

MULTIFUNCTIONAL MOLECULES THAT BIND TO T CELLS AND USES THEREOF TO TREAT AUTOIMMUNE DISORDERS

RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application 62/808,713 filed on February 21, 2019 and U.S. Provisional Application 62/957,045 filed on January 3, 2020, the entire contents of each of which are hereby incorporated by reference.

SEQUENCE LISTING

10 The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 19, 2020, is named E2070-7024WO_SL.txt and is 1,519,578 bytes in size.

BACKGROUND

15 T cell mediated antigen recognition depends on the interaction of the T cell receptor (TCR) with the antigen-major histocompatibility complex (MHC). The heterodimeric TCRs consist of a combination of α and β chains ($\alpha\beta$ TCR) expressed by the majority of T cells, or $\gamma\delta$ chains ($\gamma\delta$ TCR) present only in about 1-5% of the T cells. A highly diverse TCR repertoire is a fundamental property of an effective immune system. However, the immune repertoire can change greatly with the onset
20 and progression of diseases, such as cancer, autoimmune, inflammatory, and infectious diseases.

Autoimmunity may result from abnormal regulation of the immune system. This may be manifested by autoreactive TCR clones that attack a patient's own cells. There is a need for improved therapies for autoimmune diseases.

SUMMARY

25 In one aspect, the present disclosure provides a use of a pharmaceutical composition comprising a multifunctional molecule in the manufacture of a medicament for treating an autoimmune disease [associated with a biased TCR](#) in a subject in need thereof, wherein the [multifunctional](#)~~multispecific~~ molecule comprises:

(i) an antigen binding domain that selectively binds to a T cell receptor variable beta (TCRβV) of the biased TCR, and (ii) ~~one, two, or all of~~ an NK cell engager, wherein the NK cell engager binds to an antigen selected from the group consisting of NKp30, NKp46, NKG2D, and CD16 ~~a cytokine molecule, a cytokine inhibitor molecule, and a death receptor signal engager.~~

5 In another aspect, the present disclosure provides a recombinant polynucleotide comprising a sequence encoding ~~a~~ the multifunctional ~~multispecific~~ molecule, wherein the multifunctional molecule encoded by the recombinant polynucleotide comprises: (i) an antigen binding domain that selectively binds to a T cell receptor variable beta region (TCRβV) of the biased TCR, and (ii) an NK cell engager, wherein the NK cell engager binds to an antigen selected from the group consisting of NKp30, NKp46, NKG2D, and CD16 ~~of the pharmaceutical composition according to the use as described~~ herein.

10 In another aspect, the present disclosure provides a host cell comprising the recombinant polynucleotide comprising a sequence encoding a multifunctional molecule as described herein.

In yet another aspect, the present disclosure provides a method of making ~~a~~ the multifunctional molecule ~~of the pharmaceutical composition according to the use as described herein,~~ comprising culturing the host cell as described herein under suitable conditions for gene expression and/or homo- or heterodimerization, wherein the multifunctional molecule comprises: (i) an antigen binding domain that selectively binds to a T cell receptor variable beta region (TCRβV) of the biased TCR, and (ii) an NK cell engager, wherein the NK cell engager binds to an antigen selected from the group consisting of NKp30, NKp46, NKG2D, and CD16.

15 The disclosure relates, inter alia, to novel multispecific or multifunctional molecules that include (i) an antigen binding domain that binds to a TCR variable beta chain (TCRBV) antigen on a T cell (e.g., a TCRBV antigen corresponding to a biased TCRBV clonotype); and one, two or all of: (ii) an immune cell engager (e.g., chosen from an NK cell engager, a T cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager); (iii) a cytokine molecule; and/or (iv) a stromal modifying moiety. The terms “multispecific” or “multifunctional” are used interchangeably herein.

