

discovered that the agonistic activity of an antibody can be reduced by increasing the size of the antibody such that the large phosphatases are less or no longer excluded. Such antibody modifications can be used, *inter alia*, to reduce the agonistic activity of blocking antibodies.

In certain circumstances an agonist antibody can overly activate target receptor signaling, as occurred in the case of the anti-CD28 antibody TGN1412 (Attarwala, J Young Pharm. 2, 332-6, 2010). In these circumstances, the agonistic antibody is too effective at excluding phosphatases. This “superagonist” effect can be attenuated by increasing the overall dimensions of the antibody to avoid the exclusion of phosphatases and dampen the agonistic activity of the antibody.

Disclosed herein are extended antibodies that allow limited re-entry of phosphatases into the contact containing the receptor-bound antibody, such that the amount of signaling is reduced in a controlled manner. The present disclosure thus allows the design of an extended antibody with optimal therapeutic activity.

Fc receptor (FcR)-binding forms of the extended antibodies disclosed herein will actively sequester receptors away from the close contact formed by other molecules, typically eliminating completely agonistic effects, which is not possible using an approach that would only eliminate FcR binding. There may also be significant additional benefits from checkpoint blocking antibodies that bind to cell surfaces via FcRs (or other engineered ligands), in terms of specificity or half-life of blockade, or because they could be used, e.g. to exclude inhibitory FcRs from the close contact.

The disclosure is further based, *inter alia*, on the discovery that the ligand-independent agonistic activities of both activating (e.g. anti-CD28) and inhibitory (e.g. anti-PD-1) antibodies can be substantially reduced by making the antibodies larger through insertion of a spacer moiety between the antigen-binding site and the Fc receptor binding site (e.g. in the antigen-binding fragment (Fab) variable/constant region interface, Fab constant region or hinge region). In certain examples, the spacer moiety is a polypeptide that adopts a rigid conformation so as to increase the size of the antibody and spatially separate the antigen-binding and Fc receptor binding sites of the antibody. Such molecules can retain their antigen-binding and, if desired, FcR binding properties but by increasing the size/length of the antibody, these molecules typically have reduced agonistic (with antagonist antibodies) and superagonistic (with agonist antibodies) activity. Without wishing to be bound by theory, it is believed that the enhanced size of the antibody means that binding to the target receptor blocks ligand binding and results in the

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 (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) and reduces the agonistic activity of the molecule as compared to the same antibody that lacks the spacer moiety, wherein the spacer moiety comprises a polypeptide sequence of at least 20 amino acids and wherein at least 25% of the amino acids of the polypeptide sequence of the spacer moiety are serine and threonine residues.

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.

In another aspect, the present disclosure refers to a method of producing an antibody variant molecule as disclosed herein, the method comprising expressing the one or more nucleic acid molecules as disclosed herein or 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; 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 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 the antibody.

In yet another aspect, 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

comprising a sequence encoding an antibody variant in accordance with any of the first to fourth examples of the disclosure.

According to a third example of the disclosure there is provided a vector comprising the nucleic acid of the second example of the disclosure.

According to a fourth example of the disclosure there is provided a host cell comprising the nucleic acid sequence according to the second example of the disclosure or the vector according to third example of the disclosure.

According to a fifth example of the disclosure there is provided a method of producing an antibody variant/construct in accordance with the first example of the disclosure, the method comprising expressing the nucleic acid(s) in accordance with the second example of the disclosure in a host cell.

According to a sixth example of the disclosure there is provided a method for preparing an antibody variant/construct in accordance with the first example of the disclosure, comprising identifying the nucleic acid sequences encoding an antibody of interest and modifying said nucleic acid sequences 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 the first example of the disclosure.

According to a seventh example of the disclosure there is provided a method for reducing the agonistic activity of an antibody, comprising introducing a spacer moiety into the antibody that increases the distance between the Fc receptor binding site and the antigen-binding site of the molecule. In a particular example the spacer moiety is inserted in the antibody hinge region. In a particular example the spacer moiety is a rigid spacer moiety.

According to an eighth example of the disclosure there is provided a pharmaceutical composition comprising an antibody variant/construct according to the first example of the disclosure and at least one pharmaceutically acceptable excipient.

Antibody variants or pharmaceutical compositions of the disclosure may be used as medicaments.

Suitable examples of diseases for treatment with the medicaments are considered elsewhere in the specification.

According to a ninth example of the disclosure there is provided an antibody variant in accordance with the first example of the disclosure or the pharmaceutical composition in accordance with the eighth example of the disclosure for use in therapy. In a particular example, the therapy is treatment of cancer.

According to a tenth example of the disclosure there is provided a method of treating a patient in need thereof, comprising administering an antibody variant in accordance with the first example of the disclosure or the pharmaceutical composition in accordance with the eighth example of the disclosure. In a particular example, the method is for treating cancer. In another example, the method of treating cancer comprises administering to a patient in need thereof an antibody variant molecule that has been adapted to minimize/lack agonistic activity by extension of the molecule to maximize its exclusion from the close contact, or a pharmaceutical composition thereof. In a particular example, the extension is caused by inclusion of a rigid spacer moiety into the antibody.

DETAILED DESCRIPTION

As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a molecule” optionally includes a combination of two or more such molecules, and the like.

It is understood that wherever aspects are described herein with the language “comprising”, otherwise analogous aspects described in terms of “consisting of” and/or “consisting essentially of” are also provided.

It is to be understood that one, some, or all of the properties of the various embodiments described herein may be applied to any aspect unless the content clearly dictates otherwise. Furthermore, that the various embodiments may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For

Claims:

1. An antibody variant 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) and reduces the agonistic activity of the molecule as compared to the same antibody that lacks the spacer moiety, wherein the spacer moiety comprises a polypeptide sequence of at least 20 amino acids and wherein at least 25% of the amino acids of the polypeptide sequence of the spacer moiety are serine and threonine residues.
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.
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.
4. The antibody variant molecule according to any one of claims 1, 2 or 3, wherein the increased size of the antibody variant 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.
5. The antibody variant molecule according to any of the preceding claims, wherein the persistence length of the polypeptide is 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%.
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. The antibody variant molecule according to any of the preceding claims, wherein the antibody variant is a human or humanized antibody.
10. 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. 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. One or more nucleic acid molecules that encode an antibody variant molecule in accordance with any one of the preceding claims.
13. 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.
14. A method of producing an antibody variant molecule in accordance with any of claims 1 to 11, the method comprising expressing the one or more nucleic acid molecules in accordance with claim 12 or the nucleic acid molecule in accordance with claim 13 in a host cell.
15. A method for preparing an antibody variant molecule in accordance with any of claims 1 to 11, 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; (iii) introducing said modified nucleic acid from step (ii) into a host cell; 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 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 the antibody.

17. An antibody molecule produced by the method of claim 16.
18. A pharmaceutical composition comprising an antibody variant molecule according to any of claims 1 to 11 or 17 and at least one pharmaceutically acceptable excipient.
19. An antibody variant molecule according to any of claims 1 to 11, 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.