conditions leading to post-capillary PH [Simonneau (2019) Eur Respir J: 53:1801913]. Diagnosis of various types of PH typically requires a series of tests.

In general, PH treatment depends on the cause or classification of PH. Where PH is caused by a known medicine or medical condition, it is known as a secondary PH, and its treatment is usually directed at the underlying disease. Treatment of Group 2 pulmonary hypertension (*e.g.*, venous hypertension) generally involves optimizing left ventricular function by administering diuretics, beta blockers, angiotensin receptor-neprilysin inhibitors (ARNI), and ACE inhibitors, cardiac resynchronization therapy, or repairing or replacing a mitral valve or aortic valve.

There is a high, unmet need for effective therapies for treating pulmonary hypertension. Accordingly, it is an object of the present disclosure to provide methods for treating, preventing, or reducing the progression rate and/or severity of PH, particularly treating, preventing or reducing the progression rate and/or severity of one or more PH-associated complications.

SUMMARY

In one aspect, the present disclosure refers to use of an ActRIIA fusion protein in the manufacture of a medicament for treating combined pre- and postcapillary pulmonary hypertension (Cpc-PH) due to heart failure with preserved ejection fraction (HFpEF) in a patient, wherein the ActRIIA fusion protein comprises:

(i) an ActRIIa polypeptide comprising the amino acid sequence of SEQ ID NO: 2;

(ii) an Fc domain comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 32; and

(iii) a linker domain positioned between the ActRIIa polypeptide and the Fc domain, wherein the ActRIIA fusion protein is to be administered to the patient at a dose of about 0.3 mg/kg once every three weeks.

In some examples, the disclosure provides for a method of treating post-capillary pulmonary hypertension (PcPH), comprising administering to a patient in need thereof an effective amount of a polypeptide comprising 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%, 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: 1 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: 1. In some examples, the disclosure provides for a method of treating, preventing, or reducing the progression rate and/or severity of one or more complications of post-capillary pulmonary hypertension (*e.g.*, WHO Group 2 and/or Group 5 PH), comprising administering to a patient in need thereof an effective amount of a polypeptide comprising 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%, 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: 1 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: 1. In some examples, the one or more complications of post-

capillary pulmonary hypertension is selected from the group consisting of: smooth muscle and/or endothelial cell proliferation in the pulmonary artery, angiogenesis in the pulmonary artery, dyspnea, chest pain, pulmonary vascular remodeling, right ventricular hypertrophy, left ventricular hypertrophy, left atrium dilation, left ventricular fibrosis, right ventricular fibrosis, and pulmonary fibrosis. In some examples, the PcPH is isolated post-capillary pulmonary hypertension (IpcPH). In some examples, the PcPH is combined post- and pre-capillary PH (CpcPH).

In some examples, the patient has Group 2 pulmonary hypertension as recognized by the World Health Organization (WHO). In some examples, the patient has pulmonary hypertension due to heart failure with preserved left ventricular ejection fraction (LVEF). In some examples, the patient has pulmonary hypertension due to heart failure with reduced left ventricular ejection fraction (LVEF). In some examples, the patient has valvular heart disease. In some examples, the patient has congenital/acquired cardiovascular conditions leading to post-capillary PH. In some examples, the patient has Group 5 pulmonary hypertension as recognized by the WHO. In some examples, the patient has pulmonary hypertension with unclear and/or multifactorial mechanisms. In some examples, the valvular heart disease is aortic regurgitation. In some examples, the valvular heart disease is aortic stenosis. In some examples, the valvular heart disease is mitral valve regurgitation. In some examples, the valvular heart disease is mitral valve stenosis.

In some examples, the patient has a mean pulmonary arterial pressure (mPAP) selected from the group consisting of an mPAP of at least 20 mmHg; an mPAP of at least 25 mmHg; an mPAP of at least 30 mmHg; an mPAP of at least 35 mmHg; an mPAP of at least 40 mmHg; an mPAP of at least 45 mmHg; and an mPAP of at least 50 mmHg. In some examples, the method reduces mPAP in the patient. In some examples, the method reduces the mPAP in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the method reduces the mPAP by at least 3 mmHg (*e.g.*, at least 3, 5, 7, 10, 12, 15, 20, or 25 mm Hg) in the patient.

