

engage Fc receptors means that these receptors could potentially be excluded from the close contact, bi-directionally altering signaling potential.

In one aspect, the present disclosure refers to an antibody variant molecule comprising (A) a heavy chain polypeptide comprising a variable domain (V_H), three constant domains (C_{H1} , C_{H2} , and C_{H3}), and a hinge region located between C_{H1} and C_{H2} ; (B) a light chain polypeptide comprising a variable domain (V_L) and a constant domain (C_L); and (C) a spacer moiety, and, optionally, a second spacer moiety, wherein: (i) an Fc receptor binding site is located within C_{H2} or C_{H3} , (ii) V_H and V_L form an antigen-binding site, and (iii) a the spacer moiety is located within the hinge region or the spacer moiety is located in the C_{H1} and the second spacer moiety is located in the C_L between (i) and (ii), wherein the spacer moiety serves to increase the distance between (i) and (ii) and (iv) a parental antibody comprising the heavy chain polypeptide of (A) and light chain polypeptide of (B), but lacking the spacer moiety of (C), has agonistic activity and the spacer moiety reduces the agonistic activity of the antibody variant molecule as compared to the same parental antibody that lacks the spacer moiety as measured by a T-cell transfer assay or IL-2 secretion, wherein (v) the spacer moiety comprises a polypeptide sequence of at least 20 amino acids to 80 amino acids, (vi) and wherein at least 25% of the amino acids of the polypeptide sequence of the spacer moiety are serine and threonine residues; and (vii) the spacer moiety increases the length and/or overall dimensions of the molecule by at least 50Å.

In another aspect, the present disclosure refers to one or more nucleic acid molecules that encode an antibody variant molecule as disclosed herein.

In another aspect, the present disclosure refers to a nucleic acid molecule comprising a sequence encoding the heavy chain polypeptide of an antibody variant molecule as disclosed herein, wherein the spacer moiety is inserted within hinge region or C_{H1} of the heavy chain polypeptide.

In yet another aspect, the present disclosure refers to a nucleic acid molecule comprising a sequence encoding the light chain polypeptide of an antibody variant molecule as disclosed herein, wherein the second spacer moiety is inserted within the C_L of the light chain polypeptide.

In another aspect, the present disclosure refers to a method of producing an antibody variant molecule as disclosed herein, the method comprising expressing (a) the one or more nucleic acid molecules as disclosed herein; or (b) the nucleic acid molecule as disclosed herein and a nucleic acid molecule encoding the light chain polypeptide of the antibody variant molecule as disclosed herein; or (c) the nucleic acid molecule as disclosed herein in a host cell.

In yet another aspect, the present disclosure refers to a method for preparing an antibody variant molecule as disclosed herein, comprising (i) identifying the nucleic acid sequence or sequences encoding an antibody of interest; (ii) modifying said nucleic acid sequence(s) to encode a variant of the antibody of interest which variant comprises a polypeptide spacer moiety introduced so as to encode an antibody variant as disclosed herein; (iii) introducing said modified nucleic acid from step (ii) into a host cell *in vitro*; and (iv) expressing said antibody variant molecule.

In yet another aspect, the present disclosure refers to a method for reducing the agonistic activity of an antibody molecule, comprising introducing a spacer moiety into the antibody molecule ~~that increases the distance between the Fc receptor binding site and the antigen binding site of the molecule~~, wherein the spacer moiety comprises a polypeptide sequence of at least 20 to 80 amino acids and wherein at least 25% of the amino acids of the polypeptide sequence of the spacer moiety are serine and threonine residues and wherein the spacer moiety is introduced into a C_H1₁ hinge region, or C_L ~~between the Fc receptor binding site and the antigen binding site of the~~ antibody.

~~In yet another aspect~~ one example, the present disclosure refers to an antibody molecule produced by the method as disclosed herein.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising an antibody variant molecule as disclosed herein and at least one pharmaceutically acceptable excipient.

In yet another aspect, the present disclosure refers to an antibody variant molecule as disclosed herein, ~~an antibody molecule as disclosed herein~~ or a pharmaceutical composition as disclosed herein for use in therapy, such as for the treatment of cancer.

