Thus, it is an object of the present disclosure to provide stable liquid pharmaceutical formulations comprising an ActRIIa fusion protein and corresponding methods for treating, preventing, or reducing the progression rate and/or severity of PH, particular treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications.

SUMMARY

Provided herein are stable, liquid pharmaceutical formulations comprising a recombinant fusion protein comprising an extracellular domain (ECD) of human activin receptor type-IIA (ActRIIa) or a variant thereof linked to a constant domain of an immunoglobulin, such as a human IgG1 Fc domain, and one or more pharmaceutical additives and/or excipients.

In some examples, the ActRIIa protein comprises an amino acid sequence that is at least 70% (e.g., at least 70%,75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to an amino acid sequence that begins at any one of amino acids 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 of SEQ ID NO: 9 and ends at any one of amino acids 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, or 135 of SEQ ID NO: 9. In some examples, the ActRIIa protein comprises an amino acid sequence that is at least 70% (e.g., at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 10. In some examples, the ActRIIa protein comprises an amino acid sequence that is at least 70% (e.g., at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 11.

In some examples, the ActRIIa protein is an ActRIIa fusion protein comprising an ActRIIa extracellular domain and one or more protein domains heterologous to ActRIIa. In some examples, the ActRIIa protein is a fusion protein comprising an Fc domain of an immunoglobulin. In some examples, the Fc domain of the immunoglobulin is an Fc domain of an IgG1 immunoglobulin. In some examples, the ActRIIa fusion protein further comprises a linker domain positioned between the ActRIIa protein domain and the one or more heterologous domains (e.g., an Fc immunoglobulin domain). In some

examples, the linker domain is selected from the group consisting of: TGGG (SEQ ID NO: 23), TGGGG (SEQ ID NO: 21), SGGGG (SEQ ID NO: 22), GGGGS (SEQ ID NO: 25), GGG (SEQ ID NO: 19), GGGG (SEQ ID NO: 20), and SGGG (SEQ ID NO: 24). In some examples, the ActRIIa fusion protein comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 32. In some examples, the ActRIIa fusion protein comprises the amino acid sequence of SEQ ID NO: 32. In some examples, the ActRIIa fusion protein consists of the amino acid sequence of SEQ ID NO: 32. In some examples, the ActRIIa fusion protein consists of the amino acid sequence of SEQ ID NO: 41. In some examples, the ActRIIa fusion protein consists of the amino acid sequence of SEQ ID NO: 32 or 41. In an aspect, there is provided a pharmaceutical formulation comprising 50-100 mg/mL of a human ActRIIa fusion protein comprising the amino acid sequence of SEQ ID NO: 32, or SEQ ID NO: 41, a buffer, a surfactant, a stabilizing agent, and optionally one or more antioxidants; wherein the buffer is not histidine. In some examples, the ActRIIa fusion protein consists of a variant of the amino acid sequence set forth in SEQ ID NO: 32, wherein the sequence is lacking the C-terminal lysine residue of SEQ ID NO:32. In some examples, the ActRIIa fusion protein variant lacking the C-terminal lysine residue comprises or consists of the amino acid sequence of SEQ ID NO:41. In some examples, the ActRIIa fusion protein is part of a homodimer protein complex. In some examples, the ActRIIa fusion protein is glycosylated. In some examples, the ActRIIa fusion protein has a glycosylation pattern obtainable by expression in a Chinese hamster ovary cell.

In certain examples, the pharmaceutical formulations described herein, comprise a ActRIIA fusion protein, or a variant, a buffer, a surfactant, a stabilizing agent, and optionally one or more antioxidants. In certain examples, the pharmaceutical formulations described herein, comprise a ActRIIA fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32 lacking the C-terminal lysine, a buffer, a surfactant, a stabilizing agent, and optionally one or more antioxidants.

