

ANTIBODY-NKG2D LIGAND DOMAIN FUSION PROTEIN

FIELD OF THE INVENTION

[0001] The invention relates an A1-A2 domain of a non-natural NKG2D ligand that binds to non-natural NKG2D receptors and an antibody fusion protein comprising the domain.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Application No. 63/208,407, filed June 8, 2021, the entire contents of which are fully incorporated herein by reference

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: 63,727 byte ASCII (Text) file named "56867_Seqlisting.txt"; created on June 7, 2022.

BACKGROUND

[0004] The engineering of patient-derived T cells to express chimeric antigen receptors (CARs) has altered the landscape of adoptive cell therapies, providing scientists and clinicians the ability to harness the powerful cytolytic capabilities of T cells and direct them to specific antigen-expressing targets in an MHC-independent manner. Their initial application to treat hematologic malignancies has led to astounding responses and inspired a surge of research efforts to drive their effective use in non-hematologic indications. However, CAR-T cell therapies are limited by their utilization of a single-purpose targeting domain, lack of dose control which can contribute to cytokine release syndrome, inability to address tumor antigen loss leading to disease relapse, and immunogenicity of non-human targeting domains leading to lack of persistence. There is a need in the art for improved CAR-based cell therapies to address these limitations of current therapy options.

SUMMARY

[0005] In one aspect, the disclosure provides an antibody fusion protein comprising (i) heavy chains comprising variable region sequences comprising the amino acid sequence of SEQ ID NO: 1 and (ii) light chains comprising variable region sequences comprising the amino acid sequence of SEQ ID NO: 8, wherein the light chains are fused at the C-terminus to an A1-A2 domain comprising the amino acid sequence of SEQ ID NO: 11. In various examples, the heavy chains comprise constant domains comprising the amino acid sequence of SEQ ID NO: 3. Optionally, the A1-A2 domain is fused to the light chains via a linker comprising the amino acid sequence of SEQ ID NO: 10. In this regard, the light chains, in various examples, comprise the amino acid sequence of SEQ ID NO: 13. In various examples, the heavy chains comprising the amino acid sequence of SEQ ID NO: 7.

[0005a] In another aspect, the disclosure provides an antibody fusion protein comprising light chains comprising the amino acid sequence of SEQ ID NO: 13 and heavy chains comprising the amino acid sequence of SEQ ID NO: 7. In yet another aspect, the disclosure provides an antibody fusion protein comprising light chains comprising the amino acid sequence of SEQ ID NO: 13 and heavy chains comprising the amino acid sequence of SEQ ID NO: 6.

[0006] In another aspect, the disclosure further provides a nucleic acid molecule comprising a nucleotide sequence encoding the light chain of the antibody fusion protein (e.g., a light chain comprising variable region sequence comprising the amino acid sequence of SEQ ID NO: 8, wherein the light chains are fused at the C-terminus to an A1-A2

domain comprising the amino acid sequence of SEQ ID NO: 11). In yet another aspect, the disclosure further provides a composition comprising the nucleic acid molecule encoding a light chain of the antibody fusion protein and a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein described herein (e.g., a heavy chain comprising a variable region sequence comprising the amino acid sequence of SEQ ID NO: 1). In yet another aspect, the disclosure provides an expression vector comprising the nucleic acid molecule described in this disclosure. Also provided is an expression vector comprising the nucleic acid molecule encoding the light chain of the antibody fusion protein described herein, optionally further comprising a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein described herein. In yet another aspect, the disclosure further provides a host cell comprising the expression vectors described herein. In yet another aspect, the disclosure provides a host cell comprising the expression vector of this disclosure and an expression vector comprising a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein of this disclosure. The disclosure provides a host cell comprising a nucleic acid molecule comprising a nucleotide sequence encoding the light chain of the antibody fusion protein and a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein. In yet another aspect, the disclosure provides a method of producing an antibody fusion protein, the method comprising culturing a host cell comprising a nucleic acid molecule comprising a nucleotide sequence encoding the light chain of the antibody fusion protein and a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein, and recovering the antibody fusion protein.

[0007] In another aspect, the disclosure provides a kit comprising one or more containers comprising the antibody fusion protein of this disclosure and instructions for use. Also provided is a kit comprising one or more containers comprising the antibody fusion protein described herein. Optionally, the kit further comprises one or more containers comprising a mammalian cell (e.g., human lymphocyte or a human macrophage) comprising a chimeric antigen receptor comprising SEQ ID NO: 15. In various examples, the chimeric antigen receptor further comprises SEQ ID NOs: 16-18.

[0008] The disclosure further provides a method of treating a subject suffering from a CD20-positive cancer, the method comprising administering to the subject the antibody fusion protein described herein and a mammalian cell (e.g., human lymphocyte or a human macrophage) comprising a chimeric antigen receptor comprising SEQ ID NO: 15. Optionally, the chimeric antigen receptor further comprises SEQ ID NOs: 16-18. Use of the antibody fusion protein and mammalian cell to treat a CD20-positive cancer is provided, as well as use of the antibody fusion protein and mammalian cell in the preparation of medicaments to treat a CD20-positive cancer.

