

We Claim:

1. A bispecific construct comprising an anti-ILT4 binding domain linked to an anti-PD-1 binding domain, wherein:

I. (a) the anti-ILT4 binding domain comprises:

(i) a heavy chain variable region CDR1 amino acid sequence selected from the consensus sequence: G Y T (I,M) H (SEQ ID NO: 21), or conservative sequence modifications thereof;

(ii) a heavy chain variable region CDR2 amino acid sequence as set forth in SEQ ID NO:3, or conservative sequence modifications thereof;

(iii) a heavy chain variable region CDR3 amino acid sequence selected from the consensus sequence: E R P G G S Q F I Y Y Y (P,A) (M,L) D Y (SEQ ID NO:22) , or conservative sequence modifications thereof;

(iv) a light chain variable region CDR1 amino acid sequence selected from the consensus sequence: R A S (A,E) N I Y S Y L A (SEQ ID NO: 23), or conservative sequence modifications thereof;

(v) a light chain variable region CDR2 amino acid sequence selected from the consensus sequence: N A (I,D) T L A E (SEQ ID NO: 24), or conservative sequence modifications thereof; and

(vi) a light chain variable region CDR3 amino acid sequence as set forth in SEQ ID NO:8, or conservative sequence modifications thereof; and

(b) the anti-PD-1 binding domain comprises:

(i) heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 31, 36, and 41, respectively, or conservative sequence modifications thereof, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 46, 51, and 56, respectively, or conservative sequence modifications thereof; or

(ii) heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 69, 74, and 79, respectively, or conservative sequence modifications thereof, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 84, 89, and 94, respectively, or conservative sequence modifications thereof; or

II. (a) the anti-ILT4 binding domain comprises:

(i) heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:11, 13, and 15, respectively, or conservative sequence modifications thereof, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:16, 17, and 18, respectively, or conservative sequence modifications thereof, or

(ii) heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:1, 3, and 5, respectively, or conservative sequence modifications thereof, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:10, 11, and 12, respectively, or conservative sequence modifications thereof; and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:6, 7, and 8, respectively, or conservative sequence modifications thereof; and

(b) the anti-PD-1 binding domain comprises:

(i) heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 31, 36, and 41, respectively, or conservative sequence modifications thereof, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 46, 51, and 56, respectively, or conservative sequence modifications thereof; or

(ii) heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 69, 74, and 79, respectively, or conservative sequence modifications thereof, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 84, 89, and 94, respectively, or conservative sequence modifications thereof; or

III. (a) the anti-ILT4 binding domain comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:11, 13, and 15, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:16, 17, and 18, respectively; and

(b) the anti-PD-1 binding domain comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 31, 36, and 41, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 46, 51, and 56, respectively; or

IV. (a) the anti-ILT4 binding domain comprises:

(i) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:19, or a sequence at least 95% identical thereto, and a light chain variable region amino acid sequence as set forth in SEQ ID NO:20, or a sequence at least 95% identical thereto; or

(ii) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:9, or a sequence at least 95% identical thereto, and a light chain variable region amino acid sequence as set forth in SEQ ID NO:10, or a sequence at least 95% identical thereto; and

(b) the anti-PD-1 binding domain comprises:

(i) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:59, or a sequence at least 95% identical thereto, and a light chain variable region amino acid sequence as set forth in SEQ ID NO:60, or a sequence at least 95% identical thereto; or

(ii) a heavy chain variable region amino acid sequence as set forth in SEQ ID NO:61, or a sequence at least 95% identical thereto, and a light chain variable region amino acid sequence as set forth in SEQ ID NO:62, or a sequence at least 95% identical thereto.

2. The bispecific construct of claim 1, wherein

(a) the anti-ILT4 binding domain comprises a heavy chain variable region comprising SEQ ID NO:19 and a light chain variable region comprising SEQ ID NO:20; or

(b) the anti-ILT4 binding domain comprises a heavy chain variable region comprising SEQ ID NO:9 and a light chain variable region comprising SEQ ID NO:10; and/or

(c) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO: 59 and a light chain variable region comprising SEQ ID NO:60.

