

comprehensive structural and functional assessment of the molecule is typically required to identify such attributes and understand their impact on the properties of the molecule. An understanding of a molecule's attributes is important to ensure production of a consistent product.

[0006] Variants of the biologic can arise during the manufacturing process or under storage conditions. Such product variants can be categorized as product-related substances or product-related impurities. Product-related substances are those molecular variants of the desired product formed during manufacture or storage of the product which have properties comparable to those of the desired product, and are not considered impurities. Conversely, product-related impurities are molecular variants of the desired product, which do not have properties comparable to those of the desired product with respect to product efficacy and patient safety. Thus, identification and characterization of product-related impurities in particular is useful for the manufacture and quality control of the biologic drug product.

SUMMARY

[0007] The present disclosure is based, in part, on the identification and characterization of product-related impurities of erenumab, particularly variants of erenumab that have reduced functional activity. Control and monitoring of the amounts of these variants in therapeutic erenumab compositions ensures that the erenumab compositions have the requisite potency to produce the desired clinical effects. In one aspect, the present disclosure refers to a composition comprising erenumab and one or more erenumab variants, wherein the one or more erenumab variants comprises an isomerization variant and a deamidation variant, and wherein the amount of the isomerization variant and deamidation variant in the composition is less than about 30%. Accordingly, in some examples, the present disclosure provides an erenumab composition comprising erenumab and one or more erenumab variants, wherein the erenumab variants comprise isomerization variants, deamidation variants, acidic variants, disulfide isoform variants, high molecular weight (HMW) species, or combinations thereof, wherein the amounts of the erenumab variants in the composition are controlled to within specific ranges.

[0008] In certain examples, the disclosure provides compositions comprising erenumab and one or more erenumab variants, wherein the one or more erenumab variants comprises an isomerization variant and a deamidation variant. In some examples, the isomerization variant

has an isoaspartic acid residue or succinimide at amino acid position 105 in either or both heavy chains (SEQ ID NO: 1 or SEQ ID NO: 3) of erenumab. In these and other examples, the deamidation variant has the asparagine residue at amino acid position 102 in either or both heavy chains (SEQ ID NO: 1 or SEQ ID NO: 3) of erenumab converted to an aspartic acid residue, a succinimide, or an isoaspartic acid residue. The amount of the isomerization variant and deamidation variant in the composition can be less than about 30%, less than about 15%, less than about 8%, or less than about 4%, for example, as determined by hydrophobic interaction chromatography high performance liquid chromatography (HIC-HPLC).

[0009] The disclosure also provides compositions of erenumab and one or more acidic variants of erenumab. In another aspect, the present disclosure refers to a composition comprising erenumab and one or more erenumab acidic variants, wherein the amount of acidic variants in the composition is less than about 40%. The amount of acidic variants in the composition can be less than about 40%, for example, from about 25% to about 38% or from about 26% to about 34%. In some examples, the amount of acidic variants in the erenumab compositions is determined by cation exchange high performance liquid chromatography (CEX-HPLC). Acidic variants of erenumab include disulfide isoform variants, deamidation variants, fragmentation variants, non-consensus glycosylation variants, HMW species, and combinations thereof. In certain examples, the acidic variants comprise disulfide isoform variants, fragmentation variants, or combinations thereof.

[0010] In another aspect, the present disclosure refers to a composition comprising erenumab and one or more disulfide isoform variants thereof, wherein the one or more disulfide isoform variants comprise an IgG2-B isoform, and wherein the amount of the IgG2-B isoform in the composition is less than about 20%. In some examples, the present disclosure provides compositions of erenumab and disulfide isoform variants of erenumab. The disulfide isoform variants of erenumab can comprise an IgG2-B isoform and/or an IgG2-A/B isoform. The amount of the IgG2-B isoform in the composition can be less than about 20%, for example, less than about 10%, or less than about 8%, such as from about 4% to about 6%. The amount of the IgG2-A/B isoform in the composition can be from about 20% to about 40%, from about 26% to about 38%, or from about 34% to about 37%. In certain examples, the amount of the disulfide isoforms is determined by non-reduced reversed phase high performance liquid chromatography (RP-HPLC).

[0011] The disclosure also includes compositions comprising erenumab and one or more size variants of erenumab, such as low molecular weight (LMW) species, middle molecular weight (MMW) species, and high molecular weight (HMW) species. In another aspect, the present disclosure refers to a composition comprising erenumab and a high molecular weight (HMW) species of erenumab, wherein the amount of the HMW species in the composition is less than about 2.5%. In yet another aspect, the present disclosure refers to A composition comprising erenumab and one or more erenumab variants, wherein the erenumab variants comprise isomerization variants, deamidation variants, acidic variants, HMW species, or combinations thereof, wherein the composition has one or more of the following characteristics: (a) the amount of acidic variants in the composition is from about 25% to about 38% as measured by CEX-HPLC; (b) the amount of HMW species in the composition is about 2.1% or less as measured by SE-UHPLC; and (c) the amount of isomerization variants and deamidation variants in the composition is about 8% or less as measured by the pre-peaks in HIC-HPLC. In certain examples, the amount of HMW species in the composition is less than about 3.0%, for example, about 2.5% or less, about 2.1% or less, about 1.8% or less, about 1.4% or less, or about 1.2% or less. In one particular example, the HMW species of erenumab is comprised predominantly of a covalently-linked dimer of erenumab. The amount of HMW species in the erenumab compositions can be determined by size exclusion ultra-high performance liquid chromatography (SE-UHPLC).

[0012] In another aspect, the present disclosure refers to a pharmaceutical formulation comprising the composition as disclosed herein and one or more pharmaceutically acceptable excipients. In some examples, the present disclosure also provides methods of evaluating or assessing the quality of erenumab compositions. In one example, the methods comprise obtaining an erenumab composition that contains erenumab and one or more erenumab variants; measuring the amount of one or more erenumab variants in the composition; comparing the measured amount of the one or more erenumab variants to a pre-determined reference criterion; and preparing a pharmaceutical formulation or pharmaceutical product of the erenumab composition if the comparison indicates that the pre-determined reference criterion is met. The methods may comprise one, two, or three of the following: (1) measuring the amount of isomerization variants and deamidation variants in the composition (e.g. by peak area percentage of the pre-peaks in HIC-HPLC), (2) measuring the amount of acidic variants in the composition (e.g. by peak area percentage of the acidic peaks in CEX-HPLC), and/or (3) measuring the amount of HMW species in the composition (e.g. by peak area percentage of the pre-peaks in SE-UHPLC). In certain examples, all three measurements are performed on an erenumab composition. In yet another aspect, the present disclosure refers to a method for

assessing the quality of an erenumab composition, comprising: obtaining an erenumab composition that contains erenumab and one or more erenumab variants; measuring the amount of one or more erenumab variants in the composition, wherein the erenumab variants comprise isomerization variants, deamidation variants, acidic variants, HMW species, or combinations thereof; comparing the measured amount of the one or more erenumab variants to a pre-determined reference criterion; and preparing a pharmaceutical formulation or pharmaceutical product of the erenumab composition if the comparison indicates that the pre-determined reference criterion is met.

[0013] Pharmaceutical formulations comprising the erenumab compositions described herein are also included in the disclosure. In some examples, the pharmaceutical formulations comprise an erenumab composition described herein and one or more pharmaceutically acceptable excipients, such as buffers, sugars, and surfactants. The pharmaceutical formulations can be incorporated into an injection device, such as a pre-filled syringe or autoinjector. In such examples, the injection volume for the pre-filled syringes or autoinjectors is less than about 2 mL, for example, about 1 mL.

[0014] In another aspect, the present disclosure refers to an erenumab composition of any one of claims 1 to 30 for use in therapy. In yet another aspect, the present disclosure refers to a use of an erenumab composition as disclosed herein in the preparation of a medicament for treating, preventing, or reducing the occurrence of headache in a patient in need thereof. The present disclosure also includes methods of treating, preventing, or reducing the occurrence of headache in a patient in need thereof using the erenumab compositions and pharmaceutical formulations of the disclosure. In one example, the methods comprise administering to the patient a pharmaceutical formulation comprising an erenumab composition described herein. The headache can be a migraine headache, cluster headache, or other type of headache disorder, such as tension-type headache, hemiplegic migraine, menstrual migraine, and retinal migraine. In certain examples, the patient to be treated according to the methods or uses of the disclosure has or is diagnosed with migraine, such as episodic migraine or chronic migraine.

[0015] The use of the erenumab compositions in any of the methods disclosed herein or for preparation of medicaments for administration according to any of the methods disclosed herein is specifically contemplated. For instance, the present disclosure includes an erenumab composition or pharmaceutical formulation described herein for use in a method for treating, preventing, or reducing the occurrence of headache in a patient in need thereof. The present disclosure also encompasses the use of an erenumab composition or pharmaceutical formulation described herein in the preparation of a medicament for treating, preventing, or reducing the occurrence of headache in a patient in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Figures 1A and 1B show the amino acid sequences of the heavy chain (Figure 1A; SEQ ID NO: 1) and light chain (Figure 1B; SEQ ID NO: 2) for erenumab. Complementarity determining regions (CDRs) in each chain are bolded and underlined. The heavy chain contains an N-linked glycosylation site at the asparagine residue at position 306.

