

(2019); Arenas-Ramirez, N. et al., *Trends Immunol* 36, 763–77 (2015). Variant proteins that avoid the preferential activation of high-affinity IL-2R-expressing cells, such as Tregs and vascular endothelial cells, are one approach to achieving this goal. In an effort to create such a molecule, other approaches have involved mutating the IL-2RA binding interface on IL-2, attaching polyethylene glycol to the IL-2 protein, creating synthetic IL-2 proteins, and generating an antibody that blocks the IL-2RA binding domain. Silva, D.-A. et al., *Nature* 565, 186–191 (2019); Charych, D. H. et al., *Clin Cancer Res* 22, 680–690 (2016); Arenas-Ramirez, N. et al., *Sci Transl Med* 8, 367ra166–367ra166 (2016); Levin, A. M. et al., *Nature* 484, 529–533 (2012); Lopes, J. E. et al., *J Immunother Cancer* 8, e000673 (2020). As an alternative to IL-2, others have engineered IL-15 variants that bind to IL-2RB/IL-2RG subunits. The IL-15-specific receptor subunit naturally binds to IL-15 *in trans* from antigen presenting cells; therefore, an active IL-15 recombinant protein requires a single chain construct that expresses both IL-15 and the receptor subunit. Bennett, M. J. et al. Abstract 5565: Potency-reduced IL15/IL15R α heterodimeric Fc-fusions display enhanced *in vivo* activity through increased exposure. 5565–5565 (2018) doi:10.1158/1538-7445.am2018-5565. Mutated cytokines have also been fused to antibodies or Fc domains to increase the *in vivo* half-life of the molecules and localize the cytokines to the tumor site. Murer, P. et al., *New Biotechnol* 52, 42–53 (2019); Klein, C. et al., *Oncoimmunology* 6:3 e1277306 (2017); Schliemann, C. et al., *Blood* 120, 3716–3716 (2012). While some of these engineered proteins have the desired functional activity, many suffer from high levels of immunogenicity *in vivo* and present challenges with *in vivo* stability and manufacturing. Brummelen, E. M. J. van et al., *Oncotarget* 9, 24737–49 (2018); Groot, A. S. D. et al., *Trends Immunol* 28, 482–90 (2007); Schellekens, H., *Nephrol Dial Transpl* 18, 1257–59 (2003); Verhoef, J. J. F., et al., *Drug Discov Today* 19, 1945–52 (2014). Therefore, creating an anti-tumor agonist of the IL-2 pathway with the desired biological activity, safety profile, and ideal drug-like properties remains a significant challenge for the field.

SUMMARY

[0007a] In one aspect, the present disclosure refers to a heavy chain only antibody comprising: a first heavy chain variable region that binds to IL2RB comprising: (1) (a) a CDR1 sequence comprising the formula: G G S I S S X1 W (SEQ ID NO: 26) where X1 is D or N; (b) a CDR2 sequence comprising the formula: I X2 H S G S T (SEQ ID NO: 27) where X2 is D or S; and (c) a CDR3 sequence comprising the formula: X3 R G X4 W E L X5 D A F D I (SEQ ID NO: 28) where X3 is G or A; X4 is S or Q; and X5 is S or T; or (2) (a) a CDR1 sequence comprising the formula: G F T F S X1 Y G (SEQ ID NO: 29) where X1 is S or T; (b) a CDR2 sequence comprising the formula: I S Y D G S N X2 (SEQ ID NO: 30) where X2 is K or R; and (c) a CDR3 sequence comprising the formula: A R D L D Y D X3 L T G D P V G G F D I (SEQ ID NO: 31) where X3 is V or I; and a second heavy chain variable region that binds to IL2RG comprising: (1) (a) a CDR1 sequence

