

SUMMARY

[0007] Provided in accordance with the present disclosure is disclosure for the first time demonstrating that the addition of an amino acid aggregation inhibitor to an aqueous solution comprising a high concentration of an anti-RANKL antibody leads to a reduced amount of antibody aggregates formed over time, as well a slower formation rate of such aggregates. The present disclosure also provides for a pH effect on aggregate formation in concentrated aqueous solutions of anti-RANKL antibody, wherein decreased aggregate formation is observed when the pH of the aqueous solutions is in the range of about 5.0 to less than 5.2. Further suggested by the disclosure presented herein is that the stabilization of the anti-RANKL antibody occurs through interactions between the amino acid aggregation inhibitor and the antibody. Without being bound to any particular theory, it is contemplated that hydrophobic interactions, as well as other types of intermolecular interactions, between the amino acid aggregation inhibitor and the anti-RANKL antibody have a stabilizing effect on the concentrated antibody solutions. Accordingly, the disclosure of the present disclosure relates to stable aqueous pharmaceutical formulations comprising a high concentration of an anti-RANKL antibody which formulations comprise low amounts (e.g., less than about 2%) of aggregates.

[0007a] In one aspect, the present disclosure refers to an aqueous pharmaceutical formulation comprising (i) a human anti-human receptor activator of nuclear factor kappa-B ligand (anti-RANKL) monoclonal antibody, or an antigen-binding portion thereof, at a concentration greater than 70 mg/mL, (ii) an amino acid aggregation inhibitor comprising an aromatic amino acid or a hydrophobic amino acid and (iii) a pH in a range of 5.0 to less than 5.2, wherein the anti-RANKL antibody, or antigen-binding portion thereof, comprises (A) a light chain variable domain comprising a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5, a light chain variable domain comprising a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a light chain variable domain comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:7; and (B) a heavy chain variable domain comprising a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a heavy chain variable domain comprising a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and a heavy chain variable domain comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

[0007b] In another aspect, the present disclosure refers to a container, optionally, a vial, pre-filled syringe (PFS), or glass container containing an aqueous pharmaceutical formulation of any one of the preceding claims, optionally, containing 1 mL or less of the formulation, optionally, 0.5 mL.

[0007c] In another aspect, the present disclosure refers to a formulation comprising denosumab at a concentration of 108 mg/mL to 132 mg/mL, 28.8 mM to 35.2 mM acetate, 33.3 mM to 40.7 mM phenylalanine, 3.51% (w/v) to 4.29% (w/v) sorbitol, and 0.009% (w/v) to 0.011% (w/v) polysorbate 20, at pH 5.1.

[0007d] In another aspect, the present disclosure refers to an aqueous pharmaceutical formulation as described herein, container as described herein or formulation as described herein for use in therapy.

[0007e] In another aspect, the present disclosure refers to the use of an aqueous pharmaceutical formulation as described herein, container as described herein or formulation as described herein in the manufacture of a medicament for (a) treatment or prevention of an SRE in a subject with bone metastases from solid tumors, (b) treatment or prevention of an SRE in a subject who is an adult or a skeletally mature adolescent with giant cell tumor of bone that is unresectable or where surgical resection is likely to result in severe morbidity, (c) treatment of hypercalcemia of malignancy refractory to bisphosphonate therapy in a subject, (d) treatment or prevention of an SRE in a subject with multiple myeloma or with bone metastases from a solid tumor, (e) treatment of osteoporosis of postmenopausal women at high risk for fracture, (f) treatment to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer, (g) treatment to increase bone mass in men at high risk for fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer, (h) treatment to increase bone mass in men with osteoporosis at high risk for fracture, (i) therapy with calcium or vitamin D, (j) treating giant cell tumor of bone in a patient in need thereof, (k) treating hypercalcemia of malignancy in a patient in need thereof, (l) treating osteoporosis in a patient in need thereof, (m) increasing bone mass in a patient in need thereof.

[0008] Accordingly, one example of the disclosure is an aqueous pharmaceutical formulation comprising a human anti-human receptor activator of nuclear factor kappa-B ligand (anti-RANKL) monoclonal antibody or an antigen-binding portion thereof at a concentration of greater than 70 mg/mL and having a pH in a range of about 5.0 to less than 5.2.

