stabilizers, to achieve long-term protein stability. The resulting formulation may be hypertonic, which may cause injection pain due to tissue damage. Therefore, it is critical to balance the amount of stabilizers for stability and osmolality of concentrated protein formulations.

[0009] For these reasons, there is a need for development of G-CSF formulations that exhibit high protein concentrations without significantly increased protein aggregation, osmolality, and/or decreased protein stability.

[0010] BRIEF SUMMARY

[0011] In some examples, disclosed herein are compositions of G-CSF. In some examples, there is provided a pharmaceutical composition comprising (or consisting essentially of, or consisting of): (a) about 1 mg/mL to about 100 mg/mL G-CSF dimer; (b) about 1 mM to about 50 mM buffering agent; (c) about 0.1 mM to about 20 mM stabilizing agent; (d) about 1% (w/v) to about 15% (w/v) (e.g., about 1% (w/v) to about 10% (w/v)) tonicity agent; and (e) about 0.001% (w/v) to about 0.1% (w/v) surfactant. In one aspect, the present disclosure refers to a pharmaceutical composition comprising: (a) about 1 mg/mL to about 100 mg/mL granulocyte colony-stimulating factor (G-CSF) dimer; (b) about 1 mM to about 50 mM buffering agent; (c) about 0.1 mM to about 20 mM EDTA; (d) about 1% (w/v) to about 15% (w/v) sorbitol; and (e) about 0.001% (w/v) to about 0.1% (w/v) surfactant; and wherein the pH of the pharmaceutical composition is about 4.2 to about 6.2.

[0012] In some examples according to any of the pharmaceutical compositions describe above, the G-CSF dimer comprises two monomeric subunits, wherein each monomeric subunit comprises a G-CSF monomer and a dimerization domain. In some examples, the G-CSF monomer comprises the sequence of SEQ ID NO: 1. In some examples, the G-CSF monomer is connected to the dimerization domain via an optional linker (e.g., within each monomeric subunit). In some examples, the linker is about 6 to about 30 amino acids in length. In some examples, the linker comprises the sequence of SEQ ID NO: 12. In some examples, the dimerization domain comprises at least two cysteines capable of forming intermolecular disulfide bonds. In some examples, the dimerization domain comprises at least a portion of an Fc fragment. In some examples, the Fc fragment comprises CH2 and CH3 domains. In some examples, the Fc fragment is derived from IgG1 Fc, IgG2 Fc, IgG4 Fc, or a fragment or a variant thereof. In some examples, the Fc fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-5. In some examples, the G-CSF monomer is N-terminal to the dimerization domain within each monomeric subunit. In some examples, each monomeric subunit comprises an amino acid sequence of any of SEQ ID NOs: 6-10, or a variant thereof having at least about 90% (e.g., at least about 95%) sequence identity to the amino acid sequence of any of SEQ ID NOs: 6-10. In some examples, each monomeric subunit comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6-10, such as SEQ ID NO: 6.

[0013] In some examples according to any of the pharmaceutical compositions describe above, the G-CSF dimer is efbemalenograstim alfa.

[0014] In some examples according to any of the pharmaceutical compositions described above, the concentration of the G-CSF dimer is about 5 mg/mL to about 50 mg/mL, such as about 20 mg/mL.

[0015] In some examples according to any of the pharmaceutical compositions described above, the buffering agent is sodium acetate. In some examples, the concentration of the sodium acetate is about 1 mM to about 30 mM, such as about 10 mM.

[0016] In some examples according to any of the pharmaceutical compositions described above, the stabilizing agent is EDTA. In some examples, the concentration of the EDTA is about 0.1 mM to about 10 mM, such as about 1 mM.

[0017] In some examples according to any of the pharmaceutical compositions described above, the tonicity agent is sorbitol or sucrose. In some examples, the tonicity agent is sorbitol. In some examples, the pharmaceutical composition comprises about 1% (w/v) to about 8% (w/v) sorbitol, such as about 5% (w/v) sorbitol. In some examples, the tonicity agent is sucrose. In some examples, pharmaceutical composition comprises about 5% (w/v) to about 15% (w/v) sucrose, such as about 9% (w/v) sucrose.