comprising the formula: G F X1 X2 X3 X4 Y Y (SEQ ID NO: 32) where X1 is T or I; X2 is F or V; X3 is S, N, or G; and X4 is D or N; (b) a CDR2 sequence comprising the formula: I S X5 S G X6 X7 I (SEQ ID NO: 33) where X5 is S or N; X6 is D, S, G, or N; and X7 is T or I; and (c) a CDR3 sequence comprising the sequence ARGDAVSITGDY (SEQ ID NO: 20); or (2) a CDR1 sequence comprising SEQ ID NO: 15, a CDR2 sequence comprising SEQ ID NO: 19, and a CDR3 sequence comprising SEQ ID NO: 1. In another aspect, the present disclosure refers to a pharmaceutical composition comprising: a heavy chain only antibody as described herein; and a pharmaceutically acceptable excipient. In another aspect, the present disclosure refers to the use of a heavy chain only antibody as described herein in the manufacture of a medicament for treating a cancer. In another aspect, the present disclosure refers to an *in vitro* method for stimulating an IL2RB/IL2RG dimeric receptor complex on an immune cell and/or stimulating IL2R signaling in an immune cell, the method comprising contacting the immune cell with a heavy chain only antibody as described herein, or a pharmaceutical composition.

DETAILED DESCRIPTION

[0008] The molecules of the present disclosure combine the favorable drug-like properties of antibodies with the functional behavior of molecules that facilitate IL-2RB and IL-2RG association and downstream signaling. Aspects of the disclosure include antibody sequences, such as fully human antibody sequences, such as fully human multispecific (e.g., bispecific) antibodies that simultaneously bind IL-2RB and IL-2RG subunits and therefore mimic the activity of IL-2 while avoiding binding to IL-2RA. In addition to exhibiting the desired activation and expansion of immune effector cells, bispecific IL-2RB/G agonist antibodies described herein also avoid preferential expansion of suppressive Tregs, both *in vitro* and *in vivo*.

Non-Limiting Example Embodiments (Set 1)

[0009] Without limitation, some example embodiments/features of this disclosure include:

1. An antibody that binds to IL2RB, comprising a heavy chain variable region comprising:

CLAIMS:

1. A heavy chain only antibody comprising:

a first heavy chain variable region that binds to IL2RB comprising:

- (1) (a) a CDR1 sequence comprising the formula:

G G S I S S S X1 W (SEQ ID NO: 26)

where X1 is D or N;

- (b) a CDR2 sequence comprising the formula:

I X2 H S G S T (SEQ ID NO: 27)

where X2 is D or S; and

- (c) a CDR3 sequence comprising the formula:

X3 R G X4 W E L X5 D A F D I (SEQ ID NO: 28)

where X3 is G or A;

X4 is S or Q; and

X5 is S or T; or

- (2) (a) a CDR1 sequence comprising the formula:

G F T F S X1 Y G (SEQ ID NO: 29)

where X1 is S or T;

- (b) a CDR2 sequence comprising the formula:

I S Y D G S N X2 (SEQ ID NO: 30)

where X2 is K or R; and

- (c) a CDR3 sequence comprising the formula:

A R D L D Y D X3 L T G D P V G G F D I (SEQ ID NO: 31)

where X3 is V or I; and

a second heavy chain variable region that binds to IL2RG comprising:

- (1) (a) a CDR1 sequence comprising the formula:

G F X1 X2 X3 X4 Y Y (SEQ ID NO: 32)

where X1 is T or I;

X2 is F or V;

X3 is S, N, or G; and

X4 is D or N;

- (b) a CDR2 sequence comprising the formula:

I S X5 S G X6 X7 I (SEQ ID NO: 33)

where X5 is S or N;

