

BISPECIFIC ANTIBODY CAR CELL IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(d) to U.S. Provisional Application No. 62/644,343, filed March 16, 2018, the contents of which are hereby incorporated by reference into the present disclosure.

TECHNICAL FIELD

[0002] The present disclosure relates generally to the field of human immunology, specifically cancer immunotherapy.

BACKGROUND

[0003] The following discussion of the background of the invention is merely provided to aid art to the present invention.

[0004] Current cancer treatment regimens including chemotherapies, immunomodulatory drugs¹⁴, monoclonal antibodies¹⁵, and autologous or allogeneic transplantation. These therapies often lead to remission, but nearly all patients eventually relapse and succumb to death due to return of the disease. Thus, there is an unmet need for new therapies, including new combination immunotherapies for relapsed and/or refractory disease. This disclosure addresses these limitations and provides related advantages as well.

SUMMARY

[0005] Chimeric antigen receptor (CAR) T cells have been used successfully in the clinic for the treatment of both hematological malignancies and solid tumors, and have recently been approved by the U.S. FDA¹⁻⁴. Bispecific antibodies (BsAb) have also been approved by the FDA for cancer treatment and are being used as an alternative immunotherapeutic approach to CAR T cell therapy⁵. However, CAR- and BsAb-based cancer immunotherapies still need improvement for five important reasons. First, in some cases, CAR T cells cannot be expanded *in vivo* and cannot survive for a sufficient period of time to initiate tumor lysis in patients⁶. It has been reported that the efficacy of CAR T cells correlates with the quantity and duration of CAR T cell presence *in vivo*⁶⁻⁸. Second, tumor cells can shed targeted antigens to evade therapy, especially when only a single antigen is targeted. Third, in addition to being costly and time-consuming to manufacture, BsAb have a short half-life and to date have not been shown to be curative^{9,10}. Fourth, combination

therapy of CAR T cells with BsAb targeting two distinct tumor associated antigens could be a good approach; however, producing each individually *ex vivo* would be labor intensive and costly; engineering T cells to express *both* a CAR and a BsAb within a *single* construct, of which the BsAb engages *all* cytolytic effector cells has not yet been reported or shown to be additive or synergistic, or to enhance T cell survival *in vivo*. Finally, current approaches to CAR immunotherapy mainly focus on one immune cell type, i.e., T cells, excluding all other cytolytic effector cells in the innate and adaptive arms of the immune system. NKG2D is a c-lectin type of receptor that is expressed on virtually all cytolytic effector cells in both the innate and adaptive arms of the immune system^{11,12}.

[0006] This disclosure provides a platform to resolve these issues, in part or in full, by engineering T cells infected with a single vector delivering these two modes of therapy, i.e., producing a T cell whose CAR is targeting one specific tumor-associated antigen.

[0007] Thus, in one example, provided herein are chimeric antigen receptors (CARs) comprising, or alternatively consisting essentially of, or yet further consisting of: (a) an antigen binding domain of a cancer or tumor targeting antibody; (b) a hinge domain; (c) a transmembrane domain; and (d) an intracellular domain. Further examples of the disclosure relate to a chimeric antigen receptor (CAR) comprising, or alternatively consisting essentially of, or yet further consisting of: (a) an antigen binding domain of a tumor targeting antibody; (b) a CD8 α hinge domain; (c) a CD8 α transmembrane domain; (d) a CD28 costimulatory signaling region and/or a 4-1BB costimulatory signaling region; and (e) a CD3 zeta signaling domain.

[0008] In certain examples, the antigen binding domain of the tumor targeting antibody comprises, or alternatively consists essentially thereof, or further consists of a heavy chain variable region and a light chain variable region that are optionally linked by a linker peptide. In some examples, the heavy and/or light chain variable region comprises, or alternatively consists essentially thereof, or further consists of the relevant CDR regions of an antibody to any one of B-cell maturation antigen (BCMA) and/or SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof. In one example, the tumor targeting antibody targets BCMA. In some examples, the heavy chain and/or light chain variable region comprises, or alternatively consists essentially thereof, or further consists of the amino acid sequence of an antibody to any one of B-cell maturation

antigen (BCMA) and/or SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof.

[0009] In certain examples, the CAR further comprises, or alternatively further consists essentially of, or yet further consists of, a linker polypeptide located between the heavy chain variable region and the light chain variable region. In certain examples, the linker is a glycine-serine linker. In further examples, the linker polypeptide comprises, or alternatively consists essentially thereof, or further consists of the sequence (glycine-serine)_n wherein *n* is an integer from 1 to 6.

[0010] In certain examples, the CAR further comprises, or alternatively further consists essentially of, or yet further consists of, a detectable marker and/or a purification marker attached to the CAR.

[0011] Additional examples of the disclosure relate to an isolated nucleic acid sequence encoding a CAR, as described above, or its complement, or an equivalent of each thereof.

[0012] In certain examples, the isolated nucleic acid sequence further comprises, or further consists essentially of, or yet further consists of, a Kozak consensus sequence located upstream of the polynucleotide encoding the antigen binding domain of a cancer or tumor targeting antibody.

[0013] In certain examples, the isolated nucleic acid further comprises, or alternatively consists essentially thereof, or further consists of a polynucleotide encoding an antibiotic resistance polypeptide operatively coupled to the isolated nucleic acid, a promoter and/or an enhancer element.

[0014] Also provided is an isolated nucleic acid encoding a bispecific antibody which recognizes and binds NKG2D. In some examples, the nucleic acid encodes a bispecific antibody that comprises, or alternatively consists essentially thereof, or further consists of an NKG2D ligand and, optionally, a SALMF7 (also known as CS1 or CD319) ligand that are optionally codon optimized. In certain examples, the isolated nucleic acid encodes a bispecific antibody that comprises, or alternatively consists essentially thereof, or further consists of the relevant CDR regions of an antibody to NKG2D and, optionally, SLAMF7 (also known as CS1 or CD319), that are optionally codon-optimized, or an equivalent of each thereof. In some examples, the nucleic acid encodes a bispecific antibody that comprises, or alternatively consists essentially thereof, or further consists of the heavy chain

and/or light chain variable region of an antibody to NKG2D and, optionally, SLAMF7 (also known as CS1 or CD319), that are optionally codon optimized, and/or an equivalent of each thereof. In some examples, the nucleic acid encodes a bispecific antibody that comprises a single chain variable fragment (scFV) derived from an antibody to NKG2D and, optionally, a single chain variable fragment (scFV) derived from SALMF7 (also known as CS1 or CD319), that are optionally codon optimized and/or an equivalent each thereof. In certain examples, the isolated nucleic acid further comprises, or alternatively consists essentially thereof, or yet further consists of a polynucleotide encoding an antibiotic resistance gene.

[0015] In further example, provided herein are isolated nucleic acids that encode, in one construct, the CAR and bispecific antibody as disclosed above (“BsAb-CAR construct”). In one example, the isolated nucleic acid encodes an antigen binding fragment that targets BCMA and a bispecific antibody, e.g., one scFv from an anti-CS1 antibody and one scFv from an anti-NKG2D antibody, joined together by a nucleic acid encoding a non-immunogenic protein linker such as from human muscle aldose. An exemplary BsAb-CAR vector is shown in Figure 3A. The vectors optionally comprise regulatory sequences such as promoters, enhancers, and and viral LTRs.

[0016] Examples of the disclosure relate to a vector comprising one or more of the isolated nucleic acids described above. In certain examples, the vector is a plasmid or a viral vector selected from the group of a retroviral vector, a lentiviral vector, an adenoviral vector, and an adeno-associated viral vector. The isolated nucleic acids and vectors containing them are useful to prepare the CARs as described herein.

[0016a] In one aspect, the present disclosure refers to a vector comprising: a polynucleotide encoding a chimeric antigen receptor (CAR) comprising: (a) an antigen binding domain of a cancer or tumor targeting antibody; (b) a hinge domain; (c) a transmembrane domain; (d) and an intracellular domain; and a polynucleotide encoding a bispecific antibody comprising an antigen binding domain that recognizes and binds NKG2D.