[0018] In some examples according to any of the pharmaceutical compositions described above, the surfactant is selected from the group consisting of a polysorbate, a poloxamer, a polyoxyethelene alkyl ether, an alkyl phenyl polyoxyethylene ether, and a combination thereof. In some examples, the surfactant is polysorbate 20 (PS20) or polysorbate 80 (PS80). In some examples, the pharmaceutical composition comprises about 0.005% (w/v) to about 0.05% (w/v) PS20, such as about 0.01% (w/v) PS20.

[0019] In some examples according to any of the pharmaceutical compositions described above, the pH of the pharmaceutical composition is about 4.2 to about 6.2, such as about 4.8 to about 5.8, about 5.0 to about 5.4, or about 5.2.

[0020] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises (or consists essentially of, or consists of): (a) about 20 mg/mL of the G-CSF dimer; (b) about 10 mM sodium acetate; (c) about 1 mM EDTA; (d) about 5% (w/v) sorbitol; and (e) about 0.01% (w/v) PS20, wherein the pharmaceutical composition has a pH of about 5.2.

[0021] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition is stable at 25± 2°C for at least about 1 month, such as stable at 25± 2°C for at least about 2 months. In some examples, the pharmaceutical composition is stable at 25± 2°C up to about 3 months.

[0022] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition has a purity of G-CSF dimer of at

least about 90% as assessed by size exclusion–high performance liquid chromatography (SE-HPLC).

[0023] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition has an osmolality of about 268 mOsm/kg to about 360 mOsm/kg.

[0024] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises main peak at least about 85% in relation to the total area of all peaks when measured by SE-HPLC after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the pharmaceutical composition comprises main peak at least about 85% in relation to the total area of all peaks when measured by SE-HPLC after up to about 3 months of storage under 25 ± 2°C.

[0025] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises dimer less than about 2.5% in relation to the total area of all peaks when measured by SE-HPLC after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the pharmaceutical composition comprises dimer less than about 2.5% in relation to the total area of all peaks when measured by SE-HPLC after up to about 3 months of storage under 25 ± 2°C.

[0026] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises an aggregate in no more than about 2% in relation to the total area of all peaks when measured by SE-HPLC after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the pharmaceutical composition comprises an aggregate in no more than about 2% in relation to the total area of all peaks when measured by SE-HPLC after up to about 3 months of storage under 25 ± 2°C.

[0027] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises main peak at least about 85% in relation to the total area of all peaks when measured by SE-HPLC after at least about 36 months of storage under about 2°C to about 8°C. In some examples, the pharmaceutical composition comprises main peak at least about 85% in relation to the total area of all peaks when measured by SE-HPLC after up to about 36 months of storage under about 2°C to about 8°C.

[0028] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises at least about 96% of the G-CSF dimer as measured by reduced capillary electrophoresis-sodium dodecyl sulfate (rCE-SDS) after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the pharmaceutical composition comprises at least

about 96% of the G-CSF dimer as measured by rCE-SDS after up to about 3 months of storage under 25 ± 2°C.

[0029] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises at least about 95.5% of the G-CSF dimer as measured by rCE-SDS after at least about 36 months of storage under about 2°C to about 8°C. In some examples, the pharmaceutical composition comprises at least about 95.5% of the G-CSF dimer as measured by rCE-SDS after up to about 36 months of storage under about 2°C to about 8°C.

[0030] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises no more than about 1.5% high molecular weight species (HMWS) as measured by rCE-SDS after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the pharmaceutical composition comprises no more than about 1.5% HMWS as measured by rCE-SDS after up to about 3 months of storage under 25 ± 2°C.

[0031] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises no more than about 1.5% high molecular weight species as measured by rCE-SDS after at least about 36 months of storage under about 2°C to about 8°C. In some examples, the pharmaceutical composition comprises no more than about 1.5% HMWS as measured by rCE-SDS after up to about 36 months of storage under about 2°C to about 8°C.

[0032] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises at least about 94% of the G-CSF dimer as measured by non-reduced capillary electrophoresis-sodium dodecyl sulfate (nrCE-SDS) after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the pharmaceutical composition comprises at least about 94% of the G-CSF dimer as measured by nrCE-SDS after up to about 3 months of storage under 25 ± 2°C.