X6 is D, S, G, or N; and

X7 is T or I; and

- (c) a CDR3 sequence comprising the sequence ARGDAVSITGDY (SEQ ID NO: 20); or
 - (2) a CDR1 sequence comprising SEQ ID NO: 15, a CDR2 sequence comprising SEQ ID NO: 19, and a CDR3 sequence comprising SEQ ID NO: 21.
2. The heavy chain only antibody of claim 1, wherein:
- the first heavy chain variable region comprises a CDR1 sequence comprising SEQ ID NO: 26, a CDR2 sequence comprising SEQ ID NO: 27, and a CDR3 sequence comprising SEQ ID NO: 28, and the second heavy chain variable region comprises a CDR1 sequence comprising SEQ ID NO: 32, a CDR2 sequence comprising SEQ ID NO: 33, and a CDR3 sequence comprising SEQ ID NO: 20; or
- the first heavy chain variable region comprises a CDR1 sequence comprising SEQ ID NO: 26, a CDR2 sequence comprising SEQ ID NO: 27, and a CDR3 sequence comprising SEQ ID NO: 28, and the second heavy chain variable region comprises a CDR1 sequence comprising SEQ ID NO: 15, a CDR2 sequence comprising SEQ ID NO: 19, and a CDR3 sequence comprising SEQ ID NO: 21.
3. The heavy chain only antibody of claim 1, wherein:
- the first heavy chain variable region comprises a CDR1 sequence comprising SEQ ID NO: 29, a CDR2 sequence comprising SEQ ID NO: 30, and a CDR3 sequence comprising SEQ ID NO: 31, and the second heavy chain variable region comprises a CDR1 sequence comprising SEQ ID NO: 32, a CDR2 sequence comprising SEQ ID NO: 33, and a CDR3 sequence comprising SEQ ID NO: 20; or
- the first heavy chain variable region comprises a CDR1 sequence comprising SEQ ID NO: 29, a CDR2 sequence comprising SEQ ID NO: 30, and a CDR3 sequence comprising SEQ ID NO: 31, and the second heavy chain variable region comprises a CDR1 sequence comprising SEQ ID NO: 15, a CDR2 sequence comprising SEQ ID NO: 19, and a CDR3 sequence comprising SEQ ID NO: 21.
4. The heavy chain only antibody of claim 1, wherein the first heavy chain variable region comprises:
- (a) a CDR1 sequence comprising SEQ ID NO: 1 or SEQ ID NO: 2; a CDR2 sequence comprising SEQ ID NO: 4 or SEQ ID NO: 5; and a CDR3 sequence comprising any one of SEQ ID NOs: 7-9; or
 - (b) a CDR1 sequence comprising SEQ ID NO: 3; a CDR2 sequence comprising SEQ ID NO: 6; and a CDR3 sequence comprising SEQ ID NO: 10.

5. The heavy chain only antibody of claim 1, wherein the first heavy chain variable region comprises:
- (a) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 4, and a CDR3 sequence of SEQ ID NO: 7; or
 - (b) a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 4, and a CDR3 sequence of SEQ ID NO: 8; or
 - (c) a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 9; or
 - (d) a CDR1 sequence of SEQ ID NO: 3, a CDR2 sequence of SEQ ID NO: 6, and a CDR3 sequence of SEQ ID NO: 10.
6. The heavy chain only antibody of claim 1, wherein:
- the first heavy chain variable region has at least 95% sequence identity to any one of SEQ ID NOs: 11-14; and/or
 - the first heavy chain variable region sequence is selected from SEQ ID NOs: 11-14.
7. The heavy chain only antibody of claim 1, wherein the second heavy chain variable region comprises:
- (a) a CDR1 sequence comprising SEQ ID NO: 15 or SEQ ID NO: 16; a CDR2 sequence comprising SEQ ID NO: 17 or SEQ ID NO: 18; and a CDR3 sequence comprising SEQ ID NO: 20; or
 - (b) a CDR1 sequence comprising SEQ ID NO: 15; a CDR2 sequence comprising SEQ ID NO: 19; and a CDR3 sequence comprising SEQ ID NO: 21.
8. The heavy chain only antibody of claim 1, wherein the second heavy chain variable region comprises:
- (a) a CDR1 sequence of SEQ ID NO: 15, a CDR2 sequence of SEQ ID NO: 17, and a CDR3 sequence of SEQ ID NO: 20; or
 - (b) a CDR1 sequence of SEQ ID NO: 15, a CDR2 sequence of SEQ ID NO: 18, and a CDR3 sequence of SEQ ID NO: 20; or
 - (c) a CDR1 sequence of SEQ ID NO: 16, a CDR2 sequence of SEQ ID NO: 18, and a CDR3 sequence of SEQ ID NO: 20; or
 - (d) a CDR1 sequence of SEQ ID NO: 15, a CDR2 sequence of SEQ ID NO: 19, and a CDR3 sequence of SEQ ID NO: 21.
9. The heavy chain only antibody of claim 1, wherein:

the second heavy chain variable region has at least 95% sequence identity to any one of SEQ ID NOs: 22-25; and/or

the second heavy chain variable region sequence is selected from SEQ ID NOs: 22-25.

10. The heavy chain only antibody of claim 1, wherein:

the first heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 4, and a CDR3 sequence of SEQ ID NO: 7; and

the second heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 15, a CDR2 sequence of SEQ ID NO: 17, and a CDR3 sequence of SEQ ID NO: 20.

11. The heavy chain only antibody of claim 10, wherein:

the first heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 11, and the second heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 22; and/or

the first heavy chain variable region comprises SEQ ID NO: 11, and the second heavy chain variable region comprises SEQ ID NO: 22.

12. The heavy chain only antibody of claim 10 or claim 11, comprising a first polypeptide comprising SEQ ID NO: 53 and a second polypeptide comprising SEQ ID NO: 61.

13. The heavy chain only antibody of claim 1, wherein:

the first heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 4, and a CDR3 sequence of SEQ ID NO: 8; and

the second heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 15, a CDR2 sequence of SEQ ID NO: 18, and a CDR3 sequence of SEQ ID NO: 20.

14. The heavy chain only antibody of claim 13, wherein:

the first heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 12, and the second heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 23; and/or

the first heavy chain variable region comprises SEQ ID NO: 12, and the second heavy chain variable region comprises SEQ ID NO: 23.

15. The heavy chain only antibody of claim 13 or claim 14, comprising a first polypeptide comprising SEQ ID NO: 62 and a second polypeptide comprising SEQ ID NO: 63.

16. The heavy chain only antibody of claim 1, wherein:

the first heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 2, a CDR2 sequence of SEQ ID NO: 5, and a CDR3 sequence of SEQ ID NO: 9; and

the second heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 15, a CDR2 sequence of SEQ ID NO: 18, and a CDR3 sequence of SEQ ID NO: 20.

17. The heavy chain only antibody of claim 16, wherein:

the first heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 13, and the second heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 23; and/or

the first heavy chain variable region comprises SEQ ID NO: 13, and the second heavy chain variable region comprises SEQ ID NO: 23.

18. The heavy chain only antibody of claim 16 or claim 17, comprising a first polypeptide comprising SEQ ID NO: 64 and a second polypeptide comprising SEQ ID NO: 65.

19. The heavy chain only antibody of claim 1, wherein:

the first heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 3, a CDR2 sequence of SEQ ID NO: 6, and a CDR3 sequence of SEQ ID NO: 10; and

the second heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 15, a CDR2 sequence of SEQ ID NO: 17, and a CDR3 sequence of SEQ ID NO: 20.

20. The heavy chain only antibody of claim 19, wherein:

the first heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 14, and the second heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 22; and/or

the first heavy chain variable region comprises SEQ ID NO: 14, and the second heavy chain variable region comprises SEQ ID NO: 22.

21. The heavy chain only antibody of claim 19 or claim 20, comprising a first polypeptide comprising SEQ ID NO: 66 and a second polypeptide comprising SEQ ID NO: 67.

22. The heavy chain only antibody of claim 1, wherein:

the first heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 1, a CDR2 sequence of SEQ ID NO: 4, and a CDR3 sequence of SEQ ID NO: 8; and

the second heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 16, a CDR2 sequence of SEQ ID NO: 18, and a CDR3 sequence of SEQ ID NO: 20.