[0017] Further examples of the disclosure relate to an isolated cell comprising, or alternatively consisting essentially thereof, or further consisting of one or more of the above described compositions: a CAR, an isolated nucleic acid encoding a CAR or its complement, an isolated nucleic acid encoding the CAR/BsA construct, or a vector containing the isolated nucleic acids. In some examples, the CAR is expressed on the surface of the isolated cell.

[0017a] In another aspect, the present disclosure refers to an isolated cell comprising the vector as disclosed herein.

[0018] In certain examples, the isolated cell further comprises, or alternatively consists essentially of, or yet further consists of, an isolated nucleic acid comprising, or alternatively consisting essentially of, or yet further consisting of a polynucleotide encoding a bispecific antibody, which optionally recognizes and binds NKG2D. In some

examples, the bispecific antibody comprises, or alternatively consists essentially thereof, or further consists of an NKG2D ligand or an anti-NKG2 antigen binding fragment of an anti-NKG2 antibody, and, optionally, a SALMF7 (also known as CS1 or CD319) ligand. In certain examples, the bispecific antibody comprises, or alternatively consists essentially thereof, or further consists of the relevant light chain and heavy chain regions or the CDR regions of an antibody to NKG2D and, optionally, SLAMF7 (also known as CS1 or CD319), that are optionally codon-optimized, or an equivalent of each thereof. In some examples, the bispecific antibody comprises, or alternatively consists essentially thereof, or further consists of the heavy chain and/or light chain variable region of an antibody to NKG2D and, optionally, SLAMF7 (also known as CS1 or CD319), that are optionally codon optimized, and/or an equivalent of each thereof. In some examples, the bispecific antibody comprises a single chain variable fragment (scFv) derived from an antibody to NKG2D and, optionally, a single chain variable fragment (scFv) derived from SALMF7 (also known as CS1 or CD319), that are optionally codon optimized and/or an equivalent each thereof. In certain examples, the isolated nucleic acid further comprises, or alternatively consists essentially thereof, or yet further consists of a polynucleotide encoding an antibiotic resistance gene.

[0019] Non-limiting examples of an isolated cell is a prokaryotic cell such as a bacteria cell, e.g., an *E coli*, or a eukaryotic cell. In some examples the isolated eukaryotic cell is selected from an animal cell, a mammalian cell, a bovine cell, a feline cell, a canine cell, a murine cell, an equine cell or a human cell. In further examples, the isolated cell is the cell is a T-cell, a B cell, a NK cell, a dendritic cell, a myeloid cell, a monocyte, a macrophage, any subsets thereof, or any other immune cell from any of the species as disclosed herein.

[0020] Examples of the disclosure relate to a composition comprising, or alternatively consisting essentially of, or further consisting of one or more of the above described compositions, e.g., a CAR, an isolated nucleic acid, a cell, or a vector and a carrier.

[0020a] In another aspect, the present disclosure refers to a composition comprising the vector as disclosed herein and/or the isolated cell as disclosed herein, and, optionally, a pharmaceutically acceptable carrier.

[0021] In certain examples, the composition further comprises, or alternatively consists essentially of, or yet further consists of, a bispecific antibody, which optionally recognizes and binds NKG2D, and/or an isolated nucleic acid comprising a polynucleotide encoding a bispecific antibody, which optionally recognizes and binds NKG2D. In some examples, the bispecific antibody comprises, or alternatively consists essentially

thereof, or further consists of an NKG2D ligand and, optionally, a SALMF7 (also known as CS1 or CD319) ligand. In certain examples, the bispecific antibody comprises, or alternatively consists essentially thereof, or further consists of the relevant CDR regions of an antibody to NKG2D and, optionally, SLAMF7 (also known as CS1 or CD319), or an equivalent of each thereof. In some examples, the bispecific antibody comprises, or alternatively consists essentially thereof, or further consists of the heavy chain and/or light chain variable region of an antibody to NKG2D and, optionally, SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof. In some examples, the bispecific antibody comprises a single chain variable fragment (scFv) derived from an antibody to NKG2D and, optionally, a single chain variable fragment (scFv) derived from SALMF7 (also known as CS1 or CD319), and/or an equivalent each thereof.