In some examples, the patient has a pulmonary arterial wedge pressure (PAWP) of greater than 15 mmHg. In some examples, the method decreases the PAWP in the patient. In some examples, the method reduces the PAWP in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the patient has a left ventricular end diastolic pressure (LVEDP) of

greater than 15 mmHg. In some examples, the method decreases the LVEDP in the patient. In some examples, the method reduces the LVEDP in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or at least 50%). In some examples, the patient has a diastolic pressure gradient (DPG) of less than 7 mmHg. In some examples, the patient has a DPG of at least 7 mmHg. In some examples, the method decreases the DPG in the patient. In some examples, the method reduces the DPG in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the patient has a transpulmonary pressure gradient (TPG) of less than or equal to 12 mm Hg. In some examples, the patient has a TPG of greater than 12 mm Hg. In some examples, the method decreases the TPG in the patient. In some examples, the method reduces the TPG in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the patient has a pulmonary vascular resistance (PVR) greater than or equal to 3 Wood Units. In some examples, the method decreases the PVR in the patient. In some examples, the method reduces the PVR in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%).

In some examples, the method prevents the progression of IpcPH to CpcPH. In some examples, the method reduces the development of a pre-capillary component of PH. In some examples, the patient has preserved left ventricular ejection fraction. In some examples, the preserved left ventricular ejection fraction is greater than 45%. In some examples, the patient has reduced left ventricular ejection fraction. In some examples, the reduced left ventricular fraction is less than 45%. In some examples, the preserved left ventricular fraction is measured using echocardiography. In some examples, the patient has diastolic dysfunction of the left ventricle. In some examples, the patient has systolic dysfunction of the left ventricle. In some examples, the method decreases right ventricular hypertrophy in the patient. In some examples, the method decreases right ventricular hypertrophy in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the method decreases left ventricular hypertrophy in the patient. In some examples, the method decreases left ventricular hypertrophy in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the method decreases smooth muscle hypertrophy in the patient. In some

examples, the method decreases smooth muscle hypertrophy in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the method decreases pulmonary arteriole muscularity in the patient. In some examples, the method decreases pulmonary arteriole muscularity in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%).

In some examples, the patient has a right ventricular systolic pressure (RVSP) of greater than 35 mmHg. In some examples, the method decreases the RVSP in the patient. In some examples, the method reduces the RVSP in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or at least 50%). In some examples, the patient has left ventricular fibrosis. In some examples, the method decreases the left ventricular fibrosis in the patient. In some examples, the method reduces the left ventricular fibrosis in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or at least 50%). In some examples, the patient has right ventricular fibrosis. In some examples, the method decreases the right ventricular fibrosis in the patient. In some examples, the method reduces the right ventricular fibrosis in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or at least 50%). In some examples, the patient has pulmonary fibrosis. In some examples, the method decreases the pulmonary fibrosis in the patient. In some examples, the method reduces the pulmonary fibrosis in the patient by at least 10% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or at least 50%).

In some examples, the patient has a comorbidity selected from the group consisting of systemic hypertension, diabetes mellitus, obesity, coronary artery disease (CAD), heart failure, and anemia. In some examples, the method further comprises administering to the patient an additional active agent and/or supportive therapy. In some examples, the additional active agent and/or supportive therapy is selected from the group consisting of: beta-blockers, angiotensin-converting enzyme inhibitors (ACE inhibitors), angiotensin receptor blockers (ARBs), neprilysin inhibitors, angiotensin receptor-neprilysin inhibitors (ARNI), mineralocorticoid receptor antagonists (MRA), hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blockers, diuretic agents, lipid-lowering medications, endothelin blockers, PDE5 inhibitors, prostacyclins, cardiac resynchronization therapy, valve replacement, valve repair, implantable cardioverter-defibrillator (ICD), or a left ventricular assist device (LVAD). In some examples, the additional active agent and/or supportive therapy is selected from the group consisting of: prostacyclin and derivatives thereof (*e.g.*,