[0009] Another example of the disclosure is an aqueous pharmaceutical formulation comprising a mixture of a human anti-human receptor activator of nuclear factor kappa-B ligand (anti-RANKL) monoclonal antibody or an antigen-binding portion thereof and an amino acid

What is claimed is:

1. An aqueous pharmaceutical formulation comprising (i) a human anti-human receptor activator of nuclear factor kappa-B ligand (anti-RANKL) monoclonal antibody, or an antigen-binding portion thereof, at a concentration greater than 70 mg/mL, (ii) an amino acid aggregation inhibitor comprising an aromatic amino acid or a hydrophobic amino acid and (iii) a pH in a range of 5.0 to less than 5.2,

wherein the anti-RANKL antibody, or antigen-binding portion thereof, comprises (A) a light chain variable domain comprising a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5, a light chain variable domain comprising a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a light chain variable domain comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 7; and (B) a heavy chain variable domain comprising a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a heavy chain variable domain comprising a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and a heavy chain variable domain comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

2. The aqueous pharmaceutical formulation of claim 1, wherein the anti-RANKL antibody, or antigen-binding portion thereof, comprises:

(A) a light chain variable domain selected from the group consisting of:

- i. a light chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 1;
- ii. a light chain variable domain comprising an amino acid sequence encoded by a polynucleotide sequence comprising SEQ ID NO: 19;
- iii. a light chain variable domain comprising an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complement of a polynucleotide consisting of SEQ ID NO: 19; or

(B) a heavy chain variable domain selected from the group consisting of:

- i. a heavy chain variable domain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 2;

- ii. a heavy chain variable domain comprising an amino acid sequence encoded by a polynucleotide sequence comprising SEQ ID NO: 20.
- iii. a heavy chain variable domain comprising an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complement of a polynucleotide consisting of SEQ ID NO: 20; or

(C) a light chain variable domain of (A) and a heavy chain variable domain of (B).

3. The aqueous pharmaceutical formulation of claim 1 or 2, wherein the anti-RANKL antibody, or antigen-binding portion thereof, comprises a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 1 and a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 2.

4. The aqueous pharmaceutical formulation of any one of the preceding claims, wherein the anti-RANKL antibody is (A) a fully human antibody, a humanized antibody, or a chimeric antibody or (B) an a Fab, Fab', F(ab')₂, or a single chain Fv, or (C) an IgG₁, IgG₂, or IgG₄ antibody, optionally, comprising kappa light chains, optionally, wherein the anti-RANKL antibody comprises a sequence of SEQ ID NO: 15 or comprises a sequence of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

5. The aqueous pharmaceutical formulation of any one of the preceding claims, wherein the anti-RANKL antibody, or antigen-binding portion thereof, comprises:

(A) a light chain selected from the group consisting of:

- i. a light chain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 3 or SEQ ID NO: 13;
- ii. a light chain comprising an amino acid sequence encoded by a polynucleotide sequence that is at least 80% identical to SEQ ID NO: 21 or 23;
- iii. a light chain comprising an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complement of a polynucleotide consisting of SEQ ID NO: 21 or 23; or

(B) a heavy chain selected from the group consisting of:

- i. a heavy chain comprising an amino acid sequence that is at least 80% identical to SEQ ID NO: 4 or SEQ ID NO: 14;
- ii. a heavy chain comprising an amino acid sequence encoded by a polynucleotide sequence that is at least 80% identical to SEQ ID NO: 22 or 24.
- iii. a heavy chain comprising an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complement of a polynucleotide consisting of SEQ ID NO: 22 or 24; or

(C) a light chain variable domain of (A) and a heavy chain variable domain of (B).

6. The aqueous pharmaceutical formulation of any one of the preceding claims, wherein the anti-RANKL antibody, or antigen-binding portion thereof, comprises a light chain comprising an amino acid sequence of SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence of SEQ ID NO: 14.

7. The aqueous pharmaceutical formulation of any one of the preceding claims, wherein the concentration of the antibody, or antigen-binding portion thereof, is (A) greater than 70 mg/mL, optionally, in a range of 70 mg/mL to 300 mg/mL or (B) in a range of greater than 70 mg/mL to 300 mg/mL, optionally in a range of greater than 70 mg/mL to 200 mg/mL or (C) in a range of 100 to 140 mg/mL.

8. The aqueous pharmaceutical formulation of any one of the preceding claims, wherein the concentration of the antibody or antigen-binding portion thereof is 120 mg/mL $\pm$ 12 mg/mL.

9. The aqueous pharmaceutical formulation of any one of the preceding claims, wherein the aromatic amino acid comprises a phenyl or an indole, optionally, wherein the aromatic amino acid comprises a C₁-C₆ alkyl chain between the alpha carbon and the phenyl or indole.

