Claims

[Claim 1]
An antigen-binding molecule comprising:
an antibody variable region that is capable of binding to a first antigen and a second antigen different from the first antigen, but does not bind to the first antigen and the second antigen at the same time; and
an antibody variable region binding to a third antigen different from the first antigen and the second antigen,
wherein any one of the first antigen and the second antigen is a molecule specifically expressed on the surface of a T cell, and the other antigen is a molecule expressed on the surface of a T cell or any other immunocyte.

[Claim 2]
An antigen-binding molecule comprising an antibody variable region with an amino acid altered in a heavy chain variable domain which binds the first antigen or the second antigen such that the variable region is capable of binding to a first antigen and a second antigen different from the first antigen, but does not bind to the first antigen and the second antigen at the same time,
wherein any one of the first antigen and the second antigen is a molecule specifically expressed on the surface of a T cell, and the other antigen is a molecule expressed on the surface of a T cell or any other immunocyte.

[Claim 3]
The antigen-binding molecule according to claim 1 or 2, wherein the antibody variable region that does not bind to the first antigen and the second antigen at the same time is an antibody variable region that does not bind to the first antigen and the second antigen each expressed on a different cell, at the same time.

[Claim 4]
The antigen-binding molecule according to any of claims 1 to 3, further comprising an antibody Fc region.

[Claim 5]
The antigen-binding molecule according to claim 4, wherein the Fc region is an Fc region having reduced binding activity against FcγR as compared with the Fc region of a naturally occurring human IgG1 antibody.

[Claim 6]
The antigen-binding molecule according to any of claims 1 to 5, wherein the antigen-binding molecule is a multispecific antibody.

[Claim 7]
The antigen-binding molecule according to claim 1, wherein the antibody variable region capable of binding to the first antigen and the second antigen is an antibody variable region having alteration of at least one amino acid in an antibody variable region which binds to the first antigen or the second antigen.

[Claim 8]

The antigen-binding molecule according to claim 2, wherein the antibody variable region capable of binding to the first antigen and the second antigen is an antibody variable region having alteration of at least one amino acid in an antibody variable region which binds to the first antigen or the second antigen.

[Claim 9]
The antigen-binding molecule according to claim 7 or 8, wherein the alteration is substitution or insertion of at least one amino acid.

[Claim 10]
The antigen-binding molecule according to any one of claims 7 to 9, wherein the alteration is substitution of a portion of the amino acid sequence of a variable region binding to the first antigen by an amino acid sequence binding to the second antigen, or insertion of an amino acid sequence binding to the second antigen to the amino acid sequence of a variable region binding to the first antigen.

[Claim 11]
The antigen-binding molecule according to claim 9 or 10, wherein the number of amino acids to be inserted is 1 to 25.

[Claim 12]
The antigen-binding molecule according to any of claims 7 to 11, wherein the amino acid to be altered is an amino acid in a CDR1, CDR2, CDR3, or FR3 region of the antibody variable region.

[Claim 13]
The antigen-binding molecule according to any of claims 7 to 12, wherein the amino acid to be altered is an amino acid in a loop.

[Claim 14]
The antigen-binding molecule according to claim 7, wherein the amino acid to be altered is at least one amino acid selected from Kabat numbering positions 31 to 35, 50 to 65, 71 to 74, and 95 to 102 in an antibody H chain variable domain, and Kabat numbering positions 24 to 34, 50 to 56, and 89 to 97 in an L chain variable domain.

[Claim 15]
The antigen-binding molecule according to claim 2, wherein the amino acid to be altered is at least one amino acid selected from Kabat numbering positions 31 to 35, 50 to 65, 71 to 74, and 95 to 102 in an antibody H chain variable domain.

[Claim 16]
The antigen-binding molecule according to claim 1 or 2, wherein any one of the first antigen and the second antigen is CD3, and the other antigen is FcγR, TLR, lectin, IgA, an immune checkpoint molecule, a TNF superfamily molecule, a TNFR superfamily molecule, or an NK receptor molecule.

[Claim 17]
The antigen-binding molecule according to claim 1, wherein the third antigen is a molecule specifically expressed in a cancer tissue.

[Claim 18]
A pharmaceutical composition comprising an antigen-binding molecule according to any of claims 1 to 17 and a pharmaceutically acceptable carrier.