According to a first example of the disclosure there is provided an antibody variant/construct molecule comprising (i) an Fc receptor binding site, (ii) an antigen-binding site and (iii) a spacer moiety located between (i) and (ii), wherein the spacer moiety serves to increase the distance between (i) and (ii) so as to reduce the agonistic activity of the molecule as compared to the antibody variant that lacks (iii).

In a particular example, the introduction of the spacer moiety increases the overall dimensions of the molecule.

In a particular example, a spacer moiety is located in the constant region between the antigen-binding site and the Fc receptor binding site.

In a particular example, a spacer moiety is located in the hinge region of the antibody.

In particular examples, the spacer moiety is a rigid spacer moiety, such as a mucin or mucin-like polypeptide sequence, and inclusion of the spacer moiety increases the length and/or overall dimensions of the antibody.

In a particular example, the increased size of the antibody variant/construct that results from inclusion of the spacer moiety causes the Fc receptor binding site of the antibody variant to be sequestered away from the close contact when the antibody variant is bound to its antigen. In other words, when the antibody variant is bound to the antigen (e.g. receptor) it is sterically excluded from the contact between the cells which results in reduced signaling by the antibody-engaged receptor.

In a particular example, the increased size of the antibody variant/construct that results from inclusion of the spacer moiety positions the Fc receptor binding site of the antibody variant further from the membrane when the antibody variant is bound to its antigen.

According to a second example of the disclosure there is provided one or more nucleic acid molecules that encode an antibody variant/construct in accordance with any of the first to fourth examples of the disclosure. In a variant of this example there is provided a nucleic acid molecule

Claims:

1. An antibody variant molecule comprising:

(A) a heavy chain polypeptide comprising a variable domain (V_H), three constant domains (C_H1, C_H2, and C_H3), and a hinge region located between C_H1 and C_H2;

(B) a light chain polypeptide comprising a variable domain (V_L) and a constant domain (C_L);
and

(C) a spacer moiety, and, optionally, a second spacer moiety,

wherein:

(i) ~~(i)~~ an Fc receptor binding site is located within C_H2 or C_H3,

(ii) ~~(ii)~~ V_H and V_L form an antigen-binding site, ~~and (iii)~~

(iii) ~~the~~ a spacer moiety is located within the hinge region or the spacer moiety is
located in the C_H1 and the second spacer moiety is located in the C_L ~~between (i)~~
and (ii), wherein the spacer moiety serves to increase the distance between (i) and
(ii) and,

(iv) a parental antibody comprising the heavy chain polypeptide of (A) and light chain
polypeptide of (B), but lacking the spacer moiety of (C), has agonistic activity and
the spacer moiety reduces the agonistic activity of the antibody variant molecule
as compared to the ~~same parental~~ antibody that lacks the spacer moiety ~~as~~
measured by a T-cell transfer assay or IL-2 secretion, ~~wherein~~

(v) the spacer moiety comprises a polypeptide sequence of ~~at least 20~~ amino acids ~~to~~
80 amino acids,

(vi) ~~and wherein~~ at least 25% of the amino acids of the polypeptide sequence of the
spacer moiety are serine and threonine residues; ~~and~~

(vii) the spacer moiety increases the length and/or overall dimensions of the molecule
by at least 50Å.

2. The antibody variant molecule according to claim 1, wherein the spacer moiety
increases the length and/or overall dimensions of the molecule by at least ~~40Å~~60Å, 70Å, or
75Å.

3. The antibody variant molecule according to claim 1 or 2, wherein the spacer moiety is located in the constant domain between the antigen binding site and the Fc receptor binding site, optionally, wherein the spacer moiety is located in the hinge region of the antibody the C_H1 or hinge region.

4. The antibody variant molecule according to any one of claims 1, 2 or 3, wherein the increased size of the antibody variant molecule that results from inclusion of the spacer moiety positions the Fc receptor binding site of the antibody variant molecule further from the membrane when the antibody variant molecule is bound to its antigen.