20 Without wishing to be bound by theory, a TCR bias may exist in autoimmune diseases. This bias may be associated with dominant autoreactive TCR clones responsible for disease or associated with symptoms. Rebalancing the TCR repertoire, e.g., by eliminating or depleting T cells comprising an autoreactive clonotype, may treat the associated autoimmune disease and/or reduce symptoms of the autoimmune disease. Accordingly, the multispecific or multifunctional molecules disclosed herein are expected to target (e.g., localize, bridge and/or activate) an immune cell (e.g., an immune effector cell chosen from an NK cell, a T cell, a B cell, a dendritic cell or a macrophage), at a target cell (e.g., a T cell comprising a biased TCRBV clonotype or comprising a TCRBV antigen corresponding to a biased TCRBV clonotype). Increasing the proximity and/or activity of the immune cell using the multispecific molecules described herein is expected to enhance an immune response against the target cell (e.g., the T cell comprising a

We claim:

1. Use of a pharmaceutical composition comprising a multifunctional molecule in the manufacture of a medicament for treating an autoimmune disease associated with a biased TCR in a subject in need thereof,

wherein the multifunctional multispecific molecule comprises:

(i) an antigen binding domain that selectively binds to a T cell receptor variable beta region (TCR β V) of the biased TCR, and

(ii) ~~one, two, or all of~~ an NK cell engager, wherein the NK cell engager binds to an antigen selected from the group consisting of NKp30, NKp46, NKG2D, and CD16a-cytokine molecule, a cytokine inhibitor molecule, and a death receptor signal engager.

~~2. The use of claim 1, wherein the multifunctional molecule comprises:~~

~~(i) the antigen binding domain that selectively binds to a T cell receptor variable beta (TCR β V), and~~

~~(ii) the NK cell engager.~~

3.2. The use of claim ~~1 or 2~~, wherein the NK cell engager binds to ~~an antigen selected from the group consisting of~~ NKp30, NKp40, NKp44, NKp46, NKG2D, DNAM1, DAP10, CD16, CRTAM, CD27, PSGL1, CD96, CD100 (SEMA4D), NKp80, CD244 (also known as SLAMF4 or 2B4), SLAMF6, SLAMF7, KIR2DS2, KIR2DS4, KIR3DS1, KIR2DS3, KIR2DS5, KIR2DS1, CD94, NKG2C, NKG2E, and CD160.

4.3. The use of claim 1 or 23, wherein the NK cell engager binds to and activates an NK cell~~the antigen is~~ NKp30, NKp46, NKG2D, or CD16.

5.4. The use of any one of claims 1-3 or 4, wherein the antigen is NKp30, and the NK cell engager comprises ~~(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (HC CDR1):~~

(i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (HC CDR1), a heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity determining region 3 (HC CDR3) comprising the sequences of SEQ ID NO: 6000, SEQ ID NO: 6001, and SEQ ID NO: 6002, and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2), and a light chain complementarity determining region 3 (LC CDR3)

comprising the sequences of SEQ ID NO: 6063, SEQ ID NO: 6064, and SEQ ID NO: 7293, respectively;

(ii) a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3 comprising the sequences of SEQ ID NO: 7313, SEQ ID NO: 6001, and SEQ ID NO: 6002, respectively, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3 comprising the sequences of SEQ ID NO: 6063, SEQ ID NO: 6064, and SEQ ID NO: 7293, respectively;

(iii) a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3 comprising the sequences of SEQ ID NO: 6007, SEQ ID NO: 6008, and SEQ ID NO: 6009, respectively, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3 comprising the sequences of SEQ ID NO: 6070, SEQ ID NO: 6071, and SEQ ID NO: 6072, respectively;

(iv) a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3 comprising the sequences of SEQ ID NO: 7313, SEQ ID NO: 6008, and SEQ ID NO: 6009, respectively, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3 comprising the sequences of SEQ ID NO: 6070, SEQ ID NO: 6071, and SEQ ID NO: 6072, respectively;

(v) a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3 comprising the sequences of SEQ ID NO: 7313, SEQ ID NO: 7385, and SEQ ID NO: 7315, respectively, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3 comprising the sequences of SEQ ID NO: 6070, SEQ ID NO: 6064, and SEQ ID NO: 7321, respectively;

