

[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 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 variant of claim 1, comprising the step of subjecting a sample containing Emicizumab and the variant according to claim 1 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 variant.

[0010b]

In yet another aspect, the present disclosure refers to a method of producing a composition comprising Emicizumab and the variant of claim 1, the method comprising the step of culturing cells producing Emicizumab at a pH of 6.70-7.10, and/or at a culture temperature of 36-38°C, 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 variant of claim 1, comprising the step of culturing cells producing Emicizumab at a pH of 6.70-7.10, and/or at a culture temperature of 36-38°C.

[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 Emicizumab, wherein
 - (a) the amino acid residue R at the 12th position from the N terminus side of the
5 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.
- 10 2. A method for detecting the variant of claim 1, comprising the step of subjecting a
sample containing Emicizumab and the variant according to claim 1 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
15 chromatography (GPC), or combinations thereof, and detecting the variant.
3. The method of claim 2, wherein the sample is subjected to ion exchange
chromatography or reverse phase chromatography.
- 20 4. The method for detecting of claim 2 or 3, which uses the variant of claim 1 as a
reference standard.
5. A method of producing a composition comprising Emicizumab and the variant of claim
1, the method comprising the step of culturing cells producing Emicizumab at a pH of 6.70-7.10,

and/or at a culture temperature of 36-38°C, 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.

5 6. The method of claim 5, further comprising a purification process comprising purification by cation exchange chromatography (CEX).

7. A method for suppressing production of the variant of claim 1, comprising the step of culturing cells producing Emicizumab at a pH of 6.70-7.10, and/or at a culture temperature of
10 36-38°C.