5. The antibody variant molecule according to any of the preceding claims, wherein ~~the persistence length of the polypeptide~~ of the spacer moiety has a persistence length of at least 4nm.

6. The antibody variant molecule according to any one of the preceding claims, wherein the polypeptide sequence is or comprises a mucin or mucin-like polypeptide sequence, optionally wherein the polypeptide sequence comprises a sequence selected from the group consisting of SEQ ID NOs: 3-6.

7. The antibody variant molecule according to any of the preceding claims, wherein the presence of the spacer moiety reduces the agonistic activity of the antibody variant relative to the same antibody that lacks the spacer moiety by at least 25% as measured by a T-cell transfer assay or IL-2 secretion.

~~8. The antibody variant molecule according to any of the preceding claims, which is sterically excluded from the close contact when bound to its antigen.~~

~~9~~8. The antibody variant molecule according to any of the preceding claims, wherein the antibody variant is a human or humanized antibody.

~~10~~9. The antibody variant molecule according to any of the preceding claims, wherein the antigen binding region recognizes a receptor that has an immunoreceptor tyrosine-based activation motif or an immunoreceptor tyrosine-based inhibition motif.

~~11~~10. The antibody variant molecule according to any one of the preceding claims, which binds to an immune checkpoint pathway molecule, such as one selected from the group consisting of: PD-1, CTLA-4, TIGIT, BTLA, PD-1H, TLT2 and TIM-3.

~~12~~11. One or more nucleic acid molecules that encode an antibody variant molecule in accordance with any one of the preceding claims.

~~13~~12. A nucleic acid molecule comprising a sequence encoding the heavy chain polypeptide of an antibody variant molecule in accordance with any of claims 1-~~11~~10, wherein the spacer moiety is inserted within hinge region or C_{H1} of the heavy chain polypeptide.

13. A nucleic acid molecule comprising a sequence encoding the light chain polypeptide of an antibody variant molecule in accordance with any of claims 1 to 10, wherein the second spacer moiety is inserted within the C_L of the light chain polypeptide.

14. A method of producing an antibody variant molecule in accordance with any of claims 1 to ~~11~~10, the method comprising expressing (a) the one or more nucleic acid molecules in accordance with claim ~~12~~11; ~~or~~ (b) the nucleic acid molecule in accordance with claim 12 and a nucleic acid molecule encoding the light chain polypeptide of the antibody variant molecule in accordance with any of claims 1 to 10; or (c) the nucleic acid molecule in accordance with claim 13 and the nucleic acid molecule in accordance with claim 12 in a host cell.

15. A method for preparing an antibody variant molecule in accordance with any of claims 1 to ~~11~~10, comprising (i) identifying the nucleic acid sequence or sequences encoding an antibody of interest; (ii) modifying said nucleic acid sequence(s) to encode a variant of the antibody of interest which variant comprises a polypeptide spacer moiety introduced so as to encode an antibody variant according to any of claims 1 to ~~11~~10; (iii) introducing said modified nucleic acid from step (ii) into a host cell *in vitro*; and (iv) expressing said antibody variant molecule.

16. A method for reducing the agonistic activity of an antibody molecule, comprising introducing a spacer moiety into the antibody molecule ~~that increases the distance between the Fc receptor binding site and the antigen binding site of the molecule,~~ wherein the spacer moiety comprises a polypeptide sequence of ~~at least 20~~ to 80 amino acids and wherein at least 25% of the amino acids of the polypeptide sequence of the spacer moiety are serine and threonine residues and wherein the spacer moiety is introduced ~~between the Fc receptor binding site and the antigen binding site of~~ into a C_{H1}, hinge region, or C_L of the antibody.

~~17. An antibody molecule produced by the method of claim 16.~~

~~18~~17. A pharmaceutical composition comprising an antibody variant molecule according to any of claims 1 to ~~11~~10 ~~or 17~~ and at least one pharmaceutically acceptable excipient.

~~19~~18. An antibody variant molecule according to any of claims 1 to ~~11~~10, ~~an antibody molecule according to claim 17~~ or a pharmaceutical composition according to claim 18 for use in therapy, such as for the treatment of cancer.