In some examples, the pharmaceutical formulations provided herein comprise 10-100 mg/mL of a recombinant fusion protein comprising an extracellular domain (ECD) of human activin receptor type-IIA (ActRIIA) proteins or derivatives thereof linked to a constant domain of an immunoglobulin, such as human IgG1 Fc domain. In other

examples the pharmaceutical formulations comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 mg/mL of a recombinant fusion protein comprising an extracellular domain (ECD) of human activin receptor type-IIA (ActRIIA) proteins or derivatives thereof linked to a constant domain of an immunoglobulin, such as human IgG1 Fc domain. In other examples the pharmaceutical formulations comprise 40-50 mg/mL of a recombinant

fusion protein comprising an extracellular domain (ECD) of human activin receptor type-IIA (ActRIIA) proteins or derivatives thereof linked to a constant domain of an immunoglobulin, such as human IgG1 Fc domain. In other examples the pharmaceutical formulations comprise 50 mg/mL of a recombinant fusion protein comprising an extracellular domain (ECD) of human activin receptor type-IIA (ActRIIA) proteins or derivatives thereof linked to a constant domain of an immunoglobulin, such as human IgG1 Fc domain.

In certain examples, the pharmaceutical formulation provided herein comprises 10-100 mg/mL of a human ActRIIA fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32 lacking the C-terminal lysine. In certain examples, the pharmaceutical formulation provided herein comprise 50-100 mg/mL of a human ActRIIA fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32 lacking the C-terminal lysine. In certain examples, the pharmaceutical formulation provided herein comprises 40-50 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32 lacking the C-terminal lysine. In certain examples, the pharmaceutical formulation provided herein comprises 100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32 lacking the C-terminal lysine. In certain examples, the pharmaceutical formulation provided herein comprises 50 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32 lacking the C-terminal lysine.

In certain examples, the pharmaceutical formulation provided herein comprises 10-100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32 and/or SEQ ID NO:41. In certain examples, the pharmaceutical formulation provided herein comprises 50-100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32 and/or SEQ ID NO:41. In certain examples, the pharmaceutical formulation provided herein comprises 40-50 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32 and/or SEQ ID NO:41. In certain examples, the pharmaceutical formulation provided herein comprises 50 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32 and/or SEQ ID NO:41. In certain examples, the pharmaceutical formulation provided herein comprises 100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32 and/or SEQ ID NO:41.

In certain examples, the pharmaceutical formulation provided herein comprises 10-100 mg/mL of a human ActRIIa fusion protein, a buffer, a surfactant, a stabilizing agent, and optionally one or more antioxidants, wherein the buffer is not histidine.

In certain examples, the pharmaceutical formulation provided herein comprises 10-100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32 and/or SEQ ID NO:41,

a buffer, a surfactant, a stabilizing agent, and optionally one or more antioxidants, wherein the buffer is not histidine.

In certain examples, the pharmaceutical formulation provided herein comprises 10-100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, a buffer, a surfactant, a stabilizing agent, and optionally one or more antioxidants, wherein the buffer is not histidine.

In certain examples, the pharmaceutical formulations provided herein comprise 50-100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32 lacking the C-terminal lysine, a buffer, a surfactant, a stabilizing agent, and optionally one or more antioxidants, wherein the buffer is not histidine.

In some examples, the buffering agent comprises organic acids, succinate, phosphate, acetate, citrate, citric acid, Tris, HEPES, glutamate, amino acids, MES (2-(N-morpholino)ethanesulfonic acid), lactate or mixtures of amino acids. In some examples, the buffering agent comprises organic acids, succinate, phosphate, acetate, citrate, citric acid, Tris, HEPES, glutamate, amino acids, or mixtures of amino acids. In some examples, the buffering agent comprises tri-sodium citrate dihydrate. In some examples, the buffering agent comprises succinate, phosphate, acetate, citrate, lactate or glutamate. In some examples, the buffering agent comprises succinate, phosphate, acetate, citrate or glutamate. In some examples, the buffering agent comprises tri-sodium citrate dihydrate. In some examples, the buffering agent comprises citric acid monohydrate. In some examples, the buffering agent comprises citrate. In some examples, the buffering agent comprises tri-sodium citrate dihydrate and citric acid monohydrate. In certain examples, wherein the protein has a negative charge due to the presence of glycans, the buffer is not histidine.