3. A bispecific construct comprising an anti-ILT4 binding domain linked to an anti-PD-1 binding domain, wherein:

I. (a) the anti-ILT4 binding domain comprises a heavy chain variable region comprising SEQ ID NO:19 and a light chain variable region comprising SEQ ID NO:20; and

(b) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO:59 and a light chain variable region comprising SEQ ID NO:60; or

II. (a) the anti-ILT4 binding domain comprises a heavy chain variable region comprising SEQ ID NO:9 and a light chain variable region comprising SEQ ID NO:10; and

(b) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO:59 and a light chain variable region comprising SEQ ID NO:60; or

III. (a) the anti-ILT4 binding domain comprises a heavy chain variable region comprising SEQ ID NO:19 and a light chain variable region comprising SEQ ID NO:20; and

(b) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO:61 and a light chain variable region comprising SEQ ID NO:62; or

IV. (a) the anti-ILT4 binding domain comprises a heavy chain variable region comprising SEQ ID NO:9 and a light chain variable region comprising SEQ ID NO:10; and

(b) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO:61 and a light chain variable region comprising SEQ ID NO:62.

4. The bispecific construct of any one of the preceding claims wherein the anti-PD-1 binding domain further comprises a human IgG1 constant domain; and/or

wherein the anti-ILT4 binding domain is linked to the C-terminus of the heavy chain of the anti-PD-1 binding domain; and / or

wherein the anti-ILT4 binding domain is a scFv.

5. The bispecific construct of any one of the preceding claims, wherein the anti-ILT4 binding domain and the anti-PD-1 binding domain are genetically fused or wherein the anti-ILT4 binding domain and the anti-PD-1 binding domain are chemically conjugated.

6. A bispecific construct comprising an anti-PD-1 binding domain linked to an anti-ILT4 scFv, wherein:

I. (a) the anti-ILT4 scFv comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:11, 13, and 15, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:16, 17, and 18, respectively; and

(b) the anti-PD-1 binding domain comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 31, 36, and 41, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 46, 51, and 56, respectively, and a human IgG1 constant domain; or

II. (a) the anti-ILT4 scFv comprises a heavy chain variable region comprising SEQ ID NO:19 and a light chain variable region comprising SEQ ID NO:20; and

(b) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO:59 and a light chain variable region comprising SEQ ID NO:60.

7. A bispecific construct comprising an anti-PD-1 binding domain linked to an anti-ILT4 scFv, wherein:

I. (a) the anti-ILT4 scFv comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:11, 13, and 15, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:16, 17, and 18, respectively; and

(b) the anti-PD-1 binding domain comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 69, 74, and 79, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 84, 89, and 94, respectively, and a human IgG1 constant domain; or

II. (a) the anti-ILT4 scFv comprises a heavy chain variable region comprising SEQ ID NO:19 and a light chain variable region comprising SEQ ID NO:20; and

(b) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO:61 and a light chain variable region comprising SEQ ID NO:62.

8. A bispecific construct comprising an anti-PD-1 binding domain linked to an anti-ILT4 scFv, wherein:

I. (a) the anti-ILT4 scFv comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:1, 3, and 5, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 6, 7, and 8, respectively; and

(b) the anti-PD-1 binding domain comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 31, 36, and 41, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 46, 51, and 56, respectively, and a human IgG1 constant domain; or

II. (a) the anti-ILT4 scFv comprises a heavy chain variable region comprising SEQ ID NO:9 and a light chain variable region comprising SEQ ID NO:10; and

(b) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO:59 and a light chain variable region comprising SEQ ID NO:60.

9. A bispecific construct comprising an anti-PD-1 binding domain linked to an anti-ILT4 scFv, wherein:

I. (a) the anti-ILT4 scFv comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:1, 3, and 5, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs:6, 7, and 8, respectively; and

(b) the anti-PD-1 binding domain comprises heavy chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 69, 74, and 79, respectively, and light chain variable region CDR1, CDR2 and CDR3 as set forth in SEQ ID NOs: 84, 89, and 94, respectively, and a human IgG1 constant domain; or

II. (a) the anti-ILT4 scFv comprises a heavy chain variable region comprising SEQ ID NO:9 and a light chain variable region comprising SEQ ID NO:10; and

(b) the anti-PD-1 binding domain comprises a heavy chain variable region comprising SEQ ID NO:61 and a light chain variable region comprising SEQ ID NO:62.

