

immunomodulatory blockade results in impressive anti-tumor responses, stimulating endogenous T cells to attack tumor cells, leading to long-term cancer remissions in a fraction of patients with a variety of malignancies. Unfortunately, only a subset of patients responds to these therapies, with response rates generally ranging from 10 to 30% and sometimes higher for each monotherapy, depending on the indication and other factors.

[0005] The second approach for de-repressing tumor-reactive T cells is T cell costimulation by treating with agonistic antibodies that bind to costimulatory proteins such as ICOS, thus adding a positive signal to overcome the negative signaling of the immune checkpoints.

[0006] Accordingly, the invention is directed to bispecific antibodies that bind to costimulatory receptors (e.g. ICOS, GITR, OX40, 4-1BB) as well as checkpoint receptors (e.g. PD-1, PD-L1, CTLA-4, LAG-3, TIM-3, BTLA and TIGIT).

SUMMARY

[0007] Accordingly, in one aspect, the present disclosure refers to a bispecific antibody that monovalently binds human ICOS and monovalently binds a human immune checkpoint receptor selected from the group consisting of PD-1, PD-L1, CTLA-4, LAG-3 and TIM-3, for use in treating cancer, wherein the monovalent binding to ICOS results in agonism of ICOS and said monovalent binding to said checkpoint receptor results in inhibition of said receptor. In one aspect example, there is provided bispecific antibodies that monovalently binds a human costimulatory receptor and monovalently binds a human checkpoint receptor for use in activating T cells for the treatment of cancer.

[0008] In some embodiments, the costimulatory receptor is selected from the group consisting of ICOS, GITR, OX40 and 4-1BB.

[0009] In additional embodiments, the checkpoint receptor is selected from the group consisting of PD-1, PD-L1, CTLA-4, LAG-3, TIGIT and TIM-3.

[0010] In further embodiments, the antibody binds an antigen pair selected from: ICOS and PD-1, ICOS and CTLA-4, ICOS and LAG-3, ICOS and TIM-3, ICOS and PD-L1, ICOS and BTLA, ICOS and TIGIT, GITR and TIGIT, GITR and PD-1, GITR and CTLA-4, GITR and LAG-3, GITR and TIM-3, GITR and PD-L1, GITR and BTLA, OX40 and PD-1, OX40 and TIGIT, OX40 and CTLA-4, IC OX40 OS and LAG-3, OX40 and TIM-3, OX40 and PD-L1, OX40 and BTLA, 4-1BB and PD-1, 4-1BB and CTLA-4, 4-1BB and LAG-3, 4-1BB and TIM-3, 4-1BB and PD-L1, TIGIT and 4-1BB and 4-1BB and BTLA.

[0011] In additional embodiments, the bispecific antibody has a format selected from those outlined in Figure 2A-2N.

[0012] In another aspect, the present disclosure provides a bispecific antibody comprising: a) a first polypeptide comprising an scFv-domain linker-CH2-CH3, wherein CH2-CH3 is a first Fc domain, wherein said scFv consists of a first heavy chain variable domain, a first light chain variable domain and a scFv linker, and wherein said scFv linker covalently attaches said first heavy chain variable domain and said first light chain variable domain; b) a second polypeptide comprising a VH-CH1-hinge-CH2-CH3, wherein VH is a second heavy chain variable domain, and CH2-CH3 is a second Fc domain; and c) a light chain comprising a second variable light chain and a constant light chain; wherein said first heavy chain variable chain and said first light chain variable region form a first antigen binding domain, wherein said second heavy chain variable domain and said second light chain variable domain form a second antigen binding domain, wherein one of said first and second antigen binding domains monovalently binds human ICOS and the other of said first and second antigen binding domains monovalently binds human PD-1, wherein said monovalent binding to ICOS results in agonism of ICOS and said monovalent binding to said checkpoint receptor results in inhibition of said receptor. In another aspect example, there is provided heterodimeric antibodies comprising: a) a first heavy chain comprising a first Fc domain, an optional domain linker and a first antigen binding domain comprising an scFv that binds a first antigen; b) a second heavy chain comprising a heavy chain comprising a heavy chain constant domain comprising a second Fc domain, a hinge domain, a CH1 domain and a variable heavy domain; and c) a light chain comprising a variable light domain and a light chain constant domain; wherein said variable heavy domain and said variable light domain form a second antigen binding domain that binds a second antigen, wherein one of said first and second antigen binding domains binds human ICOS and the other binds human PD-1.