In some examples, the buffering agent is selected to be physiologically compatible and to maintain a pH above 4. In some examples, the buffering agent is selected to be physiologically compatible and to maintain a pH between 5-7. In some examples, the buffering agent is selected to be physiologically compatible and to maintain a pH of 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6 5.7, 5.8, 5.9 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, or 9.0. In some examples, the buffering agent is selected to be physiologically compatible and to maintain a pH of 5.5, 5.6 5.7, 5.8, 5.9 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6 6.7, 6.8, 6.9, or 7.0. In some examples, the buffering agent is selected to be physiologically compatible and to maintain a pH of 5.8.

In certain examples, the pharmaceutical formulations described herein include a buffer, wherein the buffer is a phosphate buffer to maintain the pH of the pharmaceutical formulation between 5-7. In certain examples, the pharmaceutical formulations described herein include a buffer, wherein the buffer is a citrate buffer to maintain the pH of the pharmaceutical formulation pH between 4.5-7. In certain examples, the pharmaceutical formulations described herein include a buffer, wherein the buffer is an acetate buffer to maintain the pH of the pharmaceutical formulation pH between 4.5-6. In certain examples, the pharmaceutical formulations described herein include a buffer, wherein the buffer is a succinate buffer to maintain the pH of the pharmaceutical formulation pH between 4.5-5. In certain examples, the pharmaceutical formulations described herein include a buffer, wherein the buffer is a glutamate buffer to maintain the pH of the pharmaceutical formulation pH between 5-7.

In some examples, the buffering agent is present at a concentration of at least 0.1, 0.5, 0.7, 0.8 0.9, 1.0, 1.2, 1.5, 1.7, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, or 500 mM. In some examples, the buffering agent is present at a concentration between 10-50 mM. In some examples, the buffering agent is present at a concentration of at least 10 mM.

In some examples, the buffering agent is 10mM of a citrate buffer that maintains a pH of the pharmaceutical formulation at a pH from about 5.5 to about pH 6.5. In some examples, the buffering agent is 10mM of a succinate buffer that maintains a pH of the pharmaceutical formulation at a pH from about 5.5 to about pH 6.5. In some examples, the buffering agent is 10mM of a histidine buffer that maintains a pH of the pharmaceutical formulation at a pH from about 5.5 to about pH 6.0. In some examples, the buffering agent is 10mM of a citrate buffer that maintains a pH of the pharmaceutical formulation at a pH of about 5.8. In some examples, the buffering agent is 10mM of a succinate buffer that maintains a pH of the pharmaceutical formulation at a pH of about 5.8.

In some examples, the stabilizing agent is selected from the group consisting of: carboxymethylcellulose (CMC), dextrose, polyethylene glycol (PEG), albumin, kelptose, proline, sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose, mannitol, sorbitol, poly-hydroxy compounds, polysaccharides, dextran, starch, hydroxyethyl starch, cyclodextrins, N-methyl pyrollidene, cellulose, and hyaluronic acid. In some examples, the stabilizing agent is selected from the group consisting of: sucrose, trehalose, mannose, maltose, lactose, glucose,

raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose, mannitol, sorbitol, poly-hydroxy compounds, polysaccharides, dextran, starch, hydroxyethyl starch, cyclodextrins, N-methyl pyrollidene, cellulose, and hyaluronic acid. In some examples, the stabilizing agent is sucrose.