[0033] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises at least about 94% of G-CSF dimer as measured by nrCE-SDS after at least about 36 months of storage under about 2°C to about 8°C. In some examples, the pharmaceutical composition comprises at least about 94% of G-CSF dimer as measured by nrCE-SDS after up to about 36 months of storage under about 2°C to about 8°C.

[0034] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises no more than about 1.5% high molecular weight species as measured by nrCE-SDS after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples,

the pharmaceutical composition comprises no more than about 1.5% HMWS as measured by nrCE-SDS after up to about 3 months of storage under 25 ± 2°C.

[0035] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises no more than about 1.5% high molecular weight species as measured by nrCE-SDS after at least about 36 months of storage under about 2°C to about 8°C. In some examples, the pharmaceutical composition comprises no more than about 1.5% high molecular weight species as measured by nrCE-SDS after up to about 36 months of storage under about 2°C to about 8°C.

[0036] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises about 27.2% to about 39.0% acidic region, about 36.5% to about 60.1% main peak, and about 7.2% to about 30% basic region as measured by imaged capillary isoelectric focusing (icIEF) after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the pharmaceutical composition comprises about 27.2% to about 39.0% acidic region, about 36.5% to about 60.1% main peak, and about 7.2% to about 30% basic region as measured by icIEF after up to about 3 months of storage under 25 ± 2°C.

[0037] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition comprises about 27.2% to about 39.0% acidic region, about 36.5% to about 60.1% main peak, and about 7.2% to about 30% basic region as measured by icIEF after at least about 36 months of storage under about 2°C to about 8°C. In some examples, the pharmaceutical composition comprises about 27.2% to about 39.0% acidic region, about 36.5% to about 60.1% main peak, and about 7.2% to about 30% basic region as measured by icIEF after up to about 36 months of storage under about 2°C to about 8°C.

[0038] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition retains at least about 70% potency after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the pharmaceutical composition retains at least about 70% potency after up to about 3 months of storage under 25 ± 2°C.

[0039] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition retains at least about 70% potency after at least about 36 months of storage under about 2°C to about 8°C. In some examples, the pharmaceutical composition retains at least about 70% potency after up to about 36 months of storage under about 2°C to about 8°C.

[0040] In some examples according to any of the pharmaceutical compositions described above, the concentration of the pharmaceutical composition varies by less

than about 0.5 mg/mL after at least about 1 month (e.g., at least about 2 months) of storage under 25 ± 2°C. In some examples, the concentration of the pharmaceutical composition varies by less than about 0.5 mg/mL after up to about 3 months of storage under 25 ± 2°C.

[0041] In some examples according to any of the pharmaceutical compositions described above, the concentration of pharmaceutical composition varies by less than about 0.5 mg/mL after at least about 36 months of storage under about 2°C to about 8°C. In some examples, the concentration of pharmaceutical composition varies by less than about 0.5 mg/mL after up to about 36 months of storage under about 2°C to about 8°C.

[0042] In some examples according to any of the pharmaceutical compositions described above, the pharmaceutical composition is contained in a syringe. In some examples, the volume of the pharmaceutical composition within the syringe is about 1 mL. In some examples, the syringe is for single use. In some examples, the syringe is sterile.

[0043] In another example, there is provided a syringe comprising any of the pharmaceutical compositions described above. In some examples, the syringe is for single use. In some examples, the volume of the liquid pharmaceutical composition within the syringe is about 1 mL. In some examples, the syringe is sterile.

[0044] In a further example, there is provided a method of treating or preventing a disease or condition in an individual (e.g., human), comprising administering to the individual an effective amount of any of the pharmaceutical compositions (e.g., liquid pharmaceutical composition) provided herein. In one aspect, the present disclosure refers to use of an effective amount of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating or preventing a disease or condition in an individual. In some examples, the pharmaceutical composition is administered at a dosage of from about 0.01 mg/kg to about 1 mg/kg. In some examples, the pharmaceutical composition is administered once every four weeks. In some examples, the pharmaceutical composition is administered once every three weeks. In some examples, the pharmaceutical composition is administered subcutaneously. In some examples, the disease or condition is selected from the group consisting of neutropenia, stroke, spinal injury, neurological disorders accompanied with blood brain barrier injury, Parkinson's disease, Alzheimer's disease, Huntington disease, amyotrophic lateral sclerosis, spinal muscular atrophy, primary lateral sclerosis, spinal cerebellar ataxias, and mobilization of hematopoietic stem cells into peripheral blood in allogeneic blood stem cell transplantation. In some examples, the disease or condition is neutropenia. In some examples, the neutropenia is chemotherapy-induced neutropenia or radiotherapy-induced neutropenia. In some examples, the method comprises administering to the individual the effective amount of the pharmaceutical composition after administration of a chemotherapeutic agent to the individual. In some examples,