[0013] In another aspect, the present disclosure refers to a bispecific antibody comprising: a) a first polypeptide comprising: i) a first variant human IgG1 Fc domain; and ii) a single chain Fv region (scFv), wherein said scFv region comprises a first heavy chain variable domain, a first light chain variable domain and a charged scFv linker, wherein said charged scFv linker covalently attaches said first heavy chain variable domain and said first light chain variable domain; and b) a second polypeptide comprising a VH-CH1-hinge-CH2-CH3 polypeptide, wherein VH is a second heavy chain variable domain and CH2-CH3 is a second variant human IgG1 Fc domain; and c) a light chain comprising a second light chain variable domain; wherein said first heavy chain variable chain and said first light chain variable region form a first antigen binding domain, wherein said second heavy chain variable domain and said second light chain variable domain form a second antigen binding domain, wherein said second variant Fc domain comprises amino acid substitutions N208D/Q295E/N384D/Q418E/N421D, wherein said first and second variant Fc domains each comprise amino acid substitutions E233P/L234V/L235A/G236del/S267K; and wherein said first variant human IgG1 Fc domain comprises amino acid substitutions S364K/E357Q and second variant human IgG1 Fc domain comprises amino acid substitutions L368D/K370S, wherein one of said first and second antigen binding domains monovalently binds human ICOS and the other of said first and second antigen binding domains monovalently binds human PD-1, wherein said monovalent binding to ICOS results in agonism of ICOS and said monovalent binding to said checkpoint receptor results in inhibition of said receptor, and wherein numbering is according to the EU index as in Kabat.

In another [aspectexample](#), there is provided heterodimeric bispecific antibodies comprising: a) a first heavy chain comprising: i) a first variant Fc domain; and ii) a single chain Fv region (scFv) that binds a first antigen, wherein said scFv region comprises a first variable heavy chain, a variable light chain and a charged scFv linker, wherein said charged scFv linker covalently attaches said first variable heavy chain and said variable light chain; and b) a second heavy chain comprising a VH-CH1-hinge-CH2-CH3 monomer, wherein VH is a second variable heavy chain and CH2-CH3 is a second variant Fc domain; and c) a light chain; wherein said second variant Fc domain comprises amino acid substitutions N208D/Q295E/N384D/Q418E/N241D, wherein said first and second variant Fc domains each comprise amino acid substitutions E233P/L234V/L235A/G236del/S267K; and wherein said first variant Fc domain comprises amino acid substitutions S364K/E357Q and second variant Fc domain comprises amino acid substitutions L368D/K370S, wherein one of said first and second antigen binding domains binds human ICOS and the other binds human PD-1, and wherein numbering is according to the EU index as in Kabat.

[0014] In some embodiments the heterodimeric antibodies have first and second variant Fc domains that each comprise M428L/N434S.

[0015] [In one aspect, the present disclosure refers to a bispecific antibody comprising: a\) a first polypeptide comprising: i\) a first variant human IgG1 Fc domain; and ii\) a single chain Fv region \(scFv\), wherein said scFv region comprises a first heavy chain variable domain, a first light chain variable domain, and a charged scFv linker, wherein said charged scFv linker covalently attaches said first heavy chain variable domain and said first light chain variable domain; and b\) a second polypeptide comprising a VH-CH1-hinge-CH2-CH3 polypeptide, wherein VH is a second heavy chain variable domain and CH2-CH3 is a second variant human IgG1 Fc domain; and c\) a light chain comprising a second light chain variable domain; wherein said first heavy chain variable chain and said first light chain variable region form a first antigen binding domain, wherein said second heavy chain variable domain and said second light chain variable domain form a second antigen binding domain, wherein said first and second variant human IgG1 Fc domains comprise a set of heterodimerization variants selected from the group consisting of L368D/K370S : S364K/E357Q; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; and T366S/L368A/Y407V : T366W, wherein one of said first and second antigen binding domains monovalently binds human ICOS and the other of said first and second antigen binding domains monovalently binds human PD-1, wherein said monovalent binding to ICOS results in agonism of ICOS and said monovalent binding to said checkpoint receptor results in inhibition of said receptor, and wherein numbering is according to the EU index as in Kabat.](#) In another [aspectexample](#), there is provided heterodimeric antibodies comprising: a) a first heavy chain comprising: i) a first variant Fc domain; and ii) a single chain Fv region (scFv) that binds a first antigen, wherein said scFv region comprises a first variable heavy chain, a variable light chain and a charged scFv linker, wherein said charged scFv linker covalently attaches said first variable heavy chain and said variable light chain; and b) a second heavy

chain comprising a VH-CH1-hinge-CH2-CH3 monomer, wherein VH is a second variable heavy chain and CH2-CH3 is a second variant Fc domain; and c) a light chain; wherein said first and second variant Fc domains comprises a set of heterodimerization variants selected from the group consisting of L368D/K370S : S364K/E357Q; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; and T366S/L368A/Y407V : T366W, and wherein one of said first and second antigen binding domains binds human ICOS and the other binds human PD-1, and wherein numbering is according to the EU index as in Kabat.