In some examples, the stabilizing agent is present in the formulation at a concentration between 2-16% weight/volume. In some examples, the stabilizing agent is present at a concentration between 6-10% weight/volume. In some examples, the stabilizing agent is present at a concentration of at least 0.005% weight/volume, 0.01% weight/volume, 0.02% weight/volume, 0.03% weight/volume, 0.05% weight/volume, 0.06% weight/volume, 0.07% weight/volume, 0.08% weight/volume, 0.09% weight/volume, 0.1% weight/volume, 0.5% weight/volume, 0.7% weight/volume, 0.8% weight/volume, 0.9% weight/volume, 1.0% weight/volume, 1.2% weight/volume, 1.5% weight/volume, 1.7% weight/volume, 2% weight/volume, 3% weight/volume, 4% weight/volume, 5% weight/volume, 6% weight/volume, 7% weight/volume, 8% weight/volume, 9% weight/volume, 10% weight/volume, 11% weight/volume, 12% weight/volume, 13% weight/volume, 14% weight/volume, 15% weight/volume, 16% weight/volume, 17% weight/volume, 18% weight/volume, 19% weight/volume, or 20% weight/volume. In some examples, the stabilizing agent is present at a concentration of at least 8% weight/volume.

In some examples, the surfactant is selected from the group consisting of: sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate, chenodeoxycholic acid, N-lauroylsarcosine sodium salt, lithium dodecyl sulfate, 1-octanesulfonic acid sodium salt, sodium cholate hydrate, sodium deoxycholate, and glycodeoxycholic acid sodium salt, benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride monohydrate, hexadecyltrimethylammonium bromide, CHAPS, CHAPSO, SB3-10, SB3-12, digitonin, Triton X-100, Triton X-114, TWEEN-20, TWEEN-80, lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 40, 50 and 60, glycerol monostearate, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65, polysorbate 80, polaxamer188 and soy lecithin. In certain examples, the surfactant is selected from the group consisting of polaxamer188, sodium dodecyl sulfate (SDS), N -dodecyl-β-D-maltoside (DDM), polysorbate 20 and triton X. In certain examples, the surfactant is selected from the group consisting of polysorbate 20, polysorbate 80, poloxamer 124, poloxamer 127, poloxamer 188, and poloxamer 407.

In some examples, the surfactant is polysorbate 80. In some examples, the surfactant is polysorbate 20. In some examples, the surfactant is polaxamer 188.

In some examples, the surfactant is present in the formulation at a concentration of 0.02-2.0 mg/mL. In some examples, the surfactant is present in the formulation at a concentration of 0.02 mg/mL, 0.03 mg/mL, 0.04 mg/mL, 0.05 mg/mL, 0.06 mg/mL, 0.07 mg/mL, 0.08 mg/mL, 0.09 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL, 0.6 mg/mL, 0.7 mg/mL, 0.8 mg/mL, 0.9 mg/mL, 1 mg/mL, 1.2 mg/mL, 1.3 mg/mL, 1.4 mg/mL, 1.5 mg/mL, 1.6 mg/mL, 1.7 mg/mL, 1.8 mg/mL, 1.9 mg/mL, and 2.0 mg/mL. In some examples, the surfactant is present in the formulation at a concentration of 0.05-0.3 mg/mL. In some examples, the surfactant is present at a concentration of at least 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 0.9, or 1.0 mg/mL. In some examples, the pharmaceutical formulation comprises a concentration of at least 0.2 mg/mL of a surfactant. In some examples, the pharmaceutical formulation comprises a concentration of at least 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4 or 0.5 mg/mL of a surfactant. In some examples, the pharmaceutical formulation comprises a concentration of 0.05-0.5 mg/mL of a surfactant. In some examples, the pharmaceutical formulation comprises a concentration of 0.1-0.5 mg/mL of a surfactant.

In some examples, one or more of the pharmaceutical additives and/or excipients is an antioxidant. In some examples of the pharmaceutical formulations provided herein, the antioxidant concentration is between 0.001-50 mM. In some examples of the pharmaceutical formulations provided herein, the antioxidant concentration is between 7.5-50 mM. In some examples of the pharmaceutical formulations provided herein, the antioxidant concentration is between 5-20 mM. In some examples of the pharmaceutical formulations provided herein, the antioxidant concentration is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, or 50 mM.

In one example, the pharmaceutical formulation comprises an antioxidant selected from the group consisting of methionine (L or D form), tryptophan (L or D form), dimercaprol and pyridoxine. In one example, the pharmaceutical formulation comprises an antioxidant selected from the group consisting of methionine (L or D form), tryptophan (L or D form) and pyridoxine. In certain examples, the formulation includes L-methionine.

In certain examples, the formulations described herein include 1-50 mM of an antioxidant. In certain examples, the formulations described herein include 5 mM, 10 mM or 50mM of an antioxidant. In certain examples, the formulation includes 1-30 mM

of an antioxidant. In certain examples, the formulation includes 1-20 mM of an antioxidant. In certain examples, the formulation includes 5-15 mM of an antioxidant. In certain examples, the formulation includes 5-10 mM of an antioxidant. In certain examples, the formulation includes 10 mM, or at least 10 mM of an antioxidant.

In certain examples, the formulation includes 1-50 mM of L-methionine. In certain examples, the formulation includes 1-30 mM of L-methionine. In certain examples, the formulation includes 1-20 mM of L-methionine. In certain examples, the formulation includes 5-15 mM of L-methionine. In certain examples, the formulation includes 5-10 mM of L-methionine. In certain examples, the formulation includes 10 mM, or at least 10 mM of L-methionine. In certain examples, the formulations described herein include 5 mM, 10 mM or 50mM of L-methionine.

In some examples of the pharmaceutical formulations described herein, the pharmaceutical formulation optionally includes a chelating agent. In certain examples, the chelating agent is DTPA or EDTA. In certain examples, the chelating agent has a concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 μM. In one example, the liquid formulation includes 1-100 μM, 1-30 μM, 1-20 μM, 10 μM -30 μM of DTPA or EDTA. In certain examples, the chelating agent has a concentration between 7.5-100 μM. In certain examples, the chelating agent has a concentration of 10 μM.

In some examples, the pharmaceutical formulations provided herein comprise 10-100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, 10-50 mM of citrate buffer, 2-16% weight/volume of sucrose, 0.05-0.5 mg/mL of polysorbate 80, polysorbate 20 or polaxamer 188, 0-50 mM of L-methionine and 0-100 μM or DTPA or EDTA. In other examples, the pharmaceutical formulations provided herein comprise 50 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, 10 mM of citrate buffer, 8% weight/volume of sucrose, 0.2 mg/mL of polysorbate 80, and 20 mM of L-methionine. In some examples, the pharmaceutical formulations provided herein comprise 10-100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32 lacking the C-terminal lysine, 10-50 mM of citrate buffer, 2-16% weight/volume of sucrose, 0.05-0.5 mg/mL of polysorbate 80, polysorbate 20 or polaxamer 188, 0-50 mM of L-methionine and 0-100 μM or DTPA or EDTA.

In other examples, the pharmaceutical formulations provided herein comprise 50 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32, or a variant of SEQ ID NO: 32

lacking the C-terminal lysine, 10 mM of citrate buffer, 8% weight/volume of sucrose, 0.2 mg/mL of polysorbate 80, and 20 mM of L-methionine.

In some examples, the pharmaceutical formulations provided herein comprise 10-100 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32 and/or SEQ ID NO: 41, 10-50 mM of citrate buffer, 2-16% weight/volume of sucrose, 0.05-0.5 mg/mL of polysorbate 80, polysorbate 20 or polaxamer 188, 0-50 mM of L-methionine and 0-100 μM or DTPA or EDTA. In another aspect, there is provided a liquid pharmaceutical formulation comprising 50-100 mg/mL of a human ActRIIa fusion protein comprising the amino acid sequence of SEQ ID NO: 32 or SEQ ID NO:41, 10-50 mM of citrate buffer, 2-16% weight/volume of sucrose, 0.05-0.5 mg/mL of polysorbate 80, polysorbate 20 or polaxamer 188, 0-50 mM of L-methionine and 0-100 μM of DTPA or EDTA. In other examples, the pharmaceutical formulations provided herein comprise 50 mg/mL of a human ActRIIa fusion protein of SEQ ID NO: 32 and/or SEQ ID NO: 41, 10 mM of citrate buffer, 8% weight/volume of sucrose, 0.2 mg/mL of polysorbate 80, and 20 mM of L-methionine. In another aspect, there is provided a liquid pharmaceutical formulation comprising 50 mg/mL of a human ActRIIa fusion protein comprising the amino acid sequence of SEQ ID NO: 32 or SEQ ID NO:41, 10 mM of citrate buffer, 8% weight/volume of sucrose, 0.2 mg/mL of polysorbate 80, and 20 mM of L-methionine.