epoprostenol, treprostinil, and iloprost); prostacyclin receptor agonists (*e.g.*, selexipag); endothelin receptor antagonists (*e.g.*, thelin, ambrisentan, macitentan, and bosentan); calcium channel blockers (*e.g.*, amlodipine, diltiazem, and nifedipine; anticoagulants (*e.g.*, warfarin); diuretics; oxygen therapy; atrial septostomy; pulmonary thromboendarterectomy; phosphodiesterase type 5 inhibitors (*e.g.*, sildenafil and tadalafil); activators of soluble guanylate cyclase (*e.g.*, cinaciguat and riociguat); ASK-1 inhibitors (*e.g.*, CIIA; SCH79797; GS-4997; MSC2032964A; 3H-naphtho[1,2,3-de]quiniline-2,7-diones, NQDI-1; 2-thioxo-thiazolidines, 5-bromo-3-(4-oxo-2-thioxo-thiazolidine-5-ylidene)-1,3-dihydro-indol-2-one); NF-κB antagonists (*e.g.*, dh404, CDDO-epoxide; 2.2-difluoropropionamide; C28 imidazole (CDDO-Im); 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO); 3-Acetyloleanolic Acid; 3-Triflouroacetyloleanolic Acid; 28-Methyl-3-acetyloleanane; 28-Methyl-3-trifluoroacetyloleanane; 28-Methyloxyoleanolic Acid; SZC014; SCZ015; SZC017; PEGylated derivatives of oleanolic acid; 3-O-(beta-D-glucopyranosyl) oleanolic acid; 3-O-[beta-D-glucopyranosyl-(1-->3)-beta-D-glucopyranosyl] oleanolic acid; 3-O-[beta-D-glucopyranosyl-(1-->2)-beta-D-glucopyranosyl] oleanolic acid; 3-O-[beta-D-glucopyranosyl-(1-->3)-beta-D-glucopyranosyl] oleanolic acid 28-O-beta-D-glucopyranosyl ester; 3-O-[beta-D-glucopyranosyl-(1-->2)-beta-D-glucopyranosyl] oleanolic acid 28-O-beta-D-glucopyranosyl ester; 3-O-[a-L-rhamnopyranosyl-(1-->3)-beta-D-glucuronopyranosyl] oleanolic acid; 3-O-[alpha-L-rhamnopyranosyl-(1-->3)-beta-D-glucuronopyranosyl] oleanolic acid 28-O-beta-D-glucopyranosyl ester; 28-O-β-D-glucopyranosyl-oleanolic acid; 3-O-β-D-glucopyranosyl (1→3)-β-D-glucopyranosiduronic acid (CS1); oleanolic acid 3-O-β-D-glucopyranosyl (1→3)-β-D-glucopyranosiduronic acid (CS2); methyl 3,11-dioxoolean-12-en-28-olate (DIOXOL); $ZCVI_4$-2; Benzyl 3-dehydr-oxy-1,2,5-oxadiazolo[3',4':2,3]oleanolate); eplerenone, spironolactone, ivabradine, implantable cardioverter-defibrillator (ICD), a left ventricular assist device (LVAD), or lung and/or heart transplantation.

In some examples, the patient has elevated brain natriuretic peptide (BNP) levels as compared to a healthy patient. In some examples, the patient has a BNP level of at least 100 pg/mL (*e.g.*, 100, 150, 200, 300, 400, 500, 1000, 3000, 5000, 10,000, 15,000, or 20,000 pg/mL). In some examples, the method decreases BNP levels in the patient by at least 10% (*e.g.*, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the method decreases BNP levels to normal levels (i.e., <100 pg/ml). In some examples, the method decreases NT-proBNP levels in the patient. In some examples, the method decreases NT-proBNP levels in the patient by at least 10%

(e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or at least 80%). In some examples, the method decreases NT-proBNP levels in the patient by at least 30%. In some examples, the method decreases NT-proBNP levels to normal levels. In some examples, the normal level of NT-proBNP is <100 pg/ml. In some examples, the method increases exercise capacity of the patient. In some examples, the patient has a 6-minute walk distance from 150 to 400 meters. In some examples, the patient has a 6-minute walk distance from 150 to 550 meters. In some examples, the method increases the patient's 6-minute walk distance. In some examples, the method increases the patient's 6-minute walk distance by at least 10 meters (*e.g.*, at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 250, 300, or more than 400 meters). In some examples, the method reduces the patient's Borg dyspnea index (BDI). In some examples, the method reduces the patient's BDI by at least 0.5 index points (*e.g.*, at least 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 index points).

In some examples, the patient has decreased renal function. In some examples, the method further improves renal function. In some examples, the patient has Functional Class II or Class III pulmonary hypertension in accordance with the World Health Organization's functional classification system for pulmonary hypertension. In some examples, the patient has Functional Class I, Class II, Class III, or Class IV pulmonary hypertension as recognized by the World Health Organization. In some examples, the method prevents or delays pulmonary hypertension Functional Class progression (*e.g.*, prevents or delays progression from Functional Class I to Class II, Class II to Class III, or Class III to Class IV pulmonary hypertension as recognized by the World Health Organization). In some examples, the method promotes or increases pulmonary hypertension Functional Class regression (*e.g.*, promotes or increases regression from Class IV to Class III, Class III to Class II, or Class II to Class I pulmonary hypertension as recognized by the World Health Organization). In some examples, the patient has Functional Class II or Class III pulmonary hypertension in accordance with the New York Heart Association's functional classification system for pulmonary hypertension. In some examples, the patient has Functional Class I, Class II, Class III, or Class IV pulmonary hypertension as recognized by the New York Heart Association. In some examples, the method prevents or delays pulmonary hypertension Functional Class progression (e.g., prevents or delays progression from Functional Class I to Class II, Class II to Class III, or Class III to Class IV pulmonary hypertension as recognized by the New York Heart Association). In some examples, the

method promotes or increases pulmonary hypertension Functional Class regression (e.g., promotes or increases regression from Class IV to Class III, Class III to Class II, or Class II to Class I pulmonary hypertension as recognized by the New York Heart Association). In some examples, the method delays clinical worsening of PcPH. In some examples, the method delays clinical worsening of PcPH in accordance with the World Health Organization's functional classification system for pulmonary hypertension. In some examples, the method delays clinical worsening of PcPH in accordance with the New York Heart Association's functional classification system for pulmonary hypertension. In some examples, the method reduces the risk of hospitalization for one or more complications associated with PcPH. In some examples, the patient has a hemoglobin level from >8 and <15 g/dl.

In some examples, the patient has been treated with one or more vasodilators. In some examples, the patient has been treated with one or more agents selected from the group consisting of: phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, prostacyclin receptor agonist, and endothelin receptor antagonists. In some examples, the one or more agents is selected from the group consisting of: bosentan, sildenafil, beraprost, macitentan, selexipag, epoprostenol, treprostinil, iloprost, ambrisentan, and tadalafil. In some examples, the method further comprises administration of one or more vasodilators. In some examples, the method further comprises administration of one or more agents selected from the group consisting of: phosphodiesterase type 5 inhibitors, soluble guanylate cyclase stimulators, prostacyclin receptor agonist, and endothelin receptor antagonists. In some examples, the one or more agents is selected from the group consisting of: bosentan, sildenafil, beraprost, macitentan, selexipag, epoprostenol, treprostinil, iloprost, ambrisentan, and tadalafil.

In some examples, the ActRII polypeptide 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%, 99%, or 100% identical to the sequence of amino acids corresponding to residues 30-110 of SEQ ID NO: 1. In some examples, the ActRII polypeptide 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%, 99%, or 100% identical to the amino acid sequence SEQ ID NO: 2. In some examples, the ActRII polypeptide 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%, 99%, or 100% identical to the amino acid sequence of SEQ

