

## 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 aspect, 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 aspects, 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 aspects, comprise the amino acid sequence of SEQ ID NO: 13. In various aspects, 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 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 aspects, 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 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. 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. In yet 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.

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.

**[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.

**[0010]** All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. Only such limitations which are described herein as critical to the disclosure should be viewed as such; variations of the disclosure lacking limitations which have not been described herein as critical are intended as aspects of the disclosure.

**[0011]** Additional features and variations of the disclosure will be apparent to those skilled in the art from the entirety of this application, including the figures and detailed description, and all such features are intended as aspects of the disclosure. Likewise, features of the disclosure described herein can be re-combined into additional examples that also are intended as aspects of the disclosure, irrespective of whether the combination of features is specified as an aspect or example of the disclosure. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein (even if described in separate sections) are contemplated, even if the combination of features is not found together in the same sentence, or paragraph, or section of this document.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0012]** Figure 1 is a chart providing various sequences described herein.

**[0013]** Figure 2A illustrates octet BLI kinetic binding data for His-tagged monomeric wild-type MIC ligand interaction with either wild-type NKG2D or iNKG2D.YA. Fc-wtNKG2D or Fc-iNKG2D.YA were captured with anti-human IgG Fc capture (AHC) biosensor tips associated with a dilution series of each ligand (parenthetical value indicates highest concentration examined) after baseline establishment. ULBP4 could not be expressed and purified as a monomer so was not included in this assay. Note that all axes are to the same scale (Binding – 0 nm, 0.4 nm, 0.8 nm, 1.2 nm (y-axis); Time – 0 s, 50 s, 100 s, 150 s, 200 s, 250 s, 300 s, 350 s (x-axis)). Data is representative of a single experiment.

**[0014]** Figure 2B illustrates results from ELISA confirming inability of iNKG2D.YA to engage natural ligands. Ligand-Fc fusions (R&D Biosystems) were coated onto microtiter plates and a titration of biotinylated Fc-wtNKG2D (dashed lines) or Fc-iNKG2D.YA (solid lines) applied and detected by streptavidin-HRP. (ELISA signal (OD450) (y-axis); nM Ligand Fc (x-axis).)

**[0015]** Figures 3A-3D: Orthogonal U2S3 ligand (A1-A2 domain) selective binding to NKG2D Y152A/199F (iNKG2D.AF) (an NKG2D ectodomain of the disclosure). Library design and phage panning performed was as described for iNKG2D.YA except that biotinylated double-mutant Fc-iNKG2D.AF was used during rounds of selection against increasing concentrations of Fc-wtNKG2D competitor. Data represents a single experiment (Figure 3A) Octet BLI binding data for interaction of monomeric ligands to either Fc-wtNKG2D or Fc-iNKG2D.AF. Data are representative of two experiments. (Figure 3B) Lead variants selected from the phage display library were cloned as fusions to the C-terminus of the Rituximab light chain and differential binding to Fc-wtNKG2D, Fc-iNKG2D.YA, and Fc-iNKG2D.AF and quantified by ELISA. Shown are four variants that selectively engage Fc-

**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 combination therapy for treating a subject suffering from a CD20-positive cancer in a subject.
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.

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.

24. The use of any one of claims 21-23, wherein the mammalian cell is a human lymphocyte or a human macrophage.

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