(vi) a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3 comprising the sequences of SEQ ID NO: 7313, SEQ ID NO: 7318, and SEQ ID NO: 6009, respectively, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3 comprising the sequences of SEQ ID NO: 6070, SEQ ID NO: 6064, and SEQ ID NO: 7321, respectively;

or

(vii) a VH comprising a HC CDR1, a HC CDR2, and a HC CDR3 comprising the sequences of SEQ ID NO: 7313, SEQ ID NO: 6001, and SEQ ID NO: 7315, respectively, and a VL comprising a LC CDR1, a LC CDR2, and a LC CDR3 comprising the sequences of SEQ ID NO: 7326, SEQ ID NO: 7327, and SEQ ID NO: 7329,

respectively of any one of the sequences selected from the group consisting of SEQ ID NOs: 6000, 6007, and 7313; a heavy chain complementarity determining region 2 (HC CDR2) of any one of the sequences selected from the group consisting of SEQ ID NOs: 6001, 6008, 7385, and 7318; and a heavy chain complementarity determining region 3 (HC CDR3) of any one of the sequences selected from the group consisting of SEQ ID NOs: 6002, 6009, and 7315; and (ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (LC CDR1) of any one of the sequences

~~selected from the group consisting of SEQ ID NOs: 6063, 6070, and 7326; a light chain complementarity determining region 2 (LC CDR2) of any one of the sequences selected from the group consisting of SEQ ID NOs: 6064, 6071, and 7327; and a light chain complementarity determining region 3 (LC CDR3) of any one of the sequences selected from the group consisting of SEQ ID NOs: 7293, 6072, 7321, and 7329.~~

6.5. The use of ~~any one of claims 43-5~~, wherein the antigen is NKp30, and the NK cell engager comprises:

- (i) a VH comprising an amino acid sequence with at least 85% sequence identity to any one selected from the group consisting of SEQ ID NOs: 6121 and 6123-6128, and a VL comprising an amino acid sequence with at least 85% sequence identity to any one selected from the group consisting of SEQ ID NOs: 7294 and 6137-6141;
- (ii) a VH comprising an amino acid sequence with at least 85% sequence identity to any one selected from the group consisting of SEQ ID NOs: 6122 and 6129-6134, and a VL comprising an amino acid sequence with at least 85% sequence identity to any one selected from the group consisting of SEQ ID NOs: 6136 and 6142-6147;
- (iii) a VH comprising an amino acid sequence with at least 85% sequence identity to the sequence of SEQ ID NO: 7295 or the sequence of SEQ ID NO: 7297, and a VL comprising an amino acid sequence with at least 85% sequence identity to the sequence of SEQ ID NO: 7296;
- (iv) a VH comprising an amino acid sequence with at least 85% sequence identity to the sequence of SEQ ID NO: 6122, and a VL comprising an amino acid sequence with at least 85% sequence identity to the sequence of SEQ ID NO: 6136; or
- (v) a VH comprising an amino acid sequence with at least 85% sequence identity to any one selected from the group consisting of SEQ ID NOs: 7298 and 7300-7304, and a VL comprising an amino acid sequence with at least 85% sequence identity to any one selected from the group consisting of SEQ ID NOs: 7299 and 7305-7309~~a VH comprising an amino acid sequence with at least 80% sequence identity to any one sequence selected from the group consisting of SEQ ID NOs: 6121-6134, 7295, 7297, 7298, and 7300-7304; and/or (ii) a VL comprising an amino acid sequence with at least 80% sequence identity to any one sequence selected from the group consisting of SEQ ID NOs: 6136-6147, 7294, 7296, 7299, and 7305-7309.~~

6. The use of any one of claims 1-5, wherein the antigen is NKp30, and the NK cell engager comprises:

- (i) a VH comprises any one amino acid sequence selected from the group consisting of SEQ ID NOs: 6121 and 6123-6128, and a VL comprising any one amino acid sequence selected from the group consisting of SEQ ID NOs: 7294 and 6137-6141;
- (ii) a VH comprising any one amino acid sequence selected from the group consisting of SEQ ID NOs: 6122 and 6129-6134, and a VL comprising any one amino acid sequence selected from the group consisting of SEQ ID NOs: 6136 and 6142-6147;
- (iii) a VH comprising the sequence of SEQ ID NO: 7295 or the sequence of SEQ ID NO: 7297, and a VL comprising the sequence of SEQ ID NO: 7296;
- (iv) a VH comprising the sequence of SEQ ID NO: 6122, and a VL comprising the sequence of SEQ ID NO: 6136; or
- (v) a VH comprising any one amino acid sequence selected from the group consisting of SEQ ID NOs: 7298 and 7300-7304, and a VL comprising any one amino acid sequence selected from the group consisting of SEQ ID NOs: 7299 and 7305-7309.