ID NO: 3. In some examples, the ActRII polypeptide is a fusion protein further 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 Fc fusion protein further comprises a linker domain positioned between the ActRII polypeptide domain and the Fc domain of the immunoglobulin. In some examples, the linker domain is selected from the group consisting of: TGGG (SEQ ID NO: 20), TGGGG (SEQ ID NO: 18), SGGGG (SEQ ID NO: 22), GGGGS (SEQ ID NO: 22), GGG (SEQ ID NO: 16), GGGG (SEQ ID NO: 17), and SGGG (SEQ ID NO: 21). In some examples, the ActRII polypeptide 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%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 23. In some examples, the polypeptide comprises an amino acid sequence that is at least 90% identical to an amino acid sequence corresponding to residues 30-110 of SEQ ID NO: 1, wherein the polypeptide binds to activing and/or GDF11. In some examples, the polypeptide comprises an amino acid sequence that is at least 90% identical to an amino acid sequence corresponding to residues 21-135 of SEQ ID NO: 1, wherein the polypeptide binds to activing and/or GDF11. In some examples, the polypeptide is lyophilized. In some examples, the polypeptide is soluble. In some examples, the polypeptide is administered using subcutaneous injection. In some examples, the polypeptide is administered every 4 weeks. In some examples, the polypeptide is part of a homodimer protein complex. In some examples, the polypeptide is glycosylated. In some examples, the polypeptide has a glycosylation pattern obtainable by expression in a Chinese hamster ovary cell. In some examples, the ActRII polypeptide binds to one or more ligands selected from the group consisting of: activin A, activin B, and GDF11. In some examples, the ActRII polypeptide further binds to one or more ligands selected from the group consisting of: BMP10, GDF8, and BMP6.

In certain aspects, the disclosure relates to a kit comprising a lyophilized polypeptide and an injection device, wherein the polypeptide is an ActRII polypeptide comprising 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%, 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: 1 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: 1. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that

is at least 90% identical to an amino acid sequence corresponding to residues 30-110 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence corresponding to residues 30-110 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 99% identical to the amino acid sequence corresponding to residues 30-110 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide comprising the amino acid sequence corresponding to residues 30-110 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide consisting of the amino acid sequence corresponding to residues 30-110 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 90% identical to the amino acid sequence corresponding to residues 21-135 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence corresponding to residues 21-135 of SEQ ID NO: 1.

In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 99% identical to the amino acid sequence corresponding to residues 21-135 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide comprising the amino acid sequence corresponding to residues 21-135 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide consisting of the amino acid sequence corresponding to residues 21-135 of SEQ ID NO: 1. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 2. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 2. In some examples, the polypeptide is a polypeptide comprising the amino acid sequence of SEQ ID NO: 2. In some examples, the polypeptide is a polypeptide consisting of the amino acid sequence of SEQ ID NO: 2. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 3. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 3. In some examples, the polypeptide is a polypeptide comprising an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 3. In some examples, the polypeptide is a polypeptide comprising the amino acid sequence of SEQ ID NO: 3. In

some examples, the polypeptide is a polypeptide consisting of the amino acid sequence of SEQ ID NO: 3.

In some examples, the polypeptide is a fusion protein further comprising an Fc domain of an immunoglobulin. In some examples, the immunoglobulin is an Fc domain of an IgG1 immunoglobulin. In some examples, the fusion protein further comprises a linker domain positioned between the polypeptide domain and the Fc domain of the immunoglobulin. In some examples, the linker domain is selected from the group consisting of: TGGG (SEQ ID NO: 20), TGGGG (SEQ ID NO: 18), SGGGG (SEQ ID NO: 19), GGGGS (SEQ ID NO: 22), GGG (SEQ ID NO: 16), GGGG (SEQ ID NO: 17), and SGGG (SEQ ID NO: 21). In some examples, the linker domain comprises TGGG (SEQ ID NO: 20). In some examples, the ActRII polypeptide 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%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 23. In some examples, the ActRII polypeptide comprises the amino acid sequence of SEQ ID NO: 23. In some examples, the ActRII polypeptide consists of the amino acid sequence of SEQ ID NO: 23. In some examples, the polypeptide is part of a homodimer protein complex. In some examples, the polypeptide is glycosylated. In some examples, the polypeptide binds to one or more ligands selected from the group consisting of: activin A, activin B, and GDF11. In some examples, the polypeptide further binds to one or more ligands selected from the group consisting of: BMP10, GDF8, and BMP6. In some examples, the polypeptide binds to activin and/or GDF11.