[0016] In a further embodiment, the disclosure provides nucleic acid compositions comprising nucleic acids that encode the heterodimeric antibodies of the disclosure, expression vector compositions comprising the nucleic acids, and host cells comprising the expression vector compositions.

[0016a] In another aspect, there is provided a nucleic acid composition comprising:

- a) a first nucleic acid encoding a first ~~heavy chain~~[polypeptide](#) as disclosed herein;
- b) a second nucleic acid encoding a second ~~heavy chain~~[polypeptide](#) as disclosed herein; and
- c) a third nucleic acid encoding a light chain as disclosed herein, respectively.

[0016b] In another aspect, there is provided an expression vector composition comprising:

- a) a first expression vector comprising said first nucleic acid as disclosed herein;
- b) a second expression vector comprising said second nucleic acid as disclosed herein; and
- c) a third expression vector comprising said third nucleic acid as disclosed herein.

[0016c] In another aspect, there is provided a host cell comprising the expression vector composition as disclosed herein.

[0016d] In another aspect, there is provided a method of making a ~~heterodimeric~~[bispecific](#) antibody comprising culturing a host cell as described herein under conditions wherein said heterodimeric antibody is expressed, and recovering said antibody.

[\[0016e\] In another aspect, there is provided a bispecific antibody comprising: a\) a first polypeptide having the amino acid sequence of SEQ ID NO:26362; b\) a second polypeptide having the amino acid sequence of SEQ ID NO:26367; and c\) a third polypeptide having the amino acid sequence of SEQ ID NO:26377.](#)

[0017] In an additional embodiment, the disclosure provides heterodimeric antibodies for use in the activation of T cells for the treatment of cancer.

[0017a]- In another ~~aspect~~[example](#), there is provided use of a heterodimeric antibody as disclosed herein in the manufacture of a medicament for activating cytotoxic T cells (CTLs) in a patient. In another ~~aspect~~[example](#), there is provided a heterodimeric antibody as disclosed herein in the manufacture of a medicament for increasing IFN γ levels in a patient. In another ~~aspect~~[example](#), there is provided use of a heterodimeric antibody as disclosed herein in the manufacture of a medicament for increasing IL-2 levels in a patient.

CLAIMS

1. A bispecific antibody that monovalently binds human ICOS ~~a human costimulatory receptor~~ and monovalently binds a human immune checkpoint receptor selected from the group consisting of PD-1, PD-L1, CTLA-4, LAG-3 and TIM-3, ~~for use in activating T cells for the treatment of treating cancer,~~ wherein the monovalent binding to ICOS results in agonism of ICOS and said monovalent binding to said checkpoint receptor results in inhibition of said receptor.

~~2. A bispecific antibody according to claim 1 wherein said costimulatory receptor is selected from the group consisting of ICOS, GITR, OX40 and 4-1BB~~

~~3. A bispecific antibody according to claim 1 or 2 wherein said checkpoint receptor is selected from the group consisting of PD-1, PD-L1, CTLA-4, LAG-3 and TIM-3.~~

~~4. A bispecific antibody according to claim 1, 2 or 3 wherein said costimulatory receptor is human ICOS.~~

~~52.~~ A bispecific antibody for use according to claim 41, wherein said antibody binds ICOS and PD-1.

~~63.~~ A bispecific antibody for use according to claim 41, wherein said antibody binds ICOS and PD-L1.

~~74.~~ A bispecific antibody for use according to claim 41, wherein said antibody binds ICOS and CTLA-4.

~~85.~~ A bispecific antibody for use according to claim 41, wherein said antibody binds ICOS and LAG-3.

~~96.~~ A bispecific antibody for use according to claim 41, wherein said antibody binds ICOS and TIM-3.