[0022] Examples of the disclosure relate to an isolated complex comprising a CAR or a cell comprising the CAR bound to a cancer or tumor antigen or a fragment thereof, and/or a cell expressing the cancer or tumor antigen. In one example, the antigen binding domain is expressed on the surface of the cell. In another example, the cancer or tumor antigen is B-cell maturation antigen (BCMA), SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof. In one example the cell containing or expressing the CAR is a T-cell, a B cell, a NK cell, a dendritic cell, a myeloid cell, a monocyte, a macrophage, any subsets thereof, or any other immune cell. The tumors or cells can be from any animal, e.g., mammalian such as a human cell.

[0022a] In another aspect, the present disclosure refers to an isolated complex comprising an isolated cell as disclosed herein bound to a cell expressing a cancer or tumor antigen, optionally, wherein the cancer or tumor antigen is NKG2D and/or SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof.

[0022b] In another aspect, the present disclosure refers to an isolated complex comprising the isolated cell as disclosed herein bound to a cancer or tumor antigen or a fragment thereof, optionally, wherein the cancer or tumor antigen is BCMA or SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof.

[0023] Some examples of the disclosure relate to a method of producing a CAR expressing cell or a CAR expressing cell that secretes BsA, the method comprising, or alternatively consisting essentially thereof, or yet further consisting of transducing an isolated cell with the nucleic acid sequence encoding a CAR and Bsa or the isolated nucleic acid encoding the BsAb-CAR, as described herein.

[0023a] In another aspect, the present disclosure refers to a method of producing a CAR expressing cell comprising transducing an isolated cell with vector as disclosed herein.

[0024] In a further example, the method further comprises selecting and isolating the cell expressing the CAR or BsAb-CAR. In a further example, the cell is a eukaryotic cell such as a mammalian cell, e.g., a human cell such as a T-cell, a B cell, a NK cell, a dendritic cell, a myeloid cell, a monocyte, a macrophage, any subsets thereof, or any other immune cell. The cells can be transduced using the viral vectors as described herein or alternatively using technology described in Riet et al. (2013) *Meth. Mol. Biol.* 969:187-201 entitled “Nonviral

RNA transfection to transiently modify T cell with chimeric antigen receptors for adoptive therapy.”

[0025] In certain examples, the method further comprises, or alternatively consists essentially of, or yet further consists of transducing the cell with an isolated nucleic acid comprising, or alternatively consisting essentially of, or yet further consisting of a polynucleotide encoding a bispecific antibody, which optionally recognizes and binds NKG2D. In some examples, the bispecific antibody comprises, or alternatively consists essentially thereof, or further consists of an NKG2D ligand and, optionally, a SALMF7 (also known as CS1 or CD319), each optionally codon optimized ligand. In certain examples, the bispecific antibody comprises, or alternatively consists essentially thereof, or further consists of the relevant CDR regions of an antibody to NKG2D and, optionally, SLAMF7 (also known as CS1 or CD319), optionally codon optimized, or an equivalent of each thereof. In some examples, the bispecific antibody comprises, or alternatively consists essentially thereof, or further consists of the heavy chain and/or light chain variable region of an antibody to NKG2D and, optionally, SLAMF7 (also known as CS1 or CD319), that are optionally codon optimized and/or an equivalent of each thereof. In some examples, the bispecific antibody comprises a single chain variable fragment (scFv) derived from an antibody to NKG2D and, optionally, a single chain variable fragment (scFv) derived from SALMF7 (also known as CS1 or CD319), that are optionally codon optimized, and/or an equivalent each thereof. The cells can be transduced using the viral vectors, e.g., lentiviral vectors, as described herein or alternatively using technology described in Riet et al. (2013) *Meth. Mol. Biol.* 969:187-201 entitled “Nonviral RNA transfection to transiently modify T cell with chimeric antigen receptors for adoptive therapy.”

[0026] In certain examples, the method of producing a CAR or BsAb-CAR expressing cell further comprises, or alternatively consists essentially of, or yet further consists of activating and expanding the population of CAR expressing cells. Certain examples of the present disclosure relate to an isolated, activated population of cells comprising, or alternatively consisting essentially of, or yet further consisting of a CAR or BsAb-CAR. In certain examples, the cells are one or more of T-cells, B cells, NK cells, dendritic cells, myeloid cells, monocytes, macrophages, any subsets thereof, or any other immune cells.