23. The heavy chain only antibody of claim 22, wherein:
the first heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 12,
and the second heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 24;
and/or
the first heavy chain variable region comprises SEQ ID NO: 12, and the second heavy chain
variable region comprises SEQ ID NO: 24.
24. The heavy chain only antibody of claim 22 or claim 23, comprising a first polypeptide
comprising SEQ ID NO: 34 and a second polypeptide comprising SEQ ID NO: 35.
25. The heavy chain only antibody of claim 1, wherein:
the first heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 1, a CDR2
sequence of SEQ ID NO: 4, and a CDR3 sequence of SEQ ID NO: 8; and
the second heavy chain variable region comprises a CDR1 sequence of SEQ ID NO: 15, a
CDR2 sequence of SEQ ID NO: 19, and a CDR3 sequence of SEQ ID NO: 21.
26. The heavy chain only antibody of claim 25, wherein:
the first heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 12,
and the second heavy chain variable region has at least 95% sequence identity to SEQ ID NO: 25;
and/or
the first heavy chain variable region comprises SEQ ID NO: 12, and the second heavy chain
variable region comprises SEQ ID NO: 25.
27. The heavy chain only antibody of claim 25 or claim 26, comprising a first polypeptide
comprising SEQ ID NO: 36 and a second polypeptide comprising SEQ ID NO: 37.
28. The heavy chain only antibody of any one of claims 1 to 5, 7, 8 10, 13, 16, 19, 22, or 25,
wherein:
the CDR1, CDR2, and CDR3 sequences in the first heavy chain variable region are present in
a VH human framework; and/or
the CDR1, CDR2, and CDR3 sequences in the second heavy chain variable region are present
in a VH human framework.
29. The heavy chain only antibody of any one of claims 1 to 11, 13, 14, 16, 17, 19, 20, 22, 23, 25,
or 26, wherein the heavy-chain only antibody comprises a silenced Fc region.

30. The heavy chain only antibody of any one of claims 1 to 11, 13, 14, 16, 17, 19, 20, 22, 23, 25, or 26, wherein the heavy-chain only antibody comprises a heavy chain constant region sequence in the absence of a CH1 sequence.
31. The heavy chain only antibody of any one of claims 1 to 30, wherein the heavy-chain only antibody is a human antibody and/or an isolated antibody.
32. The heavy chain only antibody of any one of claims 1 to 31, wherein:
the heavy-chain only antibody has an affinity for IL2R with a K_d of from about 10^{-11} M to around about 10^{-6} M; and/or
the heavy-chain only antibody has an affinity for IL2RB with a K_d of from about 10^{-8} M to around about 2.5×10^{-7} M; and/or
the heavy-chain only antibody has an affinity for IL2RG with a K_d of from about 10^{-9} M to around about 2.5×10^{-8} M.
33. The heavy chain only antibody of any one of claims 1 to 32, wherein the heavy-chain only antibody functions as an IL2 receptor beta/gamma agonist.
34. A pharmaceutical composition comprising:
a heavy chain only antibody of any one of claims 1 to 33; and
a pharmaceutically acceptable excipient.
35. The pharmaceutical composition of claim 34, wherein the pharmaceutical composition is adapted for intravenous or subcutaneous delivery.
36. Use of a heavy chain only antibody of any one of claims 1 to 33 in the manufacture of a medicament for treating a cancer.
37. The use of claim 36, wherein the heavy chain only antibody is to be administered in conjunction with a chemotherapy regimen.
38. An *in vitro* method for stimulating an IL2RB/IL2RG dimeric receptor complex on an immune cell and/or stimulating IL2R signaling in an immune cell, the method comprising contacting the immune cell with a heavy chain only antibody of any one of claims 1 to 33, or a pharmaceutical composition of claim 34 or 35.