

CLAIMS

1. Use of an rAAV vector comprising a nucleic acid sequence encoding a phosphatase inhibitor (I-1) protein that inhibits phosphatase activity in the preparation of a medicament for the treatment of heart failure in a patient, in which at least one total dose of the rAAV is to be administered into heart cells of the patient having a classification of congestive heart failure (CHF), in which the at least one dose of the rAAV is selected from a total dose-range of about 10^{13} vg to about 10^{15} vg, in which the at least one total dose is to be administered in a series of sub-doses, in which each sub-dose is to be administered over a period of time of about 1 minute to about 5 minutes, and in which at least twelve months post-administration there is an improvement in the classification of congestive heart failure.
2. Use according to Claim 1, in which the classification is based upon a classification system used by the American Heart Association (AHA), the American College of Cardiology (ACC), Minnesota LIVING WITH HEART FAILURE® Questionnaire (MLHFQ), Kansas City Cardiomyopathy questionnaire (KCCQ), or the 2016 European Society of Cardiology guidelines (ESCG), the Japanese heart failure Society (JHFS) guidelines, The Japanese Circulation Society (JCS) Guidelines, or, the New York Heart Association (NYHA).
3. Use according to Claim 1 or 2, in which there is an improvement in the classification of at least one level within 6 or 12 months after administration of the rAAV, or in which twelve months post-administration there is an improvement of at least 2 levels in the Classification.
4. Use according to Claim 1, 2 or 3, in which the classification system is NYHA and the level of classification is selected from the group consisting of: Class I, Class II, Class III and Class IV, or in which the classification system is the American College of Cardiology/American Heart Association (ACC/AHA) complementary staging system and the level of classification is selected from the group consisting of: Stages A, Stage B, Stage C, Stage D, or in which the classification system is KCCQ and the level of classification is a KQQC overall summary score range selected from the group consisting of: KCCQ fair to excellent scores of 50 to 100, very poor to fair scores of 0 to 49, good to excellent scores of 75 to 100 and very poor to good scores of 0 to 74.
5. Use of an rAAV vector comprising a nucleic acid sequence encoding a phosphatase inhibitor (I-1) protein that inhibits phosphatase activity in the preparation of a medicament for the treatment of cardiomyopathy in a patient, in which at least one total dose of rAAV is to be

administered into heart cells of the patient, in which the at least one dose of the rAAV is selected from a total dose-range of about 10^{13} vg to about 10^{15} vg, in which the at least one total dose is to be administered in a series of sub-doses, in which each sub-dose is to be administered over a period of time of about 1 minute to about 5 minutes, in which at least
 5 twelve months post-administration there is an improvement in the at least one parameter from a baseline level in the patient and in which the at least one parameter is selected from the group consisting essentially of:

- (a) ejection fraction (EF),
- (b) end systolic volume (ESV)
- 10 (c) cardiac contractility, selected from ejection fraction (EF) and fractional shortening (FS)
- (d) cardiac volumes selected from any of: end diastolic volume (DV) and end systolic volume (ESV),
- (e) functional criteria, selected from any of: a 6-minute walk test (6MWT), exercise
 15 and VO₂max;
- (f) BNP level,
- (g) Pro-BNP level
- (h) biomarker level, in which the biomarker level is selected from the group of: troponin, serum creatinine, cystatin-C, or hepatic transaminases,
- 20 (i) Patient-reported outcomes (PROs), such as reduced symptoms, health-related quality of life (HRQOL), or patient perceived health status and
- (j) decrease in any of: mortality risk due to heart failure, reduced hospitalization due to heart failure symptoms, or therapeutic intervention for treatment of heart failure.