[0027] Examples of the disclosure relate to a method of inhibiting the growth of a tumor expressing a cancer or tumor antigen, by contacting the tumor with an effective amount of the isolated cells or compositions disclosed above. The contacting can be *in vitro* or *in vivo*. When the contacting is *in vitro*, the method can be used to test personalized therapy against a patient's tumor or to assay for combination therapies. When the contacting is *in vivo*, the method is useful to inhibit the growth of the tumor or cancer cell in a subject in need thereof, such as a human patient suffering from cancer and the patient receives an effective amount of the cells. In certain examples, the tumor is a solid tumor. An effective amount is administered alone or in combination with other therapies as described herein. In certain examples, the cancer/tumor targeted is a solid tumor or a cancer affecting the blood and/or bone marrow, e.g., multiple myeloma (MM). In certain examples the isolated cells are autologous to the subject being treated. In another example, the cells are allogeneic to the subject being treated. In another example, the method further comprises, or consists essentially of, or yet further consists of, administering to the subject an effective amount of a cytoreductive therapy. In a further example, the method further comprises the steps of isolating the cells to be administered to the subject, transducing the cells with an effective amount of an isolated nucleic acid encoding a CAR or BsAb-CAR as described herein, culturing the cells to obtain a population of CAR or BsAb-CAR encoding cells, that are optionally expanded and activated and then administering the cells to the patient.

[0027a] In another aspect, the present disclosure refers to an *in vitro* method of inhibiting the growth of a cancer cell or tumor expressing a cancer or tumor antigen, comprising contacting the cancer cell or tumor with the isolated cell as disclosed herein.

[0027b] In another aspect, the present disclosure refers to use of the isolated cell as disclosed herein in the manufacture of a medicament for inhibiting the growth of a cancer cell or tumor expressing a cancer or tumor antigen in a subject.

[0028] Also disclosed herein are kits comprising one or more of the above noted compositions and instructions for their use in the methods as disclosed herein.

[0028a] In another aspect, the present disclosure refers to a kit comprising a composition as disclosed herein and optionally, instructions for use.

[0029] The above compositions and methods are unique and overcome the limitation of the state of the art in that they provide a CAR cell that simultaneously secretes a NKG2D-based BsAb targeting a second tumor-associated antigen. The disclosed CAR NKGD2D-based BsAb is exemplary only. This approach can be modified for any number of tumor antigens, as known in the art, e.g., EGFRVIII; CD70, mesothelin, CD123, CD19, CEA, CD133, Her2, see Townsend et al. (2018) J. Exp. & Clinical Cancer Res. 37:163.

[0030] The exemplary constructs were tested in a multiple myeloma model (MM). MM is a malignancy characterized by an accumulation of clonal plasma cells¹³. As noted above, current treatment regimens including chemotherapies, immunomodulatory drugs¹⁴, monoclonal antibodies¹⁵, and autologous or allogeneic transplantation often lead to remission, but nearly all patients eventually relapse and succumb to death due to return of

WHAT IS CLAIMED IS:

1. A vector comprising:
 - a polynucleotide encoding a chimeric antigen receptor (CAR) comprising: (a) an antigen binding domain of a cancer or tumor targeting antibody; (b) a hinge domain; (c) a transmembrane domain; (d) and an intracellular domain; and
 - a polynucleotide encoding a bispecific antibody comprising an antigen binding domain that recognizes and binds NKG2D.
2. The vector of claim 1, wherein the CAR comprises: (a) an antigen binding domain of a cancer or tumor targeting antibody; (b) a CD8 α hinge domain; (c) a CD8 α transmembrane domain; (d) a CD28 costimulatory signaling region and/or a 4-1BB costimulatory signaling region; and (e) a CD3 zeta signaling domain.
3. The vector of claims 1 or 2, wherein the cancer or tumor targeting antibody targets B-cell maturation antigen (BCMA) and/or SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof.
4. The vector of any one of claims 1 to 3, wherein the bispecific antibody comprises a ligand of NKG2D or an anti-NKG2D scFv and an antigen binding domain of an anti-SLAMF7 antibody (also known as CS1 or CD319), and/or an equivalent each thereof.
5. The vector of any one of claims 1 to 3, wherein the bispecific antibody comprises CDR regions of an antibody to NKG2D and an antigen binding domain of an anti-SLAMF7 antibody (also known as CS1 or CD319), and/or an equivalent of each thereof.
6. The vector of claim 5, wherein the bispecific antibody comprises the heavy chain and light chain variable region of an antibody to NKG2D and an antigen binding domain of an anti-SLAMF7 antibody (also known as CS1 or CD319), and/or an equivalent of each thereof.
7. The vector of claim 5 or 6, wherein the bispecific antibody comprises a single chain variable fragment (scFV) derived from an antibody to NKG2D, optionally, a single chain variable fragment (scFv) derived from an anti-SALMF7 antibody (also known as CS1 or CD319), and/or an equivalent each thereof.
8. The vector of any one of claims 1 to 7, wherein the vector is a plasmid, and optionally a promoter to regulate expressson of the polynucleotide.