~~107.~~ A bispecific antibody for use according to any previous claim wherein said antibody ~~has the format of Figure 2A~~ comprises:

a) a first polypeptide comprising:

i) a first variant Fc domain; and

ii) a first antigen binding domain that is a single chain variable fragment (scFv), wherein said scFv consists of a first heavy chain variable domain, a first light chain variable domain, and an scFv linker, wherein said scFv linker covalently attaches said first heavy chain variable domain and first light chain variable domain;

b) a second polypeptide comprising a VH-CH1-hinge-CH2-CH3 polypeptide, wherein VH is a second heavy chain variable domain, and CH2-CH3 is a second variant Fc domain; and

c) a light chain comprising a second light chain variable domain, and a constant light chain,

wherein said second variable chain and said second light chain variable domain form a second antigen binding domain, and

wherein one of said first antigen binding domain and said second antigen binding domain binds human ICOS and the other of said first antigen binding domain and said second antigen binding domains binds to the human immune checkpoint receptor.

~~11.~~ A bispecific antibody according to any previous claim wherein said antibody ~~has the format of Figure 2E.~~

~~12. A bispecific antibody according to any previous claim wherein said antibody has the format of Figure 2F.~~

~~13. A bispecific antibody according to any previous claim wherein said antibody has the format of Figure 2G.~~

~~148. A~~ The bispecific antibody for use according to claim ~~52~~, wherein said antibody comprises:

- a) a first polypeptide having the amino acid sequence ~~having of~~ SEQ ID NO:26362;
- b) a second polypeptide having the amino acid sequence ~~having of~~ SEQ ID NO:26367; and
- c) a third polypeptide having the amino acid sequence ~~having of~~ SEQ ID NO:26377.

~~159. A heterodimeric-bispecific~~ antibody comprising:

a) a first polypeptide comprising an scFv-domain linker-CH2-CH3, wherein CH2-CH3 is a first Fc domain, wherein said scFv consists of a first heavy chain variable domain, a first light chain variable domain and a scFv linker, and wherein said scFv linker covalently attaches said first heavy chain variable domain and said first light chain variable domain ~~heavy chain comprising a first Fc domain, an optional domain linker and a first antigen binding domain comprising an scFv that binds a first antigen~~;

b) a second polypeptide comprising a VH-CH1-hinge-CH2-CH3, wherein VH is a second heavy chain variable domain, and CH2-CH3 is a second Fc domain ~~heavy chain comprising a heavy chain comprising a heavy chain constant domain comprising a second Fc domain, a hinge domain, a CH1 domain and a variable heavy domain~~; and

c) a light chain comprising a second variable light chain domain and a constant light chain constant domain;

wherein said first heavy chain variable chain and said first light chain variable region form a first antigen binding domain.

wherein said second heavy chain variable domain and said second light chain variable domain form a second antigen binding domain.

~~wherein said variable heavy domain and said variable light domain form a second antigen binding domain that binds a second antigen~~, wherein one of said first and second antigen binding domains monovalently binds human ICOS and the other of said first and second antigen binding domains monovalently binds human PD-1, wherein said monovalent binding to ICOS results in agonism of ICOS and said monovalent binding to said checkpoint receptor results in inhibition of said receptor.

~~1610. A heterodimeric-bispecific~~ antibody comprising:

a) a first ~~heavy chain~~ polypeptide comprising:

i) a first variant human IgG1 Fc domain; and

ii) a single chain Fv region (scFv) ~~that binds a first antigen~~, wherein said scFv region comprises a first ~~variable~~ heavy chain variable domain, a ~~variable-first~~ light chain variable domain and a charged scFv linker, wherein said charged scFv linker covalently attaches said first ~~variable~~ heavy chain variable domain and said ~~variable-first~~ light chain variable domain; and

b) a second ~~heavy chain~~ polypeptide comprising a VH-CH1-hinge-CH2-CH3 ~~monomer~~ polypeptide, wherein VH is a second ~~variable~~ heavy chain variable domain and CH2-CH3 is a second variant human IgG1 Fc domain; and

c) a light chain comprising a second light chain variable domain;

wherein said first heavy chain variable chain and said first light chain variable region form a first antigen binding domain,

wherein said second heavy chain variable domain and said second light chain variable domain form a second antigen binding domain,

wherein said second variant Fc domain comprises amino acid substitutions N208D/Q295E/N384D/Q418E/~~N241D~~N421D,

wherein said first and second variant Fc domains each comprise amino acid substitutions E233P/L234V/L235A/G236del/S267K; and

wherein said first variant human IgG1 Fc domain comprises amino acid substitutions S364K/E357Q and second variant human IgG1 Fc domain comprises amino acid substitutions L368D/K370S, wherein one of said first and second antigen binding domains monovalently binds human ICOS and the other of said first and second antigen binding domains monovalently binds human PD-1, wherein said monovalent binding to ICOS results in agonism of ICOS and said monovalent binding to said checkpoint receptor results in inhibition of said receptor, and wherein numbering is according to the EU index as in Kabat.

