

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 molecule comprises:

(i) an antigen binding domain that selectively binds to a T cell receptor variable beta (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.

In another aspect, the present disclosure provides a recombinant polynucleotide comprising a sequence encoding a multifunctional 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 herein.

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 multifunctional molecule 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 (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.

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.

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 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.
2. The use of claim 1, wherein the NK cell engager binds to NKp30.
3. The use of claim 1 or 2, wherein the NK cell engager binds to and activates an NK cell.
4. The use of any one of claims 1-3, 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), 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.

5. The use of claim 4, 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 .

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.

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

- (i) a VH comprising the amino acid sequence of SEQ ID NO: 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 .

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.

10. The use of claim 1 or 3, wherein the antigen is NKp46, and the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6182 and a VL comprising the amino acid sequence 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.
12. The use of claim 1 or 3, wherein the antigen is CD16, and the NK cell engager comprises a VH comprising the amino acid sequence of SEQ ID NO: 6185 and a VL comprising the amino acid sequence 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.
14. The use of any one of claims 1-13, wherein the multifunctional molecule comprises a cytokine inhibitor molecule.
15. The use of claim 14, wherein the cytokine inhibitor molecule is a TGF-beta inhibitor.
16. The use of claim 15, wherein the TGF-beta inhibitor comprises a portion of a TGF-beta receptor that is capable of inhibiting TGF-beta,
optionally, wherein the portion of a TGF-beta receptor comprises an extracellular domain of a TGF-beta receptor.
17. The use of claim 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.

18. The use of any one of claims 15-17, 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.
19. The use of any one of claims 1-18, wherein the multifunctional molecule comprises a death receptor signal engager.
20. The use of claim 19, 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.
21. The use of claim 20, 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.
22. The use of claim 20 or 21, 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.
23. The use of any one of claims 1-22, wherein the subject has one or more biased TCRs.
24. The use of any one of claims 1-23, wherein the biased TCR comprises one or more biased TCRBV clonotypes.
25. The use of any one of claims 1-24, wherein the multifunctional molecule depletes a T cell population expressing the biased TCR, thereby treating the autoimmune disease in the subject.
26. A recombinant polynucleotide comprising a sequence encoding a multifunctional 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.
27. A host cell comprising the recombinant polynucleotide comprising a sequence encoding a multifunctional molecule of claim 26.
28. A method of making a multifunctional molecule comprising culturing the host cell of claim 27 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.