BB-targeting fusion polypeptides” or “41BB-targeting fusion proteins.” In some embodiments, the 41BB-

[0022] In some embodiments, the isolated polypeptide includes an amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124. In some embodiments, the isolated polypeptide includes an amino acid sequence that is at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 119-124.

[0023] In some embodiments, the isolated polypeptide comprises a complementarity determining region 1 (CDR1) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 101, 105, and 109; a complementarity determining region 2 (CDR2) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 102, 106, 110, and 117; and a complementarity determining region 3 (CDR3) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 103, 107, 111, 113, 115, and 118.

[0024] In some embodiments, the present disclosure provides isolated polypeptides that specifically bind at least 41BB and PDL1. In some embodiments, each binding domain (BD) in the isolated polypeptide is derived from antibodies or antibody fragments including scFv, Fabs, single domain antibodies (sdAb), V_{NAR}, or VHHs. In some embodiments, each BD is human or humanized sdAb. The sdAb fragments can be derived from VHH, V_{NAR}, engineered VH or VK domains. VHHs can be generated from camelid heavy chain only antibodies. V_{NARS} can be generated from cartilaginous fish heavy chain only antibodies. Various methods have been implemented to generate monomeric sdAbs from conventionally heterodimeric VH and VK domains, including interface engineering and selection of specific germline families. In other embodiments, the isolated polypeptides are derived from non-antibody scaffold proteins for example but not limited to designed ankyrin repeat proteins (darpins), avimers, anticalin/lipocalins, centyrins and fynomers.

[0024a] In a first aspect, there is provided an isolated polypeptide comprising at least one VHH domain that binds PDL1 and at least one VHH domain that binds 41BB.

[0024b] In a second aspect, there is provided an isolated polypeptide comprising at least one VHH domain that binds PDL1.

[0024c] In a third aspect, there is provided an isolated polypeptide comprising at least one VHH domain that binds 41BB.

[0024d] In a fourth aspect, there is provided an isolated polynucleotide that encodes any of the polypeptide disclosed herein.

[0024e] In a fifth aspect, there is provided an isolated cell that comprises the polynucleotide of the fourth aspect.

[0024f] In a sixth aspect, there is provided an isolated cell that expresses the polypeptide of the fourth aspect.

[0024g] In a seventh aspect, there is provided use of any of the polypeptide disclosed herein in the manufacture of a medicament for treating cancer.

[0024h] In an eighth aspect, there is provided an in vitro method of increasing T cell activation and/or proliferation comprising contacting T cells with any of the polypeptide disclosed herein.

[0024i] In a ninth aspect, there is provided use of any of the polypeptide disclosed herein in the manufacture of a medicament for increasing T cell activation and/or proliferation.

[0025] In some embodiments, the isolated polypeptide includes a first amino acid sequence that binds 4B11 selected from the group consisting of SEQ ID NO: 16, 20, 23, 25, 29, 33, 39, 33-41, 43, 45-47, 49, 51, 53, 54, 56, 58-60, 62, 65, 66, 68, 70, 72, 74, 76, 78, and 80-83, and a second amino acid sequence that binds PDL1 selected from the group consisting of SEQ ID NO: 100, 104, 108, 112, 114, 116, and 119-124.

What is claimed is:

1. An isolated polypeptide comprising at least one VHH domain that binds PDL1 and at least one VHH domain that binds 41BB.
2. The isolated polypeptide of claim 1, wherein at least one VHH domain that binds PDL1 comprises a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 105, 101, and 109, a CDR2 comprising an amino acid sequence selected from SEQ ID NO: 106, 102, 110, and 117, and a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 107, 103, 111, 113, 115, and 118.
3. The isolated polypeptide of claim 1 or claim 2, wherein at least one VHH domain that binds PDL1 comprises CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 105, 106, and 107; SEQ ID NOs: 101, 102, and 103; SEQ ID NOs: 109, 110, and 111; SEQ ID NOs: 101, 110, and 113; SEQ ID NOs 101, 110, and 115; and SEQ ID NOs: 101, 117, and 118.
4. The isolated polypeptide of any one of claims 1-3, wherein at least one VHH domain that binds PDL1 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 105, a CDR2 comprising the amino acid sequence of SEQ ID NO: 106, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 107.
5. The isolated polypeptide of any one of claims 1-4, wherein at least one VHH domain that binds PDL1 is humanized.
6. The isolated polypeptide of any one of claims 1-5, wherein at least one VHH domain that binds PDL1 comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 124, 100, 104, 108, 112-114, 116, and 119-123.
7. The isolated polypeptide of any one of claims 1-6, wherein at least one VHH domain that binds PDL1 comprises an amino acid sequence selected from SEQ ID NOs: 124, 100, 104, 108, 112-114, 116, and 119-123.
8. The isolated polypeptide of any one of claims 1-7, wherein at least one VHH domain that binds PDL1 comprises the amino acid sequence of SEQ ID NO: 124.
9. The isolated polypeptide of any one of claims 1-8, wherein the polypeptide comprises one VHH domain that binds PDL1.
10. The isolated polypeptide of any one of claims 1-9, wherein at least one VHH domain that binds 41BB comprises a CDR1 comprising an amino acid sequence selected from