7. The use of any one of claims 1-5, wherein the antigen is NKp30, and the NK cell engager comprises the sequence of SEQ ID NO: 7310 or the sequence of SEQ ID NO: 7311.

7.8. The use of claim 1 or 3-or-4, wherein the antigen is NKG2D, and the NK cell engager comprises:

- (i) a VH comprising ~~thean~~ amino acid sequence ~~with at least 80% sequence identity to any one sequence selected from the group consisting of~~ SEQ ID NOs: 6176 and a VL comprising the amino acid sequence of SEQ ID NO: 6177; or
- (ii) a VH comprising the amino acid sequence of SEQ ID NO: 6179 and a VL comprising the amino acid sequence of SEQ ID NO: 6180~~and 6179; and/or (ii) a VL comprising an amino acid sequence with at least 80% sequence identity to any one sequence selected from the group consisting of SEQ ID NOs: 6177 and 6180.~~

9. The use of any one of claims 1, 3, and 8, wherein the antigen is NKG2D, and the NK cell engager comprises the amino acid sequence of SEQ ID NO: 6175 or the amino acid sequence of SEQ ID NO: 6178.

8-10. The use of claim 1 or 3-or-4, wherein the antigen is NKp46, and the NK cell engager comprises: ~~(i) a VH comprising thean amino acid sequence with at least 80% sequence identity to of~~ SEQ ID NO: 6182; ~~and/or (ii) a VL comprising thean amino acid sequence with at least 80% sequence identity to of~~ SEQ ID NO: 6183.

11. The use of any one of claims 1, 3, and 10, wherein the antigen is NKp46, and the NK cell engager comprises the amino acid sequence of SEQ ID NO: 6181.

9.12. The use of claim 1 or 3 ~~or 4~~, wherein the antigen is CD16, and the NK cell engager comprises: ~~(i) a VH comprising the~~ amino acid sequence ~~with at least 80% sequence identity to~~ of SEQ ID NO: 6185; and ~~or (ii) a VL comprising the~~ amino acid sequence ~~with at least 80% sequence identity to~~ of SEQ ID NO: 6186.

13. The use of any one of claims 1, 3, and 12, wherein the antigen is CD16, and the NK cell engager comprises the amino acid sequence of SEQ ID NO: 6184.

~~10. The use of any one of claims 1-9, wherein the multifunctional molecule comprises the cytokine molecule, and~~

~~optionally, wherein the cytokine molecule is selected from the group consisting of interleukin-2 (IL-2) or fragment or variant thereof, interleukin-7 (IL-7) or fragment or variant thereof, interleukin-12 (IL-12) or fragment or variant thereof, interleukin-15 (IL-15) or fragment or variant thereof, interleukin-18 (IL-18) or fragment or variant thereof, interleukin-21 (IL-21) or fragment or variant thereof, interferon gamma or fragment or variant thereof, and any combination thereof.~~

~~11. The use of claim 10, wherein the cytokine molecule is a monomer or a dimer.~~

~~12. The use of claim 10 or 11, wherein the cytokine molecule further comprises a receptor dimerizing domain.~~

13.14. The use of any one of claims 1-~~13~~12, wherein the multifunctional molecule comprises ~~the~~ cytokine inhibitor molecule, ~~and~~

15. The use of claim 14, ~~optionally~~, wherein the cytokine inhibitor molecule is a TGF-beta inhibitor.

14.16. The use of claim 15~~13~~, wherein the TGF-beta inhibitor comprises a portion of a TGF-beta receptor that is capable of inhibiting TGF-beta, ~~or functional fragment or variant thereof~~, optionally, wherein the portion of a TGF-beta receptor comprises an extracellular domain of a TGF-beta receptor.

15.17. The use of claim ~~13 or 14~~15 or 16, wherein the TGF-beta inhibitor comprises a portion of TGFBR1 comprising an extracellular domain of a TGF-beta receptor 1 and capable of inhibiting TGF-beta; a portion of TGFBR2 comprising an extracellular domain of a TGF-beta receptor 2 and capable of inhibiting TGF-beta; a portion of TGFBR3 comprising an extracellular domain of