In some examples, the kit comprises one or more vials containing the lyophilized polypeptide. In some examples, the injection device comprises a pre-filled syringe. In some examples, the injection device comprises a pump apparatus. In some examples, the pump apparatus comprises an electromechanical pumping assembly. In some examples, the pump apparatus is a wearable pump apparatus. In some examples, the pre-filled syringe comprises a reconstitution solution. In some examples, the reconstitution solution comprises a pharmaceutically acceptable carrier and/or excipient. In some examples, the pharmaceutically acceptable carrier is selected from saline solution, purified water, or sterile water for injection. In some examples, the pharmaceutically acceptable excipient is selected from a buffering agent [*e.g.*, citric acid (monohydrate) and/or trisodium citrate (dehydrate)], a surfactant (*e.g.*, polysorbate 80), a stabilizer (*e.g.*, sucrose), and a lyoprotectant (*e.g.*, sucrose).

In some examples, the injection device comprises a vial adapter. In some examples, the vial adapter is capable of attaching to a vial. In some examples, the vial adapter is capable of attaching to a pre-filled syringe. In some examples, the pre-filled syringe and the vial are attached to opposite ends of the vial adapter. In some examples, the reconstitution solution is transferred from the pre-filled syringe to the vial. In some examples, the lyophilized polypeptide is reconstituted into a sterile injectable solution. In some examples, the lyophilized polypeptide is reconstituted into a sterile injectable solution prior to use. In some examples, the sterile injectable solution is sterile water for injection.

In some examples, the sterile injectable solution is administered parenterally. In some examples, the injection device is used to administer the sterile injectable solution parenterally. In some examples, the sterile injectable solution is administered via subcutaneous injection. In some examples, the sterile injectable solution is administered via intradermal injection. In some examples, the sterile injectable solution is administered via intramuscular injection. In some examples, the sterile injectable solution is administered via intravenous injection. In some examples, the sterile injectable solution is self-administered. In some examples, the sterile injectable solution comprises a therapeutically effective dose. In some examples, the therapeutically effective dose comprises a weight based dose. In some examples, the lyophilized polypeptide is administered every 4 weeks.

In some examples, the kit is used to treat post-capillary pulmonary hypertension (PcPH). In some examples, the PcPH is isolated post-capillary pulmonary hypertension (IpcPH). In some examples, the PcPH is combined post- and pre-capillary PH (CpcPH). In some examples, the patient has Group 2 pulmonary hypertension as recognized by the WHO. In some examples, the patient has pulmonary hypertension due to heart failure with preserved left ventricular ejection fraction (LVEF). In some examples, the patient has pulmonary hypertension due to heart failure with reduced left ventricular ejection fraction (LVEF). In some examples, the patient has valvular heart disease. In some examples, the patient has congenital/acquired cardiovascular conditions leading to post-capillary PH. In some examples, the patient has Group 5 pulmonary hypertension as recognized by the WHO. In some examples, the patient has pulmonary hypertension with unclear and/or multifactorial mechanisms. In some examples, the shelf life of the lyophilized polypeptide is at least 1, 1.5, 2, 2.5, or 3 years. In some examples, the lyophilized polypeptide is

reconstituted. In some examples, the reconstituted polypeptide has a shelf life of at least 2 hrs, 3 hrs, or 4 hrs.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing/photograph executed in color. Copies of this patent with color drawing(s)/photograph(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 shows an alignment of extracellular domains of human ActRIIB (SEQ ID NO: 31) and human ActRIIA (SEQ ID NO: 2) with the residues that are deduced herein, based on composite analysis of multiple ActRIIB and ActRIIA crystal structures, to directly contact ligand indicated with boxes.

Figure 2 shows a multiple sequence alignment of various vertebrate ActRIIA proteins and human ActRIIA (SEQ ID NOs: 6-10 and 36-38).

Figure 3 shows multiple sequence alignment of Fc domains from human IgG isotypes using Clustal 2.1. Hinge regions are indicated by dotted underline. Double underline indicates examples of positions engineered in IgG1 Fc (SEQ ID NO: 32) to promote asymmetric chain pairing and the corresponding positions with respect to other isotypes IgG2 (SEQ ID NO: 33), IgG3 (SEQ ID NO: 34) and IgG4 (SEQ ID NO: 35).