[Claim 19]
A method for producing an antigen-binding molecule according to claim 1 or 7, the method comprising steps (i) to (iv):
(i) preparing a library of antigen-binding molecules with at least one amino acid altered in their antibody variable regions each binding to the first antigen or the second antigen, wherein the altered variable regions differ in at least one amino acid from each other;
(ii) selecting, from the prepared library, an antigen-binding molecule comprising an antibody variable region that has binding activity against the first antigen and the second antigen, but does not bind to the first antigen and the second antigen at the same time;
(iii) culturing a host cell comprising a nucleic acid encoding the antibody variable region of the antigen-binding molecule selected in the step (ii), and a nucleic acid encoding an antibody variable region of an antigen-binding molecule binding to the third antigen, to express an antigen-binding molecule comprising the antibody variable region that is capable of binding to the first antigen and the second antigen, but does not bind to the first antigen and the second antigen at the same time, and the antibody variable region binding to the third antigen; and
(iv) recovering the antigen-binding molecule from the host cell cultures,
wherein any one of the first antigen and the second antigen is a molecule specifically expressed on the surface of a T cell, and the other antigen is a molecule expressed on the surface of a T cell or any other immunocyte.

[Claim 20]
A method for producing an antigen-binding molecule according to claim 2 or 8, the method comprising steps (i) to (iv):
(i) preparing a library of antigen-binding molecules with at least one amino acid altered in their antibody variable regions each binding to the first antigen or the second antigen, wherein the altered variable regions differ in at least one amino acid from each other;
(ii) selecting, from the prepared library, an antigen-binding molecule comprising an antibody variable region that has binding activity against the first antigen and the second antigen, but does not bind to the first antigen and the second antigen at the same time;
(iii) culturing a host cell comprising a nucleic acid encoding the antibody variable region of the antigen-binding molecule selected in the step (ii) to express an antigen-binding molecule comprising the antibody variable region that is capable of binding to the first antigen and the second antigen, but does not bind to the first antigen and the second antigen at the same time; and
(iv) recovering the antigen-binding molecule from the host cell cultures,
wherein any one of the first antigen and the second antigen is a molecule specifically expressed on the surface of a T cell, and the other antigen is a molecule expressed on the surface of a T cell or any other immunocyte.

[Claim 21]
The method according to claim 19 or 20, wherein the antibody variable region that does not bind to the first antigen and the second antigen at the same time, contained in the antigen-binding molecule selected in the step (ii) is an antibody variable region that does not bind to the first antigen and the second antigen each expressed on a different cell, at the same time.

[Claim 22]
The method according to any one of claims 19 to 21, wherein an antigen-binding molecule produced by the method of any one of claims 19 to 21 further comprises an antibody Fc region.

[Claim 23]
The method according to claim 22, wherein the Fc region is an Fc region having reduced binding activity against FcγR as compared with the Fc region of a naturally occurring human IgG1 antibody.

[Claim 24]
The method according to any of claims 19 to 23, wherein the antigen-binding molecule to be produced is a multispecific antibody.

[Claim 25]
The method according to any of claims 19 to 24, wherein the at least one amino acid altered in the variable regions in the step (i) is a substituted or inserted amino acid.

[Claim 26]
The method according to claim 25, wherein the number of inserted amino acids is 1 to 25.

[Claim 27]
The method according to any of claims 19 to 26, wherein the alteration is alteration of an amino acid in a CDR1, CDR2, CDR3, or FR3 region of the antibody variable region.

[Claim 28]
The method according to any of claims 19 to 27, wherein the alteration is alteration of an amino acid in a loop.

[Claim 29]
The method according to claim 19, wherein the alteration is alteration of at least one amino acid selected from Kabat numbering positions 31 to 35, 50 to 65, 71 to 74, and 95 to 102 in an antibody H chain variable domain, and Kabat numbering positions 24 to 34, 50 to 56, and 89 to 97 in an L chain variable domain.

[Claim 30]
The method according to claim 20, wherein the alteration is alteration of at least one amino acid selected from Kabat numbering positions 31 to 35, 50 to 65, 71 to 74, and 95 to 102 in an antibody H chain variable domain.

[Claim 31]
The method according to claim 19 or 20, wherein any one of the first antigen and the second antigen is CD3, and the other antigen is FcγR, TLR, IgA, lectin, an immune checkpoint molecule, a TNF superfamily molecule, a TNFR superfamily molecule, or an NK receptor molecule.

[Claim 32]

The method according to claim 19, wherein the third antigen is a molecule specifically expressed in a cancer tissue.