~~1711.~~ A ~~heterodimeric-bispecific~~ antibody according to any ~~previous one of~~ claims 9-11, wherein said first and second variant Fc domains each comprise M428L/N434S.

~~1812.~~ A ~~bispecific~~~~heterodimeric~~ antibody comprising:

a) a first polypeptide heavy chain comprising:

i) a first variant human IgG1 Fc domain; and

ii) a single chain Fv region (scFv) ~~that binds a first antigen~~, wherein said scFv region comprises a first ~~variable~~-heavy chain variable domain, a ~~variable-first~~ light chain variable domain, and a charged scFv linker, wherein said charged scFv linker covalently attaches said first ~~variable~~ heavy chain variable domain and said first variable-light chain variable domain; and

b) a second ~~heavy chain~~polypeptide comprising a VH-CH1-hinge-CH2-CH3 ~~monomer~~polypeptide, wherein VH is a second ~~variable~~-heavy chain variable domain and CH2-CH3 is a second variant human IgG1 Fc domain; and

c) a light chain comprising a second light chain variable domain;

wherein said first heavy chain variable chain and said first light chain variable region form a first antigen binding domain,

wherein said second heavy chain variable domain and said second light chain variable domain form a second antigen binding domain,

wherein said first and second variant human IgG1 Fc domains comprises a set of heterodimerization variants selected from the group consisting of L368D/K370S : S364K/E357Q; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; and T366S/L368A/Y407V : T366W, ~~and~~ wherein one of said first and second antigen binding domains monovalently binds human ICOS and the other of said first and second antigen binding domains monovalently binds human PD-1, wherein said monovalent binding to ICOS results in agonism of ICOS and said monovalent binding to said checkpoint receptor results in inhibition of said receptor, and wherein numbering is according to the EU index as in Kabat.

~~19~~13. A ~~heterodimeric-bispecific~~ antibody according to any one of claims ~~9-125, 15, 16, 17, or 18,~~ wherein said ~~the~~ antigen binding domain that binds ICOS ~~is formed from-comprises~~ a ~~variable~~ light chain ~~variable domain~~ having SEQ ID NO:26378 and a ~~variable~~ heavy chain ~~variable domain~~ having SEQ ID NO:16363.

14. A bispecific antibody comprising:

a) a first polypeptide having the amino acid sequence of SEQ ID NO:26362;

b) a second polypeptide having the amino acid sequence of SEQ ID NO:26367; and

c) a third polypeptide having the amino acid sequence of SEQ ID NO:26377.

~~20~~15. A nucleic acid composition comprising:

a) a first nucleic acid encoding a first ~~heavy chain~~polypeptide according to any of claims ~~1-to~~199 to 14;

b) a second nucleic acid encoding a second ~~heavy chain~~polypeptide according to any of claims ~~1-to~~199 to 14, respectively; and

c) a third nucleic acid encoding a light chain according to any of claims ~~1-to~~199 to 14, respectively.

~~21~~16. An expression vector composition comprising:

a) a first expression vector comprising said first nucleic acid of claim ~~20~~15;

b) a second expression vector comprising said second nucleic acid of claim ~~20~~15; and

c) a third expression vector comprising said third nucleic acid of claim ~~20~~15.

~~22~~17. A host cell comprising the expression vector composition of claim ~~21~~16.

~~23~~18. A method of making a ~~heterodimeric-bispecific~~ antibody comprising culturing a host cell of claim ~~22~~17- under conditions wherein said heterodimeric antibody is expressed, and recovering said antibody.

~~24. Use of a heterodimeric antibody according to any of claims 1 to 19 in the manufacture of a medicament for activating cytotoxic T cells (CTLs) in a patient.~~

~~25. Use of a heterodimeric antibody according to any of claims 1 to 19 in the manufacture of a medicament for increasing IFN γ levels in a patient.~~

~~26. Use of a heterodimeric antibody according to any of claims 1 to 19 in the manufacture of a medicament for increasing IL-2 levels in a patient.~~