

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
20 fundamental property of an effective immune system. However, the immune repertoire can change greatly with the onset 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
25 improved therapies for autoimmune diseases.

SUMMARY

 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
30 autoimmune disease in a subject in need thereof, wherein the multispecific molecule comprises:

(i) an antigen binding domain that selectively binds to a T cell receptor variable beta (TCR β V), and (ii) one, two, or all of an NK cell engager, a cytokine molecule, a cytokine inhibitor molecule, and a death receptor signal engager.

In another aspect, the present disclosure provides a recombinant polynucleotide comprising a sequence encoding the multispecific molecule of the pharmaceutical composition according to the use as described herein.

In another aspect, the present disclosure provides a host cell comprising the recombinant polynucleotide as described herein.

In yet another aspect, the present disclosure provides a method of making 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.

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. Re-balancing 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

TCRBV, e.g., TCRBV antigen (e.g., a TCRBV antigen corresponding to a biased TCRBV clonotype), thereby providing a more effective therapy (e.g., a more effective autoimmune disease therapy). Without being bound by theory, a targeted, localized immune response against the target cell (e.g., a T cell comprising a biased TCRBV clonotype, e.g., and not T cells not comprising the biased TCRBV clonotype) is believed to reduce the effects of systemic toxicity of the multispecific molecules described herein. A targeted immune response against the autoreactive T cell population that targets non-autoreactive T cells to a lesser degree (e.g., does not target non-autoreactive T cells) is believed to have fewer deleterious effects than systemic ablation of all T cells.

Accordingly, provided herein are, *inter alia*, multispecific molecules (e.g., multispecific or multifunctional antibody molecules) that include the aforesaid moieties, nucleic acids encoding the same, methods of producing the aforesaid molecules, and methods of treating autoimmune disease using the aforesaid molecules. Also provided herein are anti-TCR β V antibody molecules, nucleic acids encoding the same, methods of producing the aforesaid molecules, and methods of treating autoimmune disease using the anti-TCR β V antibody molecules.

Further provided are methods for depletion (e.g., in vivo depletion) of biased TCRBV clonotypes, e.g., in the context of autoimmune disease with a multispecific molecule or an anti-TCR β V antibody molecule. In some examples, the method involves identifying in a patient a clonal bias in TCRBV usage, e.g., associated with the autoreactive subpopulation, and responsive to this analysis administering a multifunctional molecule targeted to the TCRBV antigen corresponding to the biased TCRBV clonotype to decrease, e.g., eliminate, the clonal bias and promote, e.g., establish, a normal TCRBV distribution.

Accordingly, in one example, the disclosure features a multifunctional molecule, comprising:

- (i) a first antigen binding domain that binds to, e.g., selectively binds to, T cell receptor variable beta (TCRBV), e.g., a TCRBV antigen,
- and
- (ii) one, two, or all of:

(a) an immune cell engager chosen from an NK cell engager, a T cell engager, a B cell engager, a dendritic cell engager, or a macrophage cell engager;
(b) a cytokine molecule or cytokine inhibitor molecule; and
(c) a death receptor signal engager.

5 In some examples, first antigen binding domain comprises an anti-TCR β V antibody molecule, e.g., as described herein.

 In another example, the disclosure features a nucleic acid molecule encoding a multifunctional molecule disclosed herein.

 In another example, the disclosure features a vector, e.g., an expression vector,
10 comprising the nucleic acid molecules disclosed herein.

 In another example, the disclosure features a host cell comprising a nucleic acid molecule or vector disclosed herein.

 In another example, the disclosure features a method of making, e.g., producing, a multifunctional molecule disclosed herein, comprising culturing a host cell disclosed herein
15 under suitable conditions, e.g., conditions suitable for gene expression and/or homo- or heterodimerization.

 In another example, the disclosure features a pharmaceutical composition comprising a multifunctional molecule disclosed herein.