6. Use according to Claim 5, in which the improvement is selected from any of:

- 25 (a) at least a 5% or more increase in ejection fraction from baseline,
- (b) at least a 10% decrease, or at least a 20ml decrease in end systolic volume from baseline,
- (c) at least a 50-meter increase in 6-minute walk test from baseline,

- (d) at least a 40% decrease in BNP levels (pg/ml) in the blood from baseline,
 - (e) at least a 35% decrease in pro-BNP levels (pg/ml) in the blood from baseline,
 - (f) at least a 10% reduction in a biomarker selected from: troponin, serum creatinine, cystatin-C, or hepatic transaminases from a baseline level of the same biomarker,
 - 5 (g) at least a 1.5ml/kg/min increase in myocardial oxygen consumption (MVO₂) from baseline, or
 - (h) a discharge from hospital due to improved HF symptoms, or a reduced intervention selected from a decrease in the use of any of: inotropes, vasodilators, diuretics due to improved HF symptoms in the subject.
- 10 7. Use according to any preceding claim, in which the series of sub-doses is to be administered as any of the following administration methods:
- (a) over a period of time of about 20 minutes to about 30 minutes,
 - (b) to be administered in a series of five sub-doses, each sub-dose is to be administered over a period of time of about 1 minute to about 5 minutes and in which
 - 15 the five sub-doses are to be administered over a period of about 20 minutes to about 30 minutes.
8. Use according to any of Claims 1 to 6, in which there rAAV vector comprises a capsid that detargets the liver.
9. Use according to any preceding claim, in which the rAAV is selected from the group
- 20 consisting of AAV1, AAV2, AAV6, AAV8, AAV9, AAV2i8, rh10, AAV2.5 and AAV2G9.
10. Use according to any preceding claim, in which at least one total dose of the rAAV is 10^{13} vg, 3×10^{13} vg, 10^{14} vg, 3×10^{14} vg, or, 10^{15} vg.
11. Use according to Claim 1, in which the I-1c is selected from any of:
- (a) a polypeptide comprises at least amino acid residues 1-54 of SEQ ID NO: 1, in
 - 25 which SEQ ID NO: 1 is truncated at the C-terminus at amino acid 70, 67, 66, 65, or 61, or 54 and where there is an aspartic acid at position 35 (T35D),
 - (b) a polypeptide comprising amino acids 1-54 of SEQ ID NO:1 or a functional fragment thereof, in which the functional fragment has at least 85% sequence identity to

amino acid residues 1-54 of SEQ ID NO: 1, or truncated at the C-terminus at amino acid 70, 67, 66, 65, or 61, or 54 and where there is an aspartic acid at position 35 (T35D),

(c) a constitutively active protein (I-1c), or

(d) a polypeptide encoded by a codon-optimized I-1C nucleic acid.

5 12. Use according to any preceding claim, in which the rAAV genome comprises nucleic acid sequence selected from the group consisting of: SEQ ID NO: 413-441.

13. Use according to Claim 1, in which the nucleic acid sequence encoding the I-1 polypeptide is selected from

10 (a) a nucleic acid sequence encoding a polypeptide comprising amino acids 1-65 of SEQ ID NO: 1, in which threonine (T) at position 35 of SEQ ID NO: 1 is replaced with an amino acid that is not T,

(b) a nucleic acid sequence encoding a polypeptide comprising amino acids 1-65 of SEQ ID NO: 1, in which threonine (T) at position 35 of SEQ ID NO: 1 is replaced with an acid amino acid selected from any of: aspartic acid (D), glutamic acid (E), asparagine (N), glutamine (Q),

(c) a nucleic acid sequence encoding a polypeptide comprising amino acids 1-65 of SEQ ID NO: 1, in which threonine (T) at position 35 of SEQ ID NO: 1 is replaced with aspartic acid (D), or a conservative amino acid of aspartic acid.

20 (d) any of SEQ ID NO: 385-412, or a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO: 385-412.

14. Use according to any preceding claim, in which the subject with cardiomyopathy has non-ischemic heart failure and/or non-ischemic cardiomyopathy, or in which the subject with cardiomyopathy has a congenital cardiomyopathy or a genetic disorder with a cardiac manifestation.

25 15. Use according to Claim 1, in which the subject with a congenital cardiomyopathy or a genetic disorder with a cardiac manifestation has a disease or disorder selected from the group consisting of: Arrhythmogenic right ventricular cardiomyopathy, Atrial myxoma, familial, Atrial septal defect ostium primum, Atrial septal defect sinus venosus, Barth syndrome, muscular dystrophy, Buerger disease, Cardioencephalomyopathy, Chromosome 1p36 deletion
30 syndrome, Congenital generalized lipodystrophy type 4, Congenital heart block, Dilated

cardiomyopathy, Duchenne muscular dystrophy (DMD), Fabry disease, Familial atrial fibrillation, Familial dilated cardiomyopathy, Familial hypertrophic cardiomyopathy, Familial progressive cardiac conduction defect, Familial thoracic aortic aneurysm and aortic dissection, Fibromuscular dysplasia, Friedreich ataxia, Gaucher disease, Glycogen storage disease (types 2, 3 or 4), His bundle tachycardia, Hurler syndrome, Hypoplastic left heart syndrome, Infantile histiocytoid cardiomyopathy, Intracranial arteriovenous malformation, Isobutyryl-CoA dehydrogenase deficiency, Kallikrein hypertension, Kawasaki disease, Kearns-Sayre syndrome, Left ventricular noncompaction, Limb-girdle muscular dystrophy (types 1B, 2E, 2F, 2M, 2C, 2D), Limited systemic sclerosis, Long QT syndrome 1, Lymphedema and cerebral arteriovenous anomaly, Lymphocytic vasculitis, Microcephaly-cardiomyopathy; Mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes, Mitochondrial trifunctional protein deficiency, Myotonic dystrophy type 1, Neonatal stroke, Noonan syndromes 1 -, 2-, 3-, 4-, 5- and 6, Peripartum cardiomyopathy, Peters plus syndrome, PGM1-CDG, PHACE syndrome, Phospholamban Arg 14 Deletion, Postural orthostatic tachycardia syndrome, Primary carnitine deficiency, Progressive familial heart block (types 1A, 1B and 2), Pseudohypoaldosteronism type 2, Pulmonary arterial hypertension, Pulmonary atresia with intact ventricular septum, Pulmonary atresia with ventricular septal defect, Pulmonary valve stenosis, Pulmonary vein stenosis, Pulmonic stenosis, Renoprival hypertension, Retinal arterial macroaneurysm with supra-ventricular pulmonic stenosis, Right ventricle hypoplasia, Sarcoidosis, Sengers syndrome, Situs inversus, Sudden Arrhythmia Death Syndrome, Supra-ventricular aortic stenosis, Swyer syndrome, TANGO2-Related Metabolic Encephalopathy and Arrhythmias, TARP syndrome, Tetralogy of Fallot, Timothy syndrome, Tricuspid atresia, Vici syndrome, VLCAD deficiency and Williams syndrome.

16. Use according to any preceding claim, in which at least twelve months post-administration of the rAAV there is an improvement of at least one class in a classification of heart failure from a baseline level, in which the classification of heart failure is assessed by at least one of the following:

(a) a classification system used by the American Heart Association (AHA), the American College of Cardiology (ACC), the 2016 European Society of Cardiology guidelines (ESCG), the Japanese heart failure Society (JHFS) guidelines, The Japanese Circulation Society (JCS) Guidelines, or, the New York Heart Association (NYHA); or equivalent thereof, or

- (b) a health-related quality of life (HRQL) questionnaire selected from the group consisting from any of: Minnesota LIVING WITH HEART FAILURE® Questionnaire (MLHFQ), or Kansas City Cardiomyopathy questionnaire (KCCQ), Chronic Heart Failure Questionnaire (CHFQ), Quality of Life Questionnaire for Severe Heart Failure (QLQ-SHF), Left Ventricular Dysfunction (LVD-36) questionnaire and the Left Ventricular Disease Questionnaire (LVDQ).
17. Use according to any preceding claim, in which the subject is to be administered a vasodilator concurrent with, and/or before, and/or after the administration of the at least one total dose of a rAAV vector, and/or in which the subject is to be administered an immune modulator concurrent with, or before, or after the administration of the at least one total dose of a rAAV vector.
18. A pharmaceutical composition comