

CLAIMS

WHAT IS CLAIMED IS:

1. A mesothelin (MSLN) binding 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 MSLN,
wherein the domains are linked in the order H₂N-(A)-(C)-(B)-COOH, H₂N-(B)-(A)-(C)-COOH, H₂N-(C)-(B)-(A)-COOH, or by linkers L1 and L2.
2. The MSLN binding trispecific protein of claim 1, wherein the first domain comprises a variable light domain and variable heavy domain each of which is capable of specifically binding to human CD3.
3. The MSLN binding protein of claim 1, wherein the first domain is humanized or human.
4. The MSLN binding trispecific protein of claim 1, wherein the second domain binds albumin.
5. The MSLN binding trispecific protein of claim 1, wherein the second domain comprises a scFv, a variable heavy domain (VH), a variable light domain (VL), a peptide, a ligand, or a small molecule.
6. The MSLN binding trispecific protein of claim 1, wherein the third domain comprises a VHH domain, a scFv, a VH domain, a VL domain, a non-Ig domain, a ligand, a knottin, or a small molecule entity that specifically binds to MSLN.
7. The MSLN binding trispecific protein of claim 6, wherein the third domain comprises a VHH domain.
8. The MSLN binding trispecific protein of claim 7, wherein said VHH domain comprises one or more conserved regions comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 41, 42, 43, or 44.
9. The MSLN binding trispecific protein of claim 8, wherein said VHH domain comprises a conserved region comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 41.
10. The MSLN binding trispecific protein of claim 8, wherein said VHH domain comprises a conserved region comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 42.
11. The MSLN binding trispecific protein of claim 8, wherein said VHH domain comprises a conserved domain comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 43.

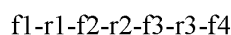
12. The MSLN binding trispecific protein of claim 8, wherein said VHH domain comprises a conserved domain comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 44.
13. The MSLN binding trispecific protein of claim 7, wherein said VHH domain comprises (i) a stretch of amino acids corresponding to SEQ ID NO: 41; (ii) a stretch of amino acids corresponding to SEQ ID NO: 42; (iii) a stretch of amino acids corresponding to SEQ ID NO: 43, and (iv) a stretch of amino acids corresponding to SEQ ID NO: 44.
14. The MSLN binding trispecific protein of claim 7, wherein said VHH domain comprises the following formula:

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

wherein, r1 is identical to or comprises one or more amino acid residue substitutions relative to SEQ ID NO: 51; r2 is identical to or comprises one or more amino acid residue substitutions relative to SEQ ID NO: 52; and r3 is identical to or comprises one or more amino acid residue substitutions relative to SEQ ID NO: 53; and wherein f1, f2, f3 and f4 are framework residues.

15. The MSLN binding trispecific protein of claim 7, wherein said VHH domain comprises a sequence that is at least 80% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-29.
16. The MSLN binding trispecific protein of claim 1, wherein the third domain comprises selected sequence from the group consisting of SEQ ID NOs: 1-29.
17. The MSLN binding trispecific protein of claim 1, wherein the third domain is a humanized VHH domain.
18. The MSLN binding trispecific protein of claim 17, wherein said humanized VHH domain comprises one or more conserved regions comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 45, 46, 47, 48, 49, or 50.
19. The MSLN binding trispecific protein of claim 18, wherein said VHH domain comprises a conserved region comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 45.
20. The MSLN binding trispecific protein of claim 18, wherein said VHH domain comprises a conserved region comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 46.

21. The MSLN binding trispecific protein of claim 18, wherein said VHH domain comprises a conserved domain comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 47.
22. The MSLN binding trispecific protein of claim 18, wherein said VHH domain comprises a conserved domain comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 48.
23. The MSLN binding trispecific protein of claim 18, wherein said VHH domain comprises a conserved domain comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 49.
24. The MSLN binding trispecific protein of claim 18, wherein said VHH domain comprises a conserved domain comprising a sequence identical to or comprising one or more amino acid residue substitutions relative to SEQ ID NO: 50.
25. The MSLN binding trispecific protein of claim 18, wherein said VHH domain comprises (i) a stretch of amino acids corresponding to SEQ ID NO: 45, (ii) a stretch of amino acids corresponding to SEQ ID NO: 46, (iii) a stretch of amino acids corresponding to SEQ ID NO: 47, (iv) a stretch of amino acids corresponding to SEQ ID NO: 48, (v) a stretch of amino acids corresponding to SEQ ID NO: 49, and (vi) a stretch of amino acids corresponding to SEQ ID NO: 50.
26. The MSLN binding trispecific protein of claim 17, wherein said humanized VHH domain comprises the following formula:



wherein, r1 is identical to or comprises one or more amino acid residue substitutions relative to SEQ ID NO: 54; r2 is identical to or comprises one or more amino acid residue substitutions relative to SEQ ID NO: 55; and r3 is identical to or comprises one or more amino acid residue substitutions relative to SEQ ID NO: 56; and wherein f1, f2, f3 and f4 are framework residues.