In some examples, the human ActRIIa fusion protein consists of the amino acid sequence of SEQ ID NO: 41. In some examples, the lyophilized formulation comprises a human ActRIIa fusion protein comprising the amino acid sequence of SEQ ID NO: 32 and a human ActRIIa fusion protein comprising the amino acid sequence of SEQ ID NO: 41. In some examples, the pharmaceutical formulation comprises sotatercept.

In certain examples, the pharmaceutical formulations described herein contain a mixture of SEQ ID NO: 32 and a variant of SEQ ID NO:32 lacking the C-terminal lysine residue (SEQ ID NO: 41). In certain examples, the pharmaceutical formulation described herein contain a mixture of SEQ ID NO: 32 and a variant of SEQ ID NO:32 lacking the C-terminal lysine residue (SEQ ID NO: 41) wherein the mixture contains 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%,

35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% by weight of SEQ ID NO:32.

In certain examples, the pharmaceutical formulation described herein contain a mixture of SEQ ID NO: 32 and a variant of SEQ ID NO:32 lacking the C-terminal lysine residue (SEQ ID NO: 41) wherein the mixture contains about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%,

46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% by weight of SEQ ID NO:32.

In certain examples, the pharmaceutical formulation described herein contain a mixture of SEQ ID NO: 32 and a variant of SEQ ID NO:32 lacking the C-terminal lysine residue (SEQ ID NO: 41) wherein the mixture contains 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% by weight of SEQ ID NO:41.

In certain examples, the pharmaceutical formulation described herein contain a mixture of SEQ ID NO: 32 and a variant of SEQ ID NO:32 lacking the C-terminal lysine residue (SEQ ID NO: 41) wherein the mixture contains about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% by weight of SEQ ID NO:41.

In certain examples, the pharmaceutical formulation described herein contain 100% of SEQ ID NO: 32 by weight. In certain examples, the pharmaceutical formulation described herein contain 100% of SEQ ID NO: 41 by weight.

The pharmaceutical formulations provided herein can be used for the treatment of pulmonary arterial hypertension in a subject in need thereof. In certain examples, the pharmaceutical formulations provided herein are liquid.

In another aspect, there is provided use of a pharmaceutical formulation as defined herein in the manufacture of a medicament for treating pulmonary arterial hypertension (PAH).

In another aspect, there is provided the pharmaceutical formulation as defined herein for use in therapy.

In certain examples, the pharmaceutical formulations provided herein are stable when stored between 2-8 °C. In certain examples, the liquid pharmaceutical formulations are stored under refrigerated conditions (temp. range: typically about 2-8 °C, but under

certain circumstances, the aqueous formulation may exhibit stability at other temperatures including at about 25 °C and about 40 °C for periods of up to about 3, 6, 9 or 12 months). In certain examples, the pharmaceutical formulation is administered via autoinjector. In certain examples, the pharmaceutical formulation is administered via subcutaneous injection. In some examples, the pharmaceutical formulation is administered parenterally. In certain examples, the formulations described herein are contained in an injection device. In certain examples, the formulations described herein are contained in an injection device, wherein the injection device is an autoinjector. In other examples, the formulations described herein are contained in a glass vial.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a multiple sequence alignment of various vertebrate ActRIIA proteins and human ActRIIA (SEQ ID NOs: 61-68).