SEQ ID NOs: 26, 17, 21, 30, 50, 65, 67, 69, 433, 437, 441, and 445; a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 61, 18, 27, 31, 42, 44, 48, 52, 63, 71, 73, 75, 77, 79, 434, 438, 442, and 446; and a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 28, 19, 22, 24, 32, 55, 57, 435, 439, 443, and 447.

11. The isolated polypeptide of any one of claims 1-10, wherein at least one VHH domain that binds 41BB comprises a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 26, 65, 67, and 69; a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 61, 27, 63, 71, 73, 75, 77, and 79; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

12. The isolated polypeptide of any one of claims 1-11, wherein at least one VHH domain that binds 41BB comprises CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 26, 61, and 28; SEQ ID NOs: 17, 18, and 19; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 30, 31, and 32; SEQ ID NOs: 21, 18, and 22; SEQ ID NOs: 17, 18, and 24; SEQ ID NOs: 21, 42, and 22; SEQ ID NOs: 21, 44, and 22; SEQ ID NOs: 21, 48, and 19; SEQ ID NOs: 21, 48, and 22; SEQ ID NOs: 50, 48, and 22; SEQ ID NOs: 21, 52, and 22; SEQ ID NOs: 50, 52, and 22; SEQ ID NOs: 21, 48, and 55; SEQ ID NOs: 21, 48, 57; SEQ ID NOs: 26, 63, and 28; SEQ ID NOs: 65, 61, and 28; SEQ ID NOs: 67, 61, and 28; SEQ ID NOs: 69, 61, and 28; SEQ ID NOs: 26, 71, and 28; SEQ ID NOs: 26, 73, and 28; SEQ ID NOs: 26, 75, and 28; SEQ ID NOs: 26, 77, and 28; SEQ ID NOs: 26, 79, and 28; SEQ ID NOs: 433, 434, and 435; SEQ ID NOs: 437, 438, and 439; SEQ ID NOs: 441, 442, and 443; and SEQ ID NOs: 445, 446, and 447.

13. The isolated polypeptide of any one of claims 1-12, wherein at least one VHH domain that binds 41BB comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a CDR2 comprising the amino acid sequence of SEQ ID NO: 61, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

14. The isolated polypeptide of any one of claim 1-13, wherein at least one VHH domain that binds 41BB is humanized.

15. The isolated polypeptide of any one of claims 1-14, wherein at least one VHH domain that binds 41BB comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 60, 16, 20, 23, 25, 29, 33, 34-41, 43, 45-47, 49, 51, 53, 54, 56, 58, 59, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 81, 82, 83, 432, 436, 440, and 444.

16. The isolated polypeptide of any one of claims 1-14, wherein at least one VHH domain that binds 41BB comprises an amino acid sequence selected from SEQ ID NOs: 60, 16, 20, 23, 25, 29, 33, 34-41, 43, 45-47, 49, 51, 53, 54, 56, 58, 59, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 81, 82, 83, 432, 436, 440, and 444.
17. The isolated polypeptide of any one of claims 1-16, wherein at least one VHH domain that binds 41BB comprises the amino acid sequence of SEQ ID NO: 60.
18. The isolated polypeptide of any one of claims 1-17, wherein the polypeptide comprises one VHH domain that binds 41BB.
19. The isolated polypeptide of any one of claims 1-18, wherein the isolated polypeptide comprises an Fc region.
20. The isolated polypeptide of claim 19, wherein the Fc region comprises an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 1-6.
21. The isolated polypeptide of claim 19, wherein the Fc region comprises an amino acid sequence selected from SEQ ID NOs: 1-6.
22. The isolated polypeptide of any one of claims 19-21, which forms a homodimer under physiological conditions.
23. The isolated polypeptide of any one of claims 19-22, wherein the polypeptide has the structure VHH-Linker-VHH-Linker-Hinge-Fc.
24. The isolated polypeptide of claim 23, wherein at least one linker is, independently, 5-20 amino acids in length.
25. The isolated polypeptide of claim 23 or claim 24, wherein the hinge comprises a sequence selected from SEQ ID NOs: 7-9.
26. The isolated polypeptide of any one of claims 1-25, comprising a first VHH domain (VHH₁) that binds PDL1 and a second VHH domain (VHH₂) that binds 41BB, wherein VHH₁ comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 105, a CDR2 comprising the amino acid sequence of SEQ ID NO: 106, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 107; and VHH₂ comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a CDR2 comprising the amino acid sequence of SEQ ID NO: 61, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28; and wherein the polypeptide has the structure VHH₁-Linker-VHH₂-Linker-Hinge-Fc.