27. The MSLN binding trispecific protein of any one of claims 17-26, wherein the third domain comprises a sequence selected from the group consisting of SEQ ID NOs: 30-40, and 102-105.
28. The MSLN binding trispecific protein of any one of claims 1-27, wherein the third domain binds to a human mesothelin protein comprising the sequence set forth as SEQ ID NO: 57.
29. The MSLN binding trispecific protein of any one of claims 1-28, wherein the third domain binds to an epitope of mesothelin, wherein said epitope is located in region I, comprising amino acid residues 296-390 of SEQ ID NO: 57, region II comprising amino acid residue 391-

486 of SEQ ID NO: 57, or region III comprising amino acid residues 487-598 of SEQ ID NO: 57.

30. The MSLN binding trispecific protein of claim 1, wherein linkers L1 and L2 are each independently selected from (GS)_n (SEQ ID NO: 87), (GGS)_n (SEQ ID NO: 88), (GGGS)_n (SEQ ID NO: 89), (GGSG)_n (SEQ ID NO: 90), (GGSGG)_n (SEQ ID NO: 91), or (GGGGS)_n (SEQ ID NO: 92), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

31. The MSLN binding trispecific protein of claim 1, wherein linkers L1 and L2 are each independently (GGGGS)₄ (SEQ ID NO: 95) or (GGGGS)₃ (SEQ ID NO: 96).

32. The MSLN binding trispecific protein of claim 1, wherein the domains are linked in the order H₂N-(C)-(B)-(A)-COOH.

33. The MSLN binding trispecific protein of claim 1, wherein the protein is less than about 80 kDa.

34. The MSLN binding trispecific protein of claim 1, wherein the protein is about 50 to about 75 kDa.

35. The MSLN binding trispecific protein of claim 1, wherein the protein is less than about 60 kDa.

36. The MSLN binding trispecific protein of claim 1, wherein the protein has an elimination half-time of at least about 50 hours.

37. The MSLN binding trispecific protein of claim 1, wherein the protein has an elimination half-time of at least about 100 hours.

38. The MSLN binding trispecific protein of claim 1, wherein the protein has increased tissue penetration as compared to an IgG to the same MSLN.

39. The MSLN binding trispecific protein of claim 1, wherein the protein comprises a sequence selected from the group consisting of SEQ ID NOs: 58-86, 98, 100, and 101.

40. A pharmaceutical composition comprising (i) the MSLN binding trispecific protein according to any one of claims 1-39 and (ii) a pharmaceutically acceptable carrier.

41. A process for the production of a mesothelin binding trispecific protein according to any one of claims 1-39, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding a mesothelin binding trispecific protein according to any one of claims 1-39 under conditions allowing the expression of the mesothelin binding trispecific protein and recovering and purifying the produced protein from the culture.

42. A method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of the mesothelin binding trispecific protein according to any one of claims 1-39, to a subject in need thereof.

43. The method of claim 42, wherein the subject is human.

44. The method of claim 43, wherein the method further comprises administration of an agent in combination with the mesothelin binding trispecific protein according to any one of claims 1-39.
45. The method of any one of claims 42-44, wherein the mesothelin binding trispecific protein selectively binds to tumor cells expressing mesothelin.
46. The method of claim 45, wherein the mesothelin binding trispecific protein mediates T cell killing of tumor cells expressing mesothelin.
47. The method of any one of claims 42-46, wherein the tumorous disease comprises a solid tumor disease.
48. The method of claim 47, wherein the solid tumor disease comprises mesothelioma, lung cancer, gastric cancer, ovarian cancer, or triple negative breast cancer.
49. The method of claim 48, wherein the solid tumor disease is metastatic.
50. A method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising administration of a mesothelin binding trispecific protein comprising a sequence selected from the group consisting of SEQ ID NOs: 58-86, 98, 100, and 101.
51. The method of claim 50, wherein the mesothelin binding trispecific protein selectively binds to tumor cells expressing mesothelin.
52. The method of claim 51, wherein the mesothelin binding trispecific protein directs T cell killing of tumor cells expressing mesothelin.
53. The method of any one of claims 50-52, wherein the tumorous disease comprises a solid tumor disease.
54. The method of claim 53, wherein the solid tumor disease comprises mesothelioma, lung cancer, gastric cancer, ovarian cancer, or triple negative breast cancer.
55. The method of claim 54, wherein the solid tumor disease is metastatic.
56. A method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising administration of a mesothelin binding trispecific protein comprising a sequence as set forth in SEQ ID NO: 98.
57. The method of any one of claims 50-56, comprising administering the mesothelin binding trispecific protein at a dose of up to 10 mg/kg.
58. The method of any one of claims 50-57, wherein the protein is administered once a week.
59. The method of any one of claims 50-57, wherein the protein is administered twice per week.
60. The method of any one of claims 50-57, wherein the protein is administered every other week.