 In another example, the disclosure features a method of treating a TCR bias, comprising
20 administering to a subject in need thereof a multifunctional molecule disclosed herein, wherein the multifunctional molecule is administered in an amount effective to treat the TCR bias.

 In another example, the disclosure features a method of treating an autoimmune disease (e.g., an autoimmune disease associated with a TCR bias), comprising administering to a subject in need thereof a multifunctional molecule disclosed herein, wherein the multifunctional
25 molecule is administered in an amount effective to treat the autoimmune disease.

 In another example, the disclosure features a method of identifying a subject in need of treatment for TCR bias or an autoimmune disease (e.g., associated with a TCR bias) using a multifunctional molecule disclosed herein, comprising determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has a TCR bias

(e.g., a biased TCRBV clonotype) and/or an autoimmune disease associated with said bias, wherein:

responsive to determining that the subject has a TCR bias (e.g., a biased TCRBV clonotype) and/or an autoimmune disease associated with said bias, identifying the subject as a candidate for treatment using a multifunctional molecule comprising an antigen binding domain that binds to the TCRBV antigen.

In another example, the disclosure features a method of evaluating a subject in need of treatment for a TCR bias (e.g., a biased TCRBV clonotype) and/or an autoimmune disease associated with said bias, comprising determining (e.g., directly determining or indirectly determining, e.g., obtaining information regarding) whether a subject has a TCR bias (e.g., a biased TCRBV clonotype).

In yet another example, disclosed herein is a method of treating an autoimmune disease (e.g., an autoimmune disease associated with a TCR bias), in a subject in need thereof, comprising administering to said subject an effective amount, *e.g.*, a therapeutically effective amount, of an antibody molecule which binds (e.g., specifically binds) to a T cell receptor beta variable region (TCR β V) (“anti-TCR β V antibody molecule”), thereby treating the disorder.

In another example, the disclosure provides a method of depleting a population of T cells in a subject having an autoimmune disorder (e.g., an autoimmune disease associated with a TCR bias), comprising, contacting the T cell population with an effective amount of an antibody molecule which binds (e.g., specifically binds) to a T cell receptor beta variable region (TCR β V) (“anti-TCR β V antibody molecule”).

In some examples, the contacting occurs in vivo or in vitro.

In some examples, the anti-TCR β V antibody molecule is not an antibody molecule disclosed in US Patent 5,861,155.

In some examples, the anti-TCR β V antibody molecule binds to TCR β V12 with an affinity and/or binding specificity that is less than (*e.g.*, less than about 10%, 20%, 30%, 40%,

50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the 16G8 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some examples, the anti-TCR β V antibody molecule binds to TCR β V12 with an affinity and/or binding specificity that is greater than (*e.g.*, greater than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the 16G8 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some examples, the anti-TCR β V antibody molecule binds to TCR β V5-5*01 or TCR β V5-1*01 with an affinity and/or binding specificity that is greater than (*e.g.*, greater than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the TM23 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some examples, the anti-TCR β V antibody molecule binds to TCR β V5-5*01 or TCR β V5-1*01 with an affinity and/or binding specificity that is greater than (*e.g.*, greater than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or about 2-, 5-, or 10- fold) the affinity and/or binding specificity of the TM23 murine antibody or a humanized version thereof as described in US Patent 5,861,155.

In some examples, the anti-TCR β V antibody molecule comprises an Fc region, *e.g.*, an Fc region having effector function, *e.g.*, antibody dependent cell-mediated cytotoxicity (ADCC), Antibody-dependent cellular phagocytosis (ADCP) and/or complement dependent cytotoxicity (CDC).

In some examples, the anti-TCR β V antibody molecule, the anti-TCR β V antibody molecule comprises an Fc region with enhanced effector function, *e.g.*, as compared to a wildtype Fc region.

In some examples, the anti-TCR β V antibody molecule, the anti-TCR β V antibody molecule comprises a human IgG1 region or a human IgG4 region.