10. The bispecific construct of claim 7, wherein the anti-PD-1 binding domain and the anti-ILT4 scFv comprise heavy and light chain sequences as set forth in SEQ ID NOs:64 and 63, respectively or wherein the anti-PD-1 binding domain and the anti-ILT4 scFv heavy and light chains are encoded by the nucleotide sequences as set forth in SEQ ID NOs:66 and 65, respectively.

11. A composition comprising the bispecific construct of any one of claims 1 to 10 and a pharmaceutically acceptable carrier.

12. The composition of claim 11, further comprising one or more therapeutic agents.

13. The composition of claim 12, wherein the therapeutic agent is another antibody.

14. The composition of claim 13, wherein the antibody is an anti-PD-L1 and/or an anti-CTLA-4 antibody.

15. A kit comprising the bispecific construct of any one of claims 1 to 10, or the composition of any one of claims 11 to 14, and instructions for use.
16. An invitro method of activating macrophages comprising contacting macrophages with the bispecific construct of any one of claims 1 to 10, or the composition of any one of claims 11 to 14.
17. The bispecific construct of any one of claims 1 to 10, or the composition of any one of claims 11 to 14, for use in inducing or enhancing an immune response in a subject.
18. The bispecific construct of any one of claims 1 to 10, or the composition of any one of claims 11 to 14, for use in treating a condition or disease in a subject.
19. The bispecific construct or composition of claim 18, wherein the subject suffers from a condition or disease in which stimulation of an immune response is desired.
20. The bispecific construct or composition of claim 17 or 18, wherein the condition or disease is cancer.
21. The bispecific construct or composition of claim 20, wherein the cancer is skin cancer, colorectal cancer, ovarian cancer, renal cell carcinoma, head and neck squamous cell carcinoma, breast cancer, lung cancer, bladder cancer, prostate cancer, melanoma, gynecological cancers, sarcoma, lymphoma, or glioblastoma.
22. The bispecific construct of any one of claims 1 to 10, or the composition of any one of claims 11 to 14, for use in treating a tumor in a subject.
23. The bispecific construct or composition of claim 22, wherein the tumor expresses ILT4, HLA-G, HLA class I, angiopoietin like 2, Nogo, an ILT4 ligand, PD-L1, or PD-L2.
24. The bispecific construct or composition of any one of claims 17 to 23, further comprising one or more therapeutic agents to be administered to the subject.

25. The bispecific construct or composition of claim 24, wherein the therapeutic agent is another antibody.

26. The bispecific construct or composition of claim 25, wherein the antibody is an anti-PD-L1 and/or an anti-CTLA-4 antibody.

27. The bispecific construct or composition of any one of claims 22 to 42, wherein the bispecific construct and the one or more therapeutic agents are to be administered concurrently, or wherein the bispecific construct and the one or more therapeutic agents are to be administered sequentially.

28. Use of the bispecific construct of any one of claims 1 to 10, or the composition of any one of claims 11 to 14, in the manufacture of a medicament for treatment of cancer.

29. The use of claim 28, further comprising one or more therapeutic agents.

30. The use of claim 29, wherein the therapeutic agent is another antibody.

31. The use of claim 30, wherein the antibody is an anti-PD-L1 and/or an anti-CTLA-4 antibody.

32. The use of any one of claims 29 or 31, wherein the bispecific construct and the one or more therapeutic agents are to be administered concurrently, or wherein the bispecific construct and the one or more therapeutic agents are to be administered sequentially.

33. The use of any one of claims 28 to 31, wherein the cancer is skin cancer, colorectal cancer, ovarian cancer, renal cell carcinoma, head and neck squamous cell carcinoma, breast cancer, lung cancer, bladder cancer, prostate cancer, melanoma, gynecological cancers, sarcoma, lymphoma, or glioblastoma.