10. The aqueous pharmaceutical formulation of claim 9, wherein the alkyl chain is a C₁-C₃ alkyl chain.
11. The aqueous pharmaceutical formulation of claim 10, wherein the aromatic amino acid is L-phenylalanine.
12. The aqueous pharmaceutical formulation of claim 10, wherein the aromatic amino acid is L-tryptophan.
13. The aqueous pharmaceutical formulation of any one of claims 1-8, wherein the hydrophobic amino acid has a score greater than 2.5 on the Kyte and Doolittle hydrophobicity scale, optionally, wherein the hydrophobic amino acid comprises a side chain comprising a C₂ to C₁₂ alkyl, branched or straight-chained, or a C₄ to C₈ cycloalkyl, a C₄ to C₈ heterocycle comprising a nitrogen heteroatom, optionally, wherein the heterocycle is an imidazole, pyrrole, or indole.
14. The aqueous pharmaceutical formulation of claim 13, wherein the hydrophobic amino acid comprises a C₃ to C₈ alkyl, optionally, a branched C₃ alkyl or branched C₄ alkyl.
15. The aqueous pharmaceutical formulation of claim 14, wherein the hydrophobic amino acid is L-valine, L-leucine, or L-isoleucine.
16. The aqueous pharmaceutical formulation of any one of the preceding claims, comprising 5 mM to 300 mM amino acid aggregation inhibitor, optionally, comprising 25 mM to 90 mM amino acid aggregation inhibitor.
17. The aqueous pharmaceutical formulation of claim 16, comprising 5 mM to 180 mM amino acid aggregation inhibitor, when the amino acid aggregation inhibitor is an aromatic amino acid, optionally, L-phenylalanine, optionally, comprising 5 mM to 100 mM amino acid aggregation inhibitor, optionally, 20 mM to 50 mM amino acid aggregation inhibitor, when the amino acid aggregation inhibitor is an aromatic amino acid, optionally, L-phenylalanine.
18. The aqueous pharmaceutical formulation of claim 16, comprising 5 mM to 300 mM amino acid aggregation inhibitor, when the amino acid aggregation inhibitor is a hydrophobic amino acid, optionally, L-valine, L-isoleucine, or L-leucine, optionally, comprising

5 mM to 200 mM amino acid aggregation inhibitor, optionally, 20 mM to 50 mM amino acid aggregation inhibitor, when the amino acid aggregation inhibitor is a hydrophobic amino acid, optionally, L-valine, L-isoleucine, or L-leucine.

19. The aqueous pharmaceutical formulation of any one of claims 16 to 18, comprising:

- a. 20 mM to 50 mM L-phenylalanine;
- b. 20 mM to 50 mM L-tryptophan;
- c. 20 mM to 50 mM L-leucine;
- d. 20 mM to 50 mM L-isoleucine;
- e. 20 mM to 50 mM L-valine; or
- f. any combination thereof.

20. The aqueous pharmaceutical formulation of any one of the preceding claims comprising only one amino acid aggregation inhibitor.

21. The aqueous pharmaceutical formulation of any one of the preceding claims, wherein the molar ratio of the amino acid aggregation inhibitor to the anti-RANKL antibody is 10 to 200, when the amino acid aggregation inhibitor is an aromatic amino acid, optionally, L-phenylalanine, optionally, wherein the molar ratio is 20 to 90.

22. The aqueous pharmaceutical formulation of any one of the preceding claims, further comprising (A) a tonicity modifier, optionally, selected from the group consisting of: sorbitol, mannitol, sucrose, trehalose, glycerol, and combinations thereof, (B) a surfactant, (C) a buffer, optionally, wherein the buffer is centered, at 25 °C, in a range of pH 4.0 to pH 5.5, or (D) any combination thereof.

23. The aqueous pharmaceutical formulation of claim 22, wherein (A) the tonicity modifier comprises sorbitol or (B) the formulation comprises 1.0 (w/w)% to 5.0 (w/w)% tonicity modifier, optionally, comprising 2.0 (w/w) % to 5.0 (w/w) % sorbitol, or 3.5 (w/w) % to 5.0 (w/w) % sorbitol, or 4.0% (w/w) to 5.0 (w/w) % sorbitol, or (C) both (A) and (B).

24. The aqueous pharmaceutical formulation of any one of preceding claims, which is free of sorbitol and, optionally, free of any tonicity modifier.

25. The aqueous pharmaceutical formulation of claim 24, wherein the surfactant is selected from the group consisting of: polyoxyethylene sorbitan fatty acid esters (e.g. polysorbate 20, polysorbate 80), or one or more alkylaryl polyethers, e.g. oxyethylated alkyl phenol (e.g. Triton® X-100), or one or more poloxamers (e.g. Pluronic®, e.g. Pluronic® F68), and combinations thereof.