the chemotherapeutic agent is a myelosuppressive chemotherapeutic agent. In some examples, the pharmaceutical composition is administered at about 20 mg once per chemotherapy cycle. In some examples, the individual is administered at least 4 cycles of the chemotherapeutic agent, and wherein the pharmaceutical composition is administered after the administration of the chemotherapeutic agent in each cycle of the at least 4 cycles. In some examples, the pharmaceutical composition is administered at about 5 mg to about 25 mg (e.g., about 20 mg) of the G-CSF dimer for each administration.

[0045] In a further example, there is provided a method of manufacturing any of the pharmaceutical compositions disclosed herein, comprising: (i) culturing a host cell under a condition suitable for expressing the G-CSF dimer; (ii) isolating the expressed G-CSF dimer from the cell culture; (iii) purifying the expressed G-CSF dimer; and (iv) formulating the purified G-CSF dimer with the buffering agent, the stabilizing agent, the tonicity agent, and the surfactant. In one aspect, the present disclosure refers to a method of manufacturing the pharmaceutical composition as disclosed herein, comprising: (i) culturing a host cell under a condition suitable for expressing the G-CSF dimer; (ii) isolating the expressed G-CSF dimer from the cell culture; (iii) purifying the expressed G-CSF dimer; and (iv) formulating the purified G-CSF dimer with the buffering agent, EDTA, sorbitol and the surfactant. In some examples, the purification step comprises one or more of: affinity chromatography, viral inactivation, ion exchange chromatography, mixed-mode chromatography, and filtration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] FIG. 1 is an illustration of the structure of an exemplary G-CSF dimer (e.g., efbemalenograstim alfa). In the figure, "-" represents the linker, the oval-shaped object labeled with "G-CSF" represents a G-CSF monomer, and the oval-shaped object labeled with "Fc" represents an Fc fragment (e.g., human IgG-derived Fc). The exemplary G-CSF dimer comprises 2 monomeric subunits. Each monomeric subunit from N' to C' comprises a G-CSF monomer, a linker, and an Fc fragment.

[0047] FIG. 2 is an exemplary chromatogram by reverse phase high-performance liquid chromatography (RP-HPLC) of an G-CSF-Fc dimer pharmaceutical composition containing 15 mg/mL G-CSF-Fc dimer, 10 mM sodium acetate, 5% (w/v) sorbitol, 0.01% (w/v) polysorbate 20, 1 mM EDTA, pH 5.2, after stored for 12 weeks at 25 ± 2 ℃. There are primarily three elution peaks, namely Impurity peak 1, Impurity peak 2, and Main peak, respectively.

[0048] FIG. 3A-3B show the change in % main band of G-CSF dimer as determined by non-reducing SDS-PAGE (FIG. 3A) and reducing SDS-PAGE (FIG. 3B) in G-CSF dimer pharmaceutical compositions comprising: (●) 10 mM sodium acetate, 5% (w/v) sorbitol, 1 mM EDTA, 0.01% (w/v) polysorbate 20, pH 4.8 (■) 10 mM sodium acetate, 5% (w/v) sorbitol, 1 mM EDTA, 0.01% (w/v) polysorbate 20, pH 5.2, (▲) 10 mM sodium acetate, 9% (w/v) sucrose, 1 mM EDTA, 0.01% (w/v) polysorbate 20, pH 4.8, or (×) 10 mM sodium acetate, 9% (w/v) sucrose, 1 mM EDTA, 0.01% (w/v) polysorbate 20, pH 5.2, after storage at 25 ± 2 ℃ for up to 12 weeks.