[0008a] In another aspect, this disclosure provides a use of the antibody fusion protein of the disclosure and a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15 in the manufacture of a medicament for treating a subject suffering from a CD20-positive cancer.

[0009] In another aspect, the disclosure also provides an A1-A2 domain peptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO: 30, wherein the peptide comprises an alanine or glutamine one or more of positions 40, 54, and/or 84 of SEQ ID NO: 30. In various examples, the peptide comprises glutamine residues at positions 40 and 54 of SEQ ID NO: 30. Optionally, the peptide comprises a glutamine at position 84 of SEQ ID NO: 30 or an alanine at position 84 of SEQ ID NO: 30.

[0010] It should be understood that, while various embodiments in the specification are presented using "comprising" language, under various circumstances, a related embodiment may also be described using "consisting of" or "consisting essentially of" language. The disclosure contemplates embodiments described as "comprising" a feature to include embodiments which "consist of" or "consist essentially of" the feature. The term "a" or "an" refers to one or more. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used

WHAT IS CLAIMED:

1. An antibody fusion protein comprising (i) heavy chains comprising variable region sequences comprising the amino acid sequence of SEQ ID NO: 1 and (ii) light chains comprising variable region sequences comprising the amino acid sequence of SEQ ID NO: 8, wherein the light chains are fused at the C-terminus to an A1-A2 domain comprising the amino acid sequence of SEQ ID NO: 11.
2. The antibody fusion protein of claim 1, wherein the A1-A2 domains are fused to the light chains via a linker comprising the amino acid sequence of SEQ ID NO: 10
3. The antibody fusion protein of claim 1 or claim 2, comprising light chains comprising the amino acid sequence of SEQ ID NO: 13.
4. The antibody fusion protein of any one of claims 1-3, wherein the heavy chains comprise constant domains comprising the amino acid sequence of SEQ ID NO: 3.
5. The antibody fusion protein of claim 4, comprising heavy chains comprising the amino acid sequence of SEQ ID NO: 7.
6. The antibody fusion protein of any one of claims 1-3, wherein the heavy chains comprise constant domains comprising the amino acid sequence of SEQ ID NO: 2.
7. The antibody fusion protein of claim 6, comprising heavy chains comprising the amino acid sequence of SEQ ID NO: 6.
8. An antibody fusion protein comprising light chains comprising the amino acid sequence of SEQ ID NO: 13 and heavy chains comprising the amino acid sequence of SEQ ID NO: 7.
9. An antibody fusion protein comprising light chains comprising the amino acid sequence of SEQ ID NO: 13 and heavy chains comprising the amino acid sequence of SEQ ID NO: 6.
10. A nucleic acid molecule comprising a nucleotide sequence encoding the light chain of the antibody fusion protein of any one of claims 1-9.
11. A composition comprising the nucleic acid molecule of claim 10 and a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein of any one of claims 1-9.

12. An expression vector comprising the nucleic acid molecule of claim 10.
13. The expression vector of claim 12, further comprising a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein of any one of claims 1-9.
14. A host cell comprising the expression vector of claim 12 or claim 13.
15. A host cell comprising the expression vector of claim 12 and an expression vector comprising a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein of any one of claims 1-9.
16. A method of producing an antibody fusion protein, the method comprising culturing a host cell comprising a nucleic acid molecule comprising a nucleotide sequence encoding the light chain of the antibody fusion protein of any one of claims 1-9 and a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein of any one of claims 1-9, and recovering said antibody fusion protein.
17. A kit comprising one or more containers comprising the antibody fusion protein of any one of claims 1-9 and instructions for use.
18. The kit of claim 17, further comprising one or more containers comprising a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15.
19. The kit of claim 18, wherein the mammalian cell is a human lymphocyte or a human macrophage.
20. The kit of claim 18 or claim 19, wherein the chimeric antigen receptor further comprises SEQ ID NOs: 16-18.
21. Use of the antibody fusion protein of any one of claims 1-9 and a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO:15 in the manufacture of a medicament for treating a subject suffering from a CD20-positive cancer.
22. The use of claim 21, wherein the mammalian cell is a human lymphocyte or a human macrophage.

23. The use of claim 21 or claim 22, wherein the chimeric antigen receptor further comprises SEQ ID NOs: 16-18.

24. An A1-A2 domain peptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO: 30, wherein the peptide comprises an alanine or glutamine at one or more of positions 40, 54, and/or 84 of SEQ ID NO: 30.

25. The A1-A2 domain peptide of claim 24, wherein the peptide comprises glutamine residues at positions 40 and 54 of SEQ ID NO: 30.

26. The A1-A2 domain of claim 24 or claim 25, wherein the peptide comprises a glutamine at position 84 of SEQ ID NO: 30.

27. The A1-A2 domain of claim 24 or claim 25, wherein the peptide comprises an alanine at position 84 of SEQ ID NO: 30.

28. The A1-A2 domain peptide of claim 24, wherein the peptide comprises SEQ ID NO: 11, SEQ ID NO: 31, or SEQ ID NO: 32.

29. The A1-A2 domain peptide of any one of claims 24-28 fused to an antibody light chain.

30. The A1-A2 domain peptide of any one of claims 24-28 fused to an antibody heavy chain.