

ANTIBODY-NKG2D LIGAND DOMAIN FUSION PROTEIN

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0001] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: 59,942 byte ASCII (Text) file named "56867_Seqlisting.txt"; created on June 8, 2021.

FIELD OF THE INVENTION

[0002] The invention relates to antibodies fused to the A1-A2 domain of a non-natural NKG2D ligand that binds to non-natural NKG2D receptors.

BACKGROUND

[0003] 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

[0004] In one example, the present disclosure refers to 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. In another aspect, the present disclosure refers to 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 another aspect, the present disclosure refers to 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.

[0005] 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 one aspect, the present disclosure refers to an antibody fusion protein comprising (i) two heavy chains comprising variable region sequences comprising the amino acid sequence of SEQ ID NO: 1 and (ii) two 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 via a linker comprising the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 14, wherein the antibody fusion protein comprises a constant region modified to reduce or inactivate antibody-dependent cell cytotoxicity (ADCC). In another aspect, the present disclosure refers to a nucleic acid molecule comprising a nucleotide sequence encoding the light chain of the antibody fusion protein as described herein. 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 present disclosure refers to a composition comprising the nucleic acid molecule as described herein and a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein as described herein. 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 another aspect, the present disclosure refers to an expression vector comprising the nucleic acid molecule as described herein. Further provided is a host cell comprising the expression vectors described herein. 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 another aspect, the present disclosure refers to a host cell comprising the expression vector as described herein. In yet another aspect, the present disclosure refers to a host cell comprising the expression vector as described herein and an expression vector comprising a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein as described herein. A method of producing an antibody fusion protein is also provided, 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. In another aspect, the present disclosure refers to 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 as described herein and a nucleic acid molecule comprising a nucleotide sequence encoding the heavy chain of the antibody fusion protein as described herein, and recovering said antibody fusion protein.

[0006] Also provided is a kit comprising one or more containers comprising the antibody fusion protein described herein. In yet another aspect, the present disclosure refers to a kit comprising one or more containers comprising the antibody fusion protein as described herein and instructions for use. 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.

[0007] 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. In another example, the present disclosure refers to the use of the antibody fusion protein as described herein and a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15 in the manufacture of a medicament for combination therapy for treating a subject suffering from a CD20-positive cancer in a subject. In another example, the present disclosure refers to the use of the antibody fusion protein as described herein in the manufacture of a first medicament and use of a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15 in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating a subject suffering from a CD20-positive cancer in a subject. In yet another example, the present disclosure refers to the use of the antibody fusion protein as described herein in the manufacture of a medicament for use in combinatorial therapy for treating a

subject suffering from a CD20-positive cancer in a subject, wherein said medicament is to be administered in combination with a mammalian cell 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.

In another aspect, the present disclosure refers to the use of the antibody fusion protein as described herein and a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15 in the manufacture of a medicament for combination therapy for treating a subject suffering from a CD20-positive cancer in a subject, wherein the mammalian cell is a T cell or an NK cell. In another aspect, the present disclosure refers to the use of the antibody fusion protein as described herein in the manufacture of a first medicament and use of a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15 in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating a subject suffering from a CD20-positive cancer in a subject, and wherein the mammalian cell is a T cell or an NK cell. In another aspect, the present disclosure refers to the use of the antibody fusion protein as described herein in the manufacture of a medicament for use in combinatorial therapy for treating a subject suffering from a CD20-positive cancer in a subject, wherein said medicament is to be administered in combination with a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15, wherein the mammalian cell is a T cell or an NK cell.

[0008] It should be understood that, while various examples in the specification are presented using "comprising" language, under various circumstances, a related example may also be described using "consisting of" or "consisting essentially of" language. The disclosure contemplates examples described as "comprising" a feature to include examples 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 interchangeably herein. The term "or" should be understood to encompass items in the alternative or together, unless context unambiguously requires otherwise.

[0009] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein. However, the description also contemplates the same ranges in which the lower and/or the higher endpoint is excluded. When the term "about" is used, it means the recited number plus or minus 5%, 10%, or more of that recited number. The actual variation intended is determinable from the context.

WHAT IS CLAIMED:

1. An antibody fusion protein comprising (i) two heavy chains comprising variable region sequences comprising the amino acid sequence of SEQ ID NO: 1 and (ii) two 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 via a linker comprising the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 14, wherein the antibody fusion protein comprises a constant region modified to reduce or inactivate antibody-dependent cell cytotoxicity (ADCC).
2. The antibody fusion protein of claim 1, wherein the A1-A2 domains are fused to the light chains via the 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 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 combination therapy for treating a subject suffering from a CD20-positive cancer in a subject, wherein the mammalian cell is a T cell or an NK cell.

22. Use of the antibody fusion protein of any one of claims 1-9 in the manufacture of a first medicament and use of a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15 in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for treating a subject suffering from a CD20-positive cancer in a subject, and wherein the mammalian cell is a T cell or an NK cell.

23. Use of the antibody fusion protein of any one of claims 1-9 in the manufacture of a medicament for use in combinatorial therapy for treating a subject suffering from a CD20-positive cancer in a subject, wherein said medicament is to be administered in combination with a mammalian cell comprising a chimeric antigen receptor comprising SEQ ID NO: 15, wherein the mammalian cell is a T cell or an NK cell.

24. The use of any one of claims 21-23, wherein the chimeric antigen receptor further comprises SEQ ID NOS: 16-18.