In another example, the disclosure features a nucleic acid molecule encoding an anti-TCR β V antibody molecule disclosed herein.

In another example, the disclosure features a vector, e.g., an expression vector, comprising the nucleic acid molecules disclosed herein.

In another example, the disclosure features a host cell comprising a nucleic acid molecule or vector disclosed herein.

5 In another example, the disclosure features a method of making, e.g., producing, an anti-TCR β V antibody molecule disclosed herein, comprising culturing a host cell disclosed herein under suitable conditions, e.g., conditions suitable for gene expression and/or homo- or heterodimerization.

10 In another example, the disclosure features a pharmaceutical composition comprising an anti-TCR β V antibody molecule disclosed herein.

Additional features of any of the aforesaid multifunctional molecules, nucleic acids, vectors, host cells, or methods include one or more of the following enumerated examples.

15 Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific examples of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated examples.

Enumerated Embodiments

20 1. A multifunctional molecule, comprising:

(i) a first antigen binding domain that binds to, e.g., selectively binds to, T cell receptor variable beta (TCRBV), e.g., a TCRBV antigen,

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We claim:

1. Use of a pharmaceutical composition comprising a multifunctional molecule in the manufacture of a medicament for treating an autoimmune disease in a subject in need thereof, wherein the multispecific molecule comprises:
 - (i) an antigen binding domain that selectively binds to a T cell receptor variable beta (TCR β V), and
 - (ii) one, two, or all of an NK cell engager, a 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. 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. The use of claim 3, wherein the antigen is NKp30, NKp46, NKG2D, or CD16.
5. The use of claim 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) 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. The use of any one of claims 3-5, wherein the NK cell engager comprises:
- (i) 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.
7. The use of claim 3 or 4, wherein the antigen is NKG2D, and the NK cell engager comprises:
- (i) 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: 6176 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.
8. The use of claim 3 or 4, wherein the antigen is NKp46, and the NK cell engager comprises:
- (i) a VH comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 6182; and/or
 - (ii) a VL comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 6183.
9. The use of claim 3 or 4, wherein the antigen is CD16, and the NK cell engager comprises:
- (i) a VH comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 6185; and/or

- (ii) a VL comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO: 6186.
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. The use of any one of claims 1-12, wherein the multifunctional molecule comprises the cytokine inhibitor molecule, and
optionally, wherein the cytokine inhibitor molecule is a TGF-beta inhibitor.
14. The use of claim 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. The use of claim 13 or 14, wherein the TGF-beta inhibitor comprises a portion of TGFBR1; a portion of TGFBR2; a portion of TGFBR3; a portion of TGFBR1 and a portion of TGFBR2; a portion of TGFBR1 and a portion of TGFBR3; a portion of TGFBR2 and a portion of TGFBR3; or a portion of TGFBR1, a portion of TGFBR2, and a portion of TGFBR3.
16. The use of any one of claims 13-15, wherein the TGF-beta inhibitor comprises an amino acid sequence with at least 93% sequence identity to SEQ ID NOs: 7254-7288.
17. The use of any one of claims 1-16, wherein the multifunctional molecule comprises a death receptor signal engager, and

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. The use of claim 17, wherein the TRAIL molecule specifically binds to Death Receptor 4 (DR4) or Death Receptor 5 (DR5).
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. The use of any one of claims 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. The use of any one of claims 1-20, wherein the subject has a TCR bias, and optionally, wherein the TCR bias comprises a biased TCRBV clonotype.
22. The use of any one of claims 1-21, wherein the multispecific molecule 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. A recombinant polynucleotide comprising a sequence encoding the multispecific molecule of the pharmaceutical composition according to the use of any one of claims 1-22.
24. A host cell comprising the recombinant polynucleotide of claim 23.
25. A method of making 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 24 under suitable conditions for gene expression and/or homo- or heterodimerization.