27. The isolated polypeptide of claim 26, wherein the first VHH domain comprises the amino acid sequence of SEQ ID NO: 124 and the second VHH domain comprises the amino acid sequence of SEQ ID NO: 60.
28. The isolated polypeptide of claim 26 or claim 27, wherein the Fc region comprises an amino acid sequence that is at least 97% identical to the sequence of SEQ ID NO: 2.
29. The isolated polypeptide of claim 26 or claim 27, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 2.
30. The isolated polypeptide of any one of claims 26-29, wherein the polypeptide comprises an amino acid sequence that is at least 97% identical to the sequence of SEQ ID NO: 449.
31. The isolated polypeptide of any one of claims 26-29, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 449.
32. The isolated polypeptide of any one of claims 26-31, which forms a dimer at physiological conditions.
33. An isolated polypeptide comprising at least one VHH domain that binds PDL1.
34. The isolated polypeptide of claim 33, wherein at least one VHH domain that binds PDL1 comprises a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 105, 101, and 109, a CDR2 comprising an amino acid sequence selected from SEQ ID NO: 106, 102, 110, and 117, and a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 107, 103, 111, 113, 115, and 118.
35. The isolated polypeptide of claim 33 or claim 34, wherein at least one VHH domain that binds PDL1 comprises CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 105, 106, and 107; SEQ ID NOs: 101, 102, and 103; SEQ ID NOs: 109, 110, and 111; SEQ ID NOs: 101, 110, and 113; SEQ ID NOs 101, 110, and 115; and SEQ ID NOs: 101, 117, and 118.
36. The isolated polypeptide of any one of claims 33-35, wherein at least one VHH domain that binds PDL1 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 105, a CDR2 comprising the amino acid sequence of SEQ ID NO: 106, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 107.
37. The isolated polypeptide of any one of claims 33-36, wherein at least one VHH domain that binds PDL1 is humanized.
38. The isolated polypeptide of any one of claims 33-37, wherein at least one VHH domain that binds PDL1 comprises an amino acid sequence that is at least 95% identical

to an amino acid sequence selected from SEQ ID NOs: 124, 100, 104, 108, 112-114, 116, and 119-123.

39. The isolated polypeptide of any one of claims 33-38, wherein at least one VHH domain that binds PDL1 comprises an amino acid sequence selected from SEQ ID NOs: 124, 100, 104, 108, 112-114, 116, and 119-123.

40. The isolated polypeptide of any one of claims 33-39, wherein at least one VHH domain that binds PDL1 comprises the amino acid sequence of SEQ ID NO: 124.

41. The isolated polypeptide of any one of claims 33-40, wherein the polypeptide comprises one VHH domain that binds PDL1.

42. The isolated polypeptide of any one of claims 33-41, wherein the isolated polypeptide comprises an Fc region.

43. The isolated polypeptide of claim 42, wherein the Fc region comprises an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 1-6.

44. The isolated polypeptide of claim 42, wherein the Fc region comprises an amino acid sequence selected from SEQ ID NOs: 1-6.

45. The isolated polypeptide of any one of claims 42-44, which forms a homodimer under physiological conditions.

46. An isolated polypeptide comprising at least one VHH domain that binds 41BB.

47. The isolated polypeptide of claim 46, wherein at least one VHH domain that binds 41BB comprises a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 26, 17, 21, 30, 50, 65, 67, 69, 433, 437, 441, and 445; a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 61, 18, 27, 31, 42, 44, 48, 52, 63, 71, 73, 75, 77, 79, 434, 438, 442, and 446; and a CDR3 comprising an amino acid sequence selected from SEQ ID NOs: 28, 19, 22, 24, 32, 55, 57, 435, 439, 443, and 447.