26. The aqueous pharmaceutical formulation of claim 25, wherein the surfactant is polysorbate 20.

27. The aqueous pharmaceutical formulation of claim 24 or 25 comprising at least 0.004 (w/v) % surfactant and, optionally, comprising less than 0.15 (w/v)% surfactant, optionally, 0.005 (w/v) % to 0.015 (w/v) % surfactant.

28. The aqueous pharmaceutical formulation of claim 22, wherein the buffer has a pKa within one pH unit of pH 5.0-5.2 at 25 °C.

29. The aqueous pharmaceutical formulation of claim 22 or 28, comprising 5 mM to 60 mM buffer, optionally, 5 mM to 50 mM buffer or 9 mM to 45 mM buffer.

30. The aqueous pharmaceutical formulation of any one of claims 22 to 29, wherein the buffer is acetate or glutamate.

31. The aqueous pharmaceutical formulation of any one of the preceding claims, having a pH in a range of 5.0 to 5.19, optionally, having a pH in a range of 5.0 to 5.15, optionally, 5.0 to 5.1.

32. The aqueous pharmaceutical formulation of any one of the preceding claims, having a pH in a range of 5.0 to 5.4, or 5.0 to 5.2, or 5.0 to less than 5.2, or 5.0 to 5.19, or 5.0 to 5.15, or 5.0 to 5.1, optionally, having a pH of 5.1.

33. The aqueous pharmaceutical formulation of any one of the preceding claims, (I) having a viscosity that is (A) not more than 6 cP at 5 °C, optionally, wherein the viscosity is 4.5 cP to 5.5 cP or (B) less than 13 cP at 25 °C or (C) in a range of 2.0 cP to 10 cP, optionally, 2.5 cP to 4 cP, (II) having a conductivity in a range of 500 µS/cm to 5500 µS/cm, optionally, wherein the conductivity is in a range of 2500 µS/cm to 5500 µS/cm, when the formulation comprises an

amino acid comprising a positive-charged side chain, or in a range of 500 $\mu\text{S}/\text{cm}$ to 2000 $\mu\text{S}/\text{cm}$, when the formulation comprises an aromatic amino acid or lacks an amino acid aggregation inhibitor or (III) having an osmolality in a range of 200 mOsm/kg to 500 mOsm/kg, or 225 mOsm/kg to 400 mOsm/kg, or 250 mOsm/kg to 350 mOsm/kg.

34. The aqueous pharmaceutical formulation of any one of the preceding claims, comprising less than 2% high molecular weight species (HMWS) and/or more than 98% of the antibody main peak, as measured by SE-UHPLC, following (A) storage at 2 °C to 8 °C for at least 12 months, 24 months, or 36 months or (B) storage at 20 °C to 30 °C for 1 month or (C) a first storage at 2 °C to 8 °C for at least 12 months, 24 months, or 36 months and a second storage at 20 °C to 30 °C for 1 month or (D) storage for one month at 37 °C or for 3 months at 30 °C.

35. A container, optionally, a vial, pre-filled syringe (PFS), or glass container containing an aqueous pharmaceutical formulation of any one of the preceding claims, optionally, containing 1 mL or less of the formulation, optionally, 0.5 mL.

36. A formulation comprising denosumab at a concentration of 108 mg/mL to 132 mg/mL, 28.8 mM to 35.2 mM acetate, 33.3 mM to 40.7 mM phenylalanine, 3.51% (w/v) to 4.29% (w/v) sorbitol, and 0.009% (w/v) to 0.011% (w/v) polysorbate 20, at pH 5.1.

37. An aqueous pharmaceutical formulation of any one of claims 1-36, container of claim 35 or formulation of claim 36 for use in therapy.

38. Use of an aqueous pharmaceutical formulation of any one of claims 1-34, container of claim 35 or formulation of claim 36 in the manufacture of a medicament for (a) treatment or prevention of an SRE in a subject with bone metastases from solid tumors, (b) treatment or prevention of an SRE in a subject who is an adult or a skeletally mature adolescent with giant cell tumor of bone that is unresectable or where surgical resection is likely to result in severe morbidity, (c) treatment of hypercalcemia of malignancy refractory to bisphosphonate therapy in a subject, (d) treatment or prevention of an SRE in a subject with multiple myeloma or with bone metastases from a solid tumor, (e) treatment of osteoporosis of postmenopausal women at high risk for fracture, (f) treatment to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer, (g) treatment to

increase bone mass in men at high risk for fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer, (h) treatment to increase bone mass in men with osteoporosis at high risk for fracture, (i) therapy with calcium or vitamin D, (j) treating giant cell tumor of bone in a patient in need thereof, (k) treating hypercalcemia of malignancy in a patient in need thereof, (l) treating osteoporosis in a patient in need thereof, (m) increasing bone mass in a patient in need thereof.