61. The method of any one of claims 50-57, wherein the protein is administered every three weeks.
62. A mesothelin (MSLN) binding trispecific protein, comprising the sequence as set forth in SEQ ID NO: 98.
63. A mesothelin (MSLN) binding 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 MSLN,
wherein the domains are linked in the order H₂N-(A)-(C)-(B)-COOH, H₂N-(B)-(A)-(C)-COOH, H₂N-(C)-(B)-(A)-COOH, or by linkers L1 and L2, wherein said third domain comprises one or more CDR sequences selected from SEQ ID Nos: 51-56 and 106-222.
64. The MSLN binding trispecific protein of claim 63, wherein said third domain comprises a CDR1 comprising a sequence as set forth in any one of SEQ ID Nos.: 51, 54, and 106-144.
65. The MSLN binding trispecific protein of claim 63 or 64, wherein said third domain comprises a CDR2 comprising a sequence as set forth in any one of SEQ ID Nos.: 52, 55, and 145-183.
66. The MSLN binding trispecific protein of any one of claims 63-65, wherein said third domain comprises a CDR2 comprising a sequence as set forth in any one of SEQ ID Nos.: 53, 56, and 184-222.
67. The MSLN binding trispecific protein of any one of claims 63-66, wherein said third domain comprises a framework region 1 (f1) comprising a sequence as set forth in any one of SEQ ID Nos.: 262-300.
68. The MSLN binding trispecific protein of any one of claims 63-67, wherein said third domain comprises a framework region (f2) sequence as set forth in any one of SEQ ID Nos.: 301-339.
69. The MSLN binding trispecific protein of any one of claims 63-68, wherein said third domain comprises a framework region (f3) a sequence as set forth in any one of SEQ ID Nos.: 340-378.
70. The MSLN binding trispecific protein of any one of claims 63-69, wherein the protein comprises a sequence selected from the group consisting of SEQ ID NOs: 58-86, 98, 100, and 101.
71. The MSLN binding trispecific protein of any one of claims 63-70, wherein the protein comprises a sequence as set forth in SEQ ID NO: 98.
72. A pharmaceutical composition comprising (i) the MSLN binding trispecific protein according to any one of claims 63-71 and (ii) a pharmaceutically acceptable carrier.

73. A process for the production of a mesothelin binding trispecific protein according to any one of claims 63-71, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding a mesothelin binding trispecific protein according to any one of claims 63-71 under conditions allowing the expression of the mesothelin binding trispecific protein and recovering and purifying the produced protein from the culture.
74. A method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising the administration of the mesothelin binding trispecific protein according to any one of claims 63-71, to a subject in need thereof.
75. The method of claim 74, wherein the subject is human.
76. The method of claim 75, wherein the method further comprises administration of an agent in combination with the mesothelin binding trispecific protein according to any one of claims 63-71.
77. The method of any one of claims 74-76, wherein the mesothelin binding trispecific protein selectively binds to tumor cells expressing mesothelin.
78. The method of claim 77, wherein the mesothelin binding trispecific protein mediates T cell killing of tumor cells expressing mesothelin.
79. The method of any one of claims 74-78, wherein the tumorous disease comprises a solid tumor disease.
80. The method of claim 79, wherein the solid tumor disease comprises mesothelioma, lung cancer, gastric cancer, ovarian cancer, or triple negative breast cancer.
81. The method of claim 80, wherein the solid tumor disease is metastatic.
82. A method for the treatment or amelioration of a proliferative disease, or a tumorous disease, comprising administration of a mesothelin binding trispecific protein according to any one of claims 63-71.
83. The method of claim 82, wherein the mesothelin binding trispecific protein selectively binds to tumor cells expressing mesothelin.
84. The method of claim 83, wherein the mesothelin binding trispecific protein directs T cell killing of tumor cells expressing mesothelin.
85. The method of any one of claims 82-84, wherein the tumorous disease comprises a solid tumor disease.
86. The method of claim 85, wherein the solid tumor disease comprises mesothelioma, lung cancer, gastric cancer, ovarian cancer, or triple negative breast cancer.
87. The method of claim 86, wherein the solid tumor disease is metastatic.