a TGF-beta receptor 3 and capable of inhibiting TGF-beta; a portion of TGFBR1 comprising an extracellular domain of a TGF-beta receptor 1 and capable of inhibiting TGF-beta and a portion of TGFBR2 comprising an extracellular domain of a TGF-beta receptor 2 and capable of inhibiting TGF-beta; a portion of TGFBR1 comprising an extracellular domain of a TGF-beta receptor 1 and capable of inhibiting TGF-beta and a portion of TGFBR3 comprising an extracellular domain of a TGF-beta receptor 3 and capable of inhibiting TGF-beta; a portion of TGFBR2 comprising an extracellular domain of a TGF-beta receptor 2 and capable of inhibiting TGF-beta and a portion of TGFBR3 comprising an extracellular domain of a TGF-beta receptor 3 and capable of inhibiting TGF-beta; or a portion of TGFBR1 comprising an extracellular domain of a TGF-beta receptor 1 and capable of inhibiting TGF-beta, a portion of TGFBR2 comprising an extracellular domain of a TGF-beta receptor 2 and capable of inhibiting TGF-beta, and a portion of TGFBR3 comprising an extracellular domain of a TGF-beta receptor 3 and capable of inhibiting TGF-beta.

16-18. The use of any one of claims 1513-1715, wherein the TGF-beta inhibitor comprises an amino acid sequence with at least 93% sequence identity to any one sequence selected from the group consisting of SEQ ID NOs: 7254-7288.

17-19. The use of any one of claims 1-~~18~~16, wherein the multifunctional molecule comprises a death receptor signal engager, ~~and~~

20. ~~The use of claim 19, optionally,~~ wherein the death receptor signal engager is selected from the group consisting of a TNF-related apoptosis-inducing ligand (TRAIL) molecule, a death receptor molecule, and an antigen binding domain that specifically binds to a death receptor.

18-21. The use of claim ~~20~~17, wherein the TRAIL molecule specifically binds to Death Receptor 4 (DR4) or Death Receptor 5 (DR5), and

optionally, wherein the TRAIL molecule comprises a truncated TRAIL polypeptide compared to a wild-type TRAIL polypeptide.

~~19. — The use of claim 17 or 18, wherein the TRAIL molecule comprises a truncated TRAIL polypeptide compared to a wild-type TRAIL polypeptide.~~

20-22. The use of ~~any one of claims 20 or 21~~17-19, wherein the death receptor signal engager comprises an amino acid sequence with at least 80% sequence identity to any one sequence selected from the group consisting of SEQ ID NOs: 6157-6165.

21-23. The use of any one of claims 1-~~22~~20, wherein the subject has one or more biased α-TCRs.
bias, and

~~24.~~ ~~The use of any one of claims 1-23, optionally,~~ wherein the biased TCR ~~bias~~ comprises one or more biased TCRBV clonotypes.

~~22.25.~~ The use of any one of claims 1-~~24~~21, wherein the multifunctional ~~multispecific~~ molecule depletes a T cell population expressing the biased TCR, thereby treating the autoimmune disease in the subject~~contacts a T cell population with an effective amount, thereby depleting the T cell population, optionally, wherein the multispecific molecule contacts the T cell population in vivo or in vitro.~~

~~23.26.~~ A recombinant polynucleotide comprising a sequence encoding a~~the~~ multifunctional ~~multispecific~~ molecule, wherein the multifunctional molecule encoded by the recombinant polynucleotide comprises:

(i) an antigen binding domain that selectively binds to a T cell receptor variable beta region (TCR β V) of the biased TCR, and

(ii) an NK cell engager, wherein the NK cell engager binds to an antigen selected from the group consisting of NKp30, NKp46, NKG2D, and CD16

~~of the pharmaceutical composition according to the use of any one of claims 1-22.~~

~~24.27.~~ A host cell comprising the recombinant polynucleotide comprising a sequence encoding a multifunctional molecule of claim 26~~of claim 23.~~

~~25.28.~~ A method of making a~~the~~ multifunctional molecule ~~of the pharmaceutical composition according to the use of any one of claims 1-22,~~ comprising culturing the host cell of claim ~~27~~24 under suitable conditions for gene expression and/or homo- or heterodimerization, wherein the multifunctional molecule comprises:

(i) an antigen binding domain that selectively binds to a T cell receptor variable beta region (TCR β V) of the biased TCR, and

(ii) an NK cell engager, wherein the NK cell engager binds to an antigen selected from the group consisting of NKp30, NKp46, NKG2D, and CD16.