Figures 2A and 2B show the purification of ActRIIA-hFc expressed in CHO cells. The protein purifies as a single, well-defined peak as visualized by sizing column (top panel) and Coomassie stained SDS-PAGE (bottom panel) (left lane: molecular weight standards; right lane: ActRIIA-hFc). See Example 1.

Figures 3A and 3B shows ActRIIA-hFc bound to activin with a dissociation constant (K_D) of 5×10^{-12} and bound to GDF11 with a K_D of 9.96×10^{-9}. See Example 1.

Figure 4 shows results of Stability pH Screening Study: Effect of pH and Buffer at t=0. See Example 3: pH and Buffer Feasibility Studies.

Figure 5 shows results of Stability pH Screening Study: Effect of pH and Buffer at 2 weeks at 40°C. See Example 3: pH and Buffer Feasibility Studies.

Figure 6 shows results of Stability pH Screening Study: Effect of pH and Buffer at 1 month at 40°C. See Example 3: pH and Buffer Feasibility Studies.

Figure 7 shows results of Stability pH Screening Study: Effect of pH and Buffer at 2 months at 40°C. See Example 3: pH and Buffer Feasibility Studies.

Figure 8 shows 1H NMR data for SEQ ID NO:32 with and without DTPA. See Example 3: Effect of Metal Chelator on SEQ ID NO: 32.

Figure 9 shows diffusion NMR data for SEQ ID NO:32 with and without DTPA. See Example 3: Effect of Metal Chelator on SEQ ID NO: 32.

Figure 10 shows results of colloidal stability studies for SEQ ID NO:32 and PS80 carried out by agitation stress. See Example 3: Colloidal Stability and Surfactant Screen.

WE CLAIM

1. A pharmaceutical formulation comprising 50-100 mg/mL of a human ActRIIa fusion protein comprising the amino acid sequence of SEQ ID NO: 32, or SEQ ID NO: 41, a buffer, a surfactant, a stabilizing agent, and optionally one or more antioxidants; wherein the buffer is not histidine.

2. The pharmaceutical formulation of claim 1, wherein the fusion protein has a concentration of 100 mg/mL.

3. The pharmaceutical formulation of claim 1, wherein the fusion protein has a concentration of 50 mg/mL.

4. The pharmaceutical formulation of anyone of claims 1-3, wherein the buffer is selected from the group consisting of succinate, phosphate, acetate, citrate, and glutamate.

5. The pharmaceutical formulation of claim 4, wherein the buffer is a phosphate buffer that maintains a pH of the formulation between 5-7.

6. The pharmaceutical formulation of claim 4, wherein the buffer is a citrate buffer that maintains a pH of the formulation between 4.5-7.

7. The pharmaceutical formulation of claim 4, wherein the buffer is an acetate buffer that maintains a pH of the formulation between 4.5-6.

8. The pharmaceutical formulation of claim 4, wherein the buffer is a succinate buffer that maintains a pH of the formulation between 4.5-5.

9. The pharmaceutical formulation of anyone of claims 1-3, wherein the buffer is a glutamate buffer that maintains a pH of the formulation between 5-7.

10. The pharmaceutical formulation of any one of claims 1-9, wherein the buffer maintains a pH of 5.8 of the formulation.

11. The pharmaceutical formulation of any one of claims 1-10, wherein the buffer is present at a concentration between 10-50 mM.

12. The pharmaceutical formulation of any one of claims 1-10, wherein the buffer is present at a concentration of at least 10 mM.

13. The pharmaceutical formulation of claim 12, wherein the stabilizing agent is sucrose.

14. The pharmaceutical formulation of any one of claims 1-13, wherein the stabilizing agent is present at a concentration between 2-16% weight/volume.

15. The pharmaceutical formulation of any one of claims 1-14, wherein the stabilizing agent is present at a concentration between 6-10 % weight/volume.

16. The pharmaceutical formulation of any one of claims 1-15, wherein the stabilizing agent is present at a concentration of at least 8% weight/volume.