9. The vector of any one of claims 1 to 7, wherein the vector is a viral vector selected from the group of a retroviral vector, a lentiviral vector, an adenoviral vector, and an adeno-associated viral vector, and optionally a promoter to regulate expressson of the polynucleotide.
- 5 10. An isolated cell comprising the vector of any one of claims 1 to 9.
11. The isolated cell of claim 10, wherein the cell is a prokaryotic cell or a eukaryotic cell.
12. The isolated cell of claim 11, wherein the cell is a eukaryotic cell.
13. The isolated of claim 12, wherein the eukaryotic cell is selected from an animal cell,
10 a mammalian cell, a bovine cell, a feline cell, a canine cell, a murine cell, an equine cell or a human cell.
14. The isolated cell of claim 12 or 13, wherein the eukaryotic cell is an immune cell, optionally a T-cell, a B cell, a NK cell, a dendritic cell, a myeloid cell, a monocyte, or a macrophage.
- 15 15. The isolated cell of any one of claims 10 to 14, wherein the isolated cell expresses the CAR and secretes the bispecific antibody.
16. A composition comprising the vector of any one of claims 1 to 9 and/or the isolated cell of any one of claims 10 to 15, and, optionally, a pharmaceutically acceptable carrier.
17. An isolated complex comprising an isolated cell comprising of any one of claims 10
20 to 15 bound to a cell expressing a cancer or tumor antigen, optionally, wherein the cancer or tumor antigen is NKG2D and/or SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof.
18. An isolated complex comprising the isolated cell of any one of claims 10 to 15 bound to a cancer or tumor antigen or a fragment thereof, optionally, wherein the cancer or
25 tumor antigen is BCMA or SLAMF7 (also known as CS1 or CD319), and/or an equivalent of each thereof.
19. A method of producing a CAR expressing cell comprising transducing an isolated cell with vector of any one of claims 1 to 9.

20. The method of claim 18, wherein the isolated cells are selected from a group consisting of T-cells, B cells, NK cells, dendritic cells, myeloid cells, monocytes, or macrophages.
21. An *in vitro* method of inhibiting the growth of a cancer cell or tumor expressing a cancer or tumor antigen, comprising contacting the cancer cell or tumor with the isolated cell of any one of claims 10 to 15.
22. Use of the isolated cell of any one of claims 10 to 15 in the manufacture of a medicament for inhibiting the growth of a cancer cell or tumor expressing a cancer or tumor antigen in a subject.
23. The use of claim 22, wherein the isolated cells are autologous or allogeneic to the subject.
24. The use of claim 22, wherein the isolated cells are allogenic to the subject.
25. The use of claim 23 or 24, wherein an effective amount of a cytoreductive therapy or chemotherapy or therapy that upregulates the expression of a target antigen is to be further administered to the subject.
26. The use of claim 25, wherein the cytoreductive therapy comprises chemotherapy, cryotherapy, hyperthermia, targeted therapy, and/or radiation therapy.
27. The use of any one of claims 22 to 26, wherein the subject is a mammal, a canine, a feline, an equine, a murine or a human patient.
28. A kit comprising a composition as disclosed herein and optionally, instructions for use.