[0017] Figures 1C and 1D show the amino acid sequences of the heavy chain (Figure 1C; SEQ ID NO: 3) and light chain (Figure 1D; SEQ ID NO: 4) with common post-translational modifications at the N- and C-termini for erenumab. CDRs in each chain are bolded and underlined. pE represents pyroglutamate.

[0018] Figures 1E and 1F show the amino acid sequences of the heavy chain variable region (Figure 1E; SEQ ID NO: 5) and light chain variable region (Figure 1F; SEQ ID NO: 6) for erenumab. CDRs in each variable region are bolded and underlined.

[0019] Figures 2A and 2B depict a representative HIC-HPLC profile of erenumab drug substance at full scale (Figure 2A) and expanded scale (Figure 2B). Erenumab drug substance was analyzed by HIC-HPLC using a sodium acetate pH 5.5 mobile phase with elution by a linear decreasing gradient of ammonium sulfate and detection at 280 nm absorbance.

[0020] **Figure 3** shows overlays of HIC-HPLC collected fractions and erenumab drug substance analyzed by HIC-HPLC. Erenumab drug substance and fractions collected from semi preparative HIC-HPLC (F1-F7) were analyzed by HIC-HPLC using a sodium acetate pH 5.5 mobile phase with elution by a linear decreasing gradient of ammonium sulfate and detection at 280 nm absorbance. Each panel is a plot of absorbance at 280 nm versus elution time in minutes. An overlay of all seven HIC-HPLC fractions with erenumab drug substance is shown in panel 8.

CLAIMS

What is claimed:

1. A composition comprising erenumab and one or more erenumab variants, wherein the one or more erenumab variants comprises an isomerization variant and a deamidation variant, and wherein the amount of the isomerization variant and deamidation variant in the composition is less than about 30%.
2. The composition of claim 1, wherein the amount of the isomerization variant and deamidation variant in the composition is less than about 15%.
3. The composition of claim 1, wherein the amount of the isomerization variant and deamidation variant in the composition is less than about 8%.
4. The composition of claim 1, wherein the amount of the isomerization variant and deamidation variant in the composition is less than about 4%.
5. The composition of claim 1, wherein the amount of the isomerization variant and deamidation variant in the composition is from about 1% to about 10%.
6. The composition of claim 1, wherein the amount of the isomerization variant and deamidation variant in the composition is from about 1% to about 4%.
7. The composition of any one of claims 1 to 6, wherein the isomerization variant has an isoaspartic acid residue or a succinimide at amino acid position 105 in either or both heavy chains (SEQ ID NO: 1 or SEQ ID NO: 3) of erenumab.
8. The composition of any one of claims 1 to 7, wherein the deamidation variant has the asparagine residue at amino acid position 102 in either or both heavy chains (SEQ ID NO: 1 or SEQ ID NO: 3) of erenumab converted to an aspartic acid residue, a succinimide, or an isoaspartic acid residue.

9. The composition of any one of claims 1 to 8, wherein the amount of the isomerization variant and deamidation variant in the composition is determined by hydrophobic interaction chromatography high performance liquid chromatography (HIC-HPLC).
10. A composition comprising erenumab and one or more erenumab acidic variants, wherein the amount of acidic variants in the composition is less than about 40%.
11. The composition of claim 10, wherein the amount of acidic variants in the composition is less than about 35%.
12. The composition of claim 10, wherein the amount of acidic variants in the composition is from about 25% to about 38%.
13. The composition of claim 10, wherein the amount of acidic variants in the composition is from about 26% to about 34%.
14. The composition of any one of claims 10 to 13, wherein one or more acidic variants comprise disulfide isoform variants, fragmentation variants, or combinations thereof.
15. The composition of any one of claims 10 to 14, wherein the amount of acidic variants in the composition is determined by cation exchange high performance liquid chromatography (CEX-HPLC).
16. A composition comprising erenumab and one or more disulfide isoform variants thereof, wherein the one or more disulfide isoform variants comprise an IgG2-B isoform, and wherein the amount of the IgG2-B isoform in the composition is less than about 20%.
17. The composition of claim 16, wherein the amount of the IgG2-B isoform in the composition is less than about 10%.