48. The isolated polypeptide of any one of claims 46-47, wherein at least one VHH domain that binds 41BB comprises a CDR1 comprising an amino acid sequence selected from SEQ ID NOs: 26, 65, 67, and 69; a CDR2 comprising an amino acid sequence selected from SEQ ID NOs: 61, 27, 63, 71, 73, 75, 77, and 79; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

49. The isolated polypeptide of any one of claims 46-48, wherein at least one VHH domain that binds 41BB comprises CDR1, CDR2, and CDR3 selected from: SEQ ID NOs: 26, 61, and 28; SEQ ID NOs: 17, 18, and 19; SEQ ID NOs: 26, 27, and 28; SEQ ID

NOs: 30, 31, and 32; SEQ ID NOs: 21, 18, and 22; SEQ ID NOs: 17, 18, and 24; SEQ ID NOs: 21, 42, and 22; SEQ ID NOs: 21, 44, and 22; SEQ ID NOs: 21, 48, and 19; SEQ ID NOs: 21, 48, and 22; SEQ ID NOs: 50, 48, and 22; SEQ ID NOs: 21, 52, and 22; SEQ ID NOs: 50, 52, and 22; SEQ ID NOs: 21, 48, and 55; SEQ ID NOs: 21, 48, 57; SEQ ID NOs: 26, 63, and 28; SEQ ID NOs: 65, 61, and 28; SEQ ID NOs: 67, 61, and 28; SEQ ID NOs: 69, 61, and 28; SEQ ID NOs: 26, 71, and 28; SEQ ID NOs: 26, 73, and 28; SEQ ID NOs: 26, 75, and 28; SEQ ID NOs: 26, 77, and 28; SEQ ID NOs: 26, 79, and 28; SEQ ID NOs: 433, 434, and 435; SEQ ID NOs: 437, 438, and 439; SEQ ID NOs: 441, 442, and 443; and SEQ ID NOs: 445, 446, and 447.

50. The isolated polypeptide of any one of claims 46-49, wherein at least one VHH domain that binds 41BB comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 26, a CDR2 comprising the amino acid sequence of SEQ ID NO: 61, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 28.

51. The isolated polypeptide of any one of claim 46-50, wherein at least one VHH domain that binds 41BB is humanized.

52. The isolated polypeptide of any one of claims 46-51, wherein at least one VHH domain that binds 41BB comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NOs: 60, 16, 20, 23, 25, 29, 33, 34-41, 43, 45-47, 49, 51, 53, 54, 56, 58, 59, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 81, 82, 83, 432, 436, 440, and 444.

53. The isolated polypeptide of any one of claims 46-52, wherein at least one VHH domain that binds 41BB comprises an amino acid sequence selected from SEQ ID NOs: 60, 16, 20, 23, 25, 29, 33, 34-41, 43, 45-47, 49, 51, 53, 54, 56, 58, 59, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 81, 82, 83, 432, 436, 440, and 444.

54. The isolated polypeptide of any one of claims 46-53, wherein at least one VHH domain that binds 41BB comprises the amino acid sequence of SEQ ID NO: 60.

55. The isolated polypeptide of any one of claims 46-54, wherein the polypeptide comprises one VHH domain that binds 41BB.

56. The isolated polypeptide of any one of claims 46-55, wherein the isolated polypeptide comprises an Fc region.

57. The isolated polypeptide of claim 56, wherein the Fc region comprises an amino acid sequence that is at least 97% identical to an amino acid sequence selected from SEQ ID NOs: 1-6.

58. The isolated polypeptide of claim 56, wherein the Fc region comprises an amino acid sequence selected from SEQ ID NOs: 1-6.
59. The isolated polypeptide of any one of claims 56-58, which forms a homodimer under physiological conditions.
60. An isolated polynucleotide that encodes the polypeptide of any one of claims 1-59.
61. An isolated cell that comprises the polynucleotide of claim 60.
62. An isolated cell that expresses the polypeptide of claim 60.
63. Use of a polypeptide of any one of claims 1-59 in the manufacture of a medicament for treating cancer.
64. The use of claim 63, wherein the cancer is selected from carcinoma, sarcoma, lymphoma, and leukemia.
65. An in vitro method of increasing T cell activation and/or proliferation comprising contacting T cells with a polypeptide of any one of claims 1-59.
66. The method of claim 65, wherein the T cells are CD4⁺ T cells and/or CD8⁺ T cells.
67. Use of a polypeptide of any one of claims 1-59 in the manufacture of a medicament for increasing T cell activation and/or proliferation.
68. The use of claim 67, wherein the T cells are CD4⁺ T cells and/or CD8⁺ T cells.