17. The pharmaceutical formulation of any one of claims 1-16, wherein the surfactant is selected from the group consisting of polysorbate 20, polysorbate 80, poloxamer 124, poloxamer 127, poloxamer 188, and poloxamer 407.

18. The pharmaceutical formulation of claim 17, wherein the surfactant is polysorbate 80.

19. The pharmaceutical formulation of claim 17, wherein the surfactant is polysorbate 20.

20. The pharmaceutical formulation of claim 17, wherein the surfactant is poloxamer 188.

21. The pharmaceutical formulation of any one of claims 1-20, wherein the surfactant is present at a concentration between 0.05- 0.5 mg/ml.

22. The pharmaceutical formulation of any one of claims 1-20, wherein the surfactant is present at a concentration 0.1-0.5 mg/ml.

23. The pharmaceutical formulation of any one of claims 1-22, wherein the surfactant is present at a concentration of at least 0.2 mg/ml.

24. The pharmaceutical formulation of any one of claims 1-23, wherein the pharmaceutical formulation further comprises an antioxidant.

25. The pharmaceutical formulation of any one of claims 1-24, wherein the pharmaceutical formulation further comprises a chelating agent, wherein the chelating agent is DTPA or EDTA.

26. The pharmaceutical formulation of claim 25, wherein the chelating agent is present at a concentration between 7.5-100 μM.

27. The pharmaceutical formulation of any one of claims 25-26, wherein the chelating agent is present at a concentration of 10 μM.

28. The pharmaceutical formulation of claim 24, wherein the antioxidant is methionine.

29. The pharmaceutical formulation of claim 28, wherein the antioxidant is L-methionine.

30. The pharmaceutical formulation of any one of claims 28-29, wherein the antioxidant is present at a concentration of between 7.5-50 mM.

31. The pharmaceutical formulation of any one of claims 28-29, wherein the antioxidant is present at a concentration between 5-20 mM.

32. A liquid pharmaceutical formulation comprising 50-100 mg/mL of a human ActRIIa fusion protein comprising the amino acid sequence of SEQ ID NO: 32 or SEQ ID NO:41, 10-50 mM of citrate buffer, 2-16% weight/volume of sucrose, 0.05-0.5 mg/mL of polysorbate 80, polysorbate 20 or polaxamer 188, 0-50 mM of L-methionine and 0-100 μM of DTPA or EDTA.

33. A liquid pharmaceutical formulation comprising 50 mg/mL of a human ActRIIa fusion protein comprising the amino acid sequence of SEQ ID NO: 32 or SEQ ID NO:41, 10 mM of citrate buffer, 8% weight/volume of sucrose, 0.2 mg/mL of polysorbate 80, and 20 mM of L-methionine.

34. The pharmaceutical formulation of any one of claims 1-33, wherein the pharmaceutical formulation is for the treatment of pulmonary arterial hypertension in a subject in need thereof.

35. The pharmaceutical formulation any one of claims 1-34, wherein the pharmaceutical formulation is to be administered via autoinjector.

36. The pharmaceutical formulation any one of claims 1-35, wherein the pharmaceutical formulation is to be administered via subcutaneous injection.

37. The pharmaceutical formulation any one of claims 1-35, wherein the pharmaceutical formulation is a liquid formulation.

38. The pharmaceutical formulation any one of claims 1-35, wherein the pharmaceutical formulation is stable when stored between 2-8 °C for at least one month.

39. The pharmaceutical formulation of any of claims 1-38, wherein the formulation is contained in an injection device.

40. The pharmaceutical formulation of claim 39, wherein the injection device is an autoinjector.

41. The pharmaceutical formulation of any of claims 1-38, wherein the formulation is contained in a glass vial.

42. Use of a pharmaceutical formulation of any one of claims 1-41 in the manufacture of a medicament for treating pulmonary arterial hypertension (PAH).

43. The pharmaceutical formulation of any one of claims 1-41 for use in therapy.