18. The composition of claim 16, wherein the amount of the IgG2-B isoform in the composition is from about 4% to about 6%.
19. The composition of any one of claims 16 to 18, wherein the one or more disulfide isoform variants further comprise an IgG2-A/B isoform.
20. The composition of claim 19, wherein the amount of the IgG2-A/B isoform in the composition is from about 34% to about 37%.
21. The composition of any one of claims 16 to 20, wherein the amount of disulfide isoform variants in the composition is determined by non-reduced reversed phase high performance liquid chromatography (RP-HPLC).
22. A composition comprising erenumab and a high molecular weight (HMW) species of erenumab, wherein the amount of the HMW species in the composition is less than about 2.5%.
23. The composition of claim 22, wherein the amount of the HMW species in the composition is about 1.8% or less.
24. The composition of claim 22, wherein the amount of the HMW species in the composition is about 1.2% or less.
25. The composition of any one of claims 22 to 24, wherein the HMW species comprises a covalently-linked dimer of erenumab.
26. The composition of any one of claims 22 to 25, wherein the amount of the HMW species in the composition is determined by size exclusion ultra-high performance liquid chromatography (SE-UHPLC).
27. A composition comprising erenumab and one or more erenumab variants, wherein the erenumab variants comprise isomerization variants, deamidation variants, acidic variants, HMW

species, or combinations thereof, wherein the composition has one or more of the following characteristics:

(a) the amount of acidic variants in the composition is from about 25% to about 38% as measured by CEX-HPLC;

(b) the amount of HMW species in the composition is about 2.1% or less as measured by SE-UHPLC; and

(c) the amount of isomerization variants and deamidation variants in the composition is about 8% or less as measured by the pre-peaks in HIC-HPLC.

28. The composition of claim 27, wherein the composition has one or more of the following characteristics:

(a) the amount of acidic variants in the composition is from about 26.5% to about 33.6% as measured by CEX-HPLC;

(b) the amount of HMW species in the composition is about 1.2% or less as measured by SE-UHPLC; and

(c) the amount of isomerization variants and deamidation variants in the composition is about 3.2% or less as measured by the pre-peaks in HIC-HPLC.

29. The composition of any one of claims 1 to 28, wherein erenumab comprises a heavy chain of SEQ ID NO: 1 and a light chain of SEQ ID NO: 2.

30. The composition of any one of claims 1 to 28, wherein erenumab comprises a heavy chain of SEQ ID NO: 3 and a light chain of SEQ ID NO: 4.

31. A pharmaceutical formulation comprising the composition of any one of claims 1 to 30 and one or more pharmaceutically acceptable excipients.

32. An erenumab composition of any one of claims 1 to 30 for use in therapy.

33. Use of an erenumab composition of any one of claims 1 to 30 in the preparation of a medicament for treating, preventing, or reducing the occurrence of headache in a patient in need thereof.
34. The use of claim 33, wherein the headache is migraine.
35. The use of claim 34, wherein the migraine is episodic migraine or chronic migraine.
36. A method for assessing the quality of an erenumab composition, comprising:
obtaining an erenumab composition that contains erenumab and one or more erenumab variants;
measuring the amount of one or more erenumab variants in the composition, wherein the erenumab variants comprise isomerization variants, deamidation variants, acidic variants, HMW species, or combinations thereof;
comparing the measured amount of the one or more erenumab variants to a pre-determined reference criterion; and
preparing a pharmaceutical formulation or pharmaceutical product of the erenumab composition if the comparison indicates that the pre-determined reference criterion is met.
37. The method of claim 36, wherein the amount of isomerization variants and deamidation variants is measured and the pre-determined reference criterion is about 10% or less.
38. The method of claim 37, wherein the amount of isomerization variants and deamidation variants in the erenumab composition is measured by HIC-HPLC.
39. The method of claim 36, wherein the amount of acidic variants is measured and the pre-determined reference criterion is about 38% or less.
40. The method of claim 39, wherein the amount of acidic variants in the erenumab composition is measured by CEX-HPLC.

41. The method of claim 36, wherein the amount of HMW species is measured and the pre-determined reference criterion is about 2.5% or less.
42. The method of claim 41, wherein the amount of HMW species is measured by SE-UHPLC.
43. The method of any one of claims 36 to 42, wherein the erenumab composition is obtained from a Chinese Hamster Ovary (CHO) cell line that expresses a nucleic acid encoding a heavy chain of SEQ ID NO: 1 and a nucleic acid encoding a light chain of SEQ ID NO: 2.