Figures 4A and 4B show the purification of ActRIIA-hFc expressed in CHO cells. The protein purifies as a single, well-defined peak as visualized by sizing column (**Figure 4A**) and Coomassie stained SDS-PAGE (**Figure 4B**) (left lane: molecular weight standards; right lane: ActRIIA-hFc).

Figures 5A and 5B show the binding of ActRIIA-hFc to activin (**Figure 5A**) and GDF-11 (**Figure 5B**), as measured by Biacore™ assay.

Figure 6 shows a schematic image of a linearized version of cardiopulmonary circulation and the regions associated with various types of PH. The difference between pre-capillary pulmonary hypertension, isolated post-capillary pulmonary hypertension, and combined post- and pre-capillary pulmonary hypertension are based on pulmonary hemodynamic parameters and the involvement of various regions of the cardiopulmonary system (pre and/or post capillary regions). Abbreviations are as follows: VC – vena cava; RA – right atrium; RV – right ventricle; PA – pulmonary artery; PC – pulmonary capillaries; PV –

We Claim:

1. Use of an ActRIIA fusion protein in the manufacture of a medicament for treating combined pre- and postcapillary pulmonary hypertension (Cpc-PH) due to heart failure with preserved ejection fraction (HFpEF) in a patient, wherein the ActRIIA fusion protein comprises:

(i) an ActRIIa polypeptide comprising the amino acid sequence of SEQ ID NO: 2;

(ii) an Fc domain comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 32; and

(iii) a linker domain positioned between the ActRIIa polypeptide and the Fc domain,

wherein the ActRIIA fusion protein is to be administered to the patient at a dose of about 0.3 mg/kg once every three weeks.

2. The use of claim 1, wherein the patient has a pulmonary vascular resistance (PVR) greater than 2 Woods Units.

3. The use of claim 1 or 2 1, wherein the Fc domain comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 32.

4. The use of any one of claims 1-3, wherein the Fc domain comprises the amino acid sequence of SEQ ID NO: 32.

5. The use of any one of claims 1-4, wherein the linker domain is selected from the group consisting of: TGGG (SEQ ID NO: 20), TGGGG (SEQ ID NO: 18), SGGGG (SEQ ID NO: 19), GGGGS (SEQ ID NO: 22), GGGG (SEQ ID NO: 17), and SGGG (SEQ ID NO: 21).

6. The use of any one of claims 1-5, wherein the linker domain comprises SEQ ID NO: 20.

7. The use of any one of claims 1-6, wherein the fusion protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NOs: 23 or 41.

8. The use of any one of claims 1-7, wherein the fusion protein comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 23.

9. The use of any one of claims 1-8, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 23.

10. The use of any one of claims 1-9, wherein the fusion protein comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of SEQ ID NO: 41.

11. The use of any one of claims 1-10, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 41.

12. The use of any one of claims 1-11, wherein the patient has Group 2 pulmonary hypertension as recognized by the World Health Organization (WHO).

13. The use of any one of claims 1-12, wherein the patient has a pulmonary vascular resistance (PVR) greater than or equal to 3 Wood Units.

14. The use of any one of claims 1-13, wherein the medicament reduces the PVR in the patient.

15. The use of any one of claims 1-14, wherein the medicament reduces the PVR in the patient by at least 10%.

16. The use of any one of claims 1-15, wherein the patient has preserved left ventricular ejection fraction.

17. The use of any one of claims 1-16, wherein the preserved left ventricular ejection fraction is greater than 45%.

18. The use of any one of claims 1-17, wherein the medicament increases the patient's 6-minute walk distance.

19. The use of any one of claims 1-18, wherein the medicament increases the patient's 6-minute walk distance by at least 10 meters.

20. The use of any one of claims 1-19, wherein the medicament delays clinical worsening of post-capillary pulmonary hypertension (PcPH).

21. The use of any one of claims 1-20, wherein the medicament delays clinical worsening of PcPH in accordance with the World Health Organization's functional classification system for pulmonary hypertension.

22. The use of any one of claims 1-21, wherein the medicament delays clinical worsening of PcPH in accordance with the New York Heart Association's functional classification system for pulmonary hypertension.

23. The use of any one of claims 1-22, wherein the patient is to be administered the ActRIIA fusion protein at a dose of 0.3 mg/kg once every three weeks.