

[Technical Problem]

[0008]

The present disclosure was achieved in view of the above circumstances. An objective of the present disclosure is to provide antibody variants or isoforms with reduced FVIII mimetic activity.

[Solution to Problem]

[0009]

The present inventors performed dedicated research to solve the above-described problems and succeeded in identifying antibody variants and isoforms which are contained in a pharmaceutical composition comprising Emicizumab as an active ingredient. The present inventors also found that FVIII mimetic activity of these antibody variants and isoforms is quite low, as compared with that of Emicizumab.

[0010]

The present disclosure was made based on these findings.

In one aspect, the present disclosure refers to variant of ~~an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2) and that exhibits FVIII mimetic activity~~Emicizumab, wherein (a) the amino acid residue R at the 12th position from the N terminus side of the sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2); or (b) the amino acid residues YYR at the 10th to 12th position from the N terminus side of the sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2), is deleted and the variable region is cleaved at the deletion site.

[0010a]

In another aspect, the present disclosure refers to a method for detecting the ~~antibody~~-variant of ~~any one of claims 1 to 6~~, comprising the step of ~~separating~~subjecting a sample containing ~~an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2)~~Emicizumab and the ~~antibody~~-variant according to ~~any one of claims 1 to 6 by~~to affinity chromatography, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), separation based on charge, size exclusion chromatography (SEC), gel permeation chromatography (GPC), or combinations thereof, and detecting the ~~antibody~~-variant.

[0010b]

In yet another aspect, the present disclosure refers to a ~~pharmaceutical method of producing a~~ composition comprising Emicizumab and the ~~antibody~~-variant of ~~any one of claims 1 to 6~~, ~~wherein the percentage of the antibody variant in the total antibody molecules in the pharmaceutical composition is 5% or less and wherein production of the antibody variant is suppressed by a~~the method comprising the step of culturing cells producing ~~the antibody~~Emicizumab at a pH of ~~7.1 or more~~6.70-7.10, and/or at a culture temperature of ~~36-38°C or less~~, obtaining a culture supernatant, and measuring the ratio of the variant to obtain the composition in which the variant in the total antibody molecules in the composition is 5% or less.

[0010c]

In still another aspect, the present disclosure refers to a method for suppressing production of the ~~antibody~~ variant of ~~any one of claims 1 to 6~~, comprising the step of culturing cells producing the ~~antibody~~ Emicizumab at a pH of ~~7.1 or more~~ 6.70-7.10, and/or at a culture temperature of ~~36-38°C or less~~.
[0010d]

- 5 The present disclosure provides [1] to [21] below.
- [1] A variant of an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2), wherein
- (a) the amino acid residue R at the 12th position from the N terminus side of the sequence (the 61th position from the N terminus side of the Emicizumab Q chain: position 60 according to Kabat numbering)
- 10 or
- (b) the amino acid residues YYR at the 10th to 12th positions from the N terminus side of the sequence (the 59th to 61th positions from the N terminus side of the Emicizumab Q chain: positions 58 to 60 according to Kabat numbering)
- is deleted and the variable region is cleaved at the deletion site.
- 15 [2] The antibody variant of [1], wherein the sequence is a CDR sequence.
- [3] The antibody variant of [1], wherein the sequence is a CDR2 sequence.
- [4] The antibody variant of [1], wherein the sequence is a sequence comprised in a heavy chain.
- [5] The antibody variant of [1], which is a variant of a bispecific antibody.
- [6] The antibody variant of [1], which is a variant of Emicizumab.
- 20 [7] A method for detecting the antibody variant of any one of [1] to [6], comprising the step of separating a sample containing an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2) by affinity chromatography, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), separation based on charge, size exclusion
- 25 chromatography (SEC), gel permeation chromatography (GPC), or combinations thereof.
- [8] The method for detecting of [7], which uses the antibody variant of any one of [1] to [6] as a reference standard.
- [8-2] The method for detecting of [8], comprising the step of conducting one or more analysis selected from the group consisting of quantitative analysis, qualitative analysis, and structural analysis.
- 30 [9] A pharmaceutical composition comprising the antibody variant of any one of [1] to [6], wherein the percentage of the antibody variant in the total antibody molecules in the pharmaceutical composition is 5% or less.
- [10] The pharmaceutical composition of [9], wherein the antibody is Emicizumab.
- [11] The pharmaceutical composition of [9], which is obtained by a purification process comprising
- 35 purification by cation exchange chromatography (CEX).

[CLAIMS]

1. A variant of ~~an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2) and that exhibits FVIII-mimetic activity~~Emicizumab, wherein
 - (a) the amino acid residue R at the 12th position from the N terminus side of the sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2); or
 - (b) the amino acid residues YYR at the 10th to 12th position from the N terminus side of the sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2),
is deleted and the variable region is cleaved at the deletion site.
- ~~2. The antibody variant of claim 1, wherein the sequence is a CDR sequence.~~
- ~~3. The antibody variant of claim 1, wherein the sequence is a CDR2 sequence.~~
- ~~4. The antibody variant of claim 1, wherein the sequence is a sequence comprised in a heavy chain.~~
- ~~5. The antibody variant of claim 1, which is a variant of a bispecific antibody.~~
- ~~6. The antibody variant of claim 1, which is a variant of Emicizumab.~~
- ~~7.~~ 2. A method for detecting the ~~antibody~~ variant of ~~any one of claims 1 to 6~~, comprising the step of ~~separating~~subjecting a sample containing ~~an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2)~~Emicizumab and the ~~antibody~~ variant according to ~~any one of claims 1 to 6 by~~to affinity chromatography, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), separation based on charge, size exclusion chromatography (SEC), gel permeation chromatography (GPC), or combinations thereof, and detecting the ~~antibody~~ variant.
- ~~3. The method of claim 2, wherein the sample is subjected to ion exchange chromatography or reverse phase chromatography.~~
- ~~8.~~ 4. The method for detecting of claim ~~7~~ 2 or 3, which uses the ~~antibody~~ variant of ~~any one of claims 1 to 6~~ as a reference standard.

95. A ~~pharmaceutical method of producing a~~ composition comprising Emicizumab and the antibody-variant of ~~any one of claims 1 to 6~~, wherein the percentage of the antibody variant in the total antibody molecules in the pharmaceutical composition is 5% or less and wherein
 5 ~~production of the antibody variant is suppressed by a~~ the method comprising the step of culturing cells producing ~~the antibody~~Emicizumab at a pH of ~~7.1 or more~~6.70-7.10, and/or at a culture temperature of ~~36-38°C or less~~, obtaining a culture supernatant, and measuring the ratio of the variant to obtain the composition in which the variant in the total antibody molecules in the composition is 5% or less.

10 ~~10. The pharmaceutical composition of claim 9, wherein the antibody is Emicizumab.~~

116. The ~~pharmaceutical composition method~~ of claim ~~95~~, ~~which is obtained by further~~ comprising a purification process comprising purification by cation exchange chromatography (CEX).
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127. A method for suppressing production of the antibody-variant of ~~any one of claims 1 to 6~~, comprising the step of culturing cells producing ~~the antibody~~Emicizumab at a pH of ~~7.1 or more~~6.70-7.10, and/or at a culture temperature of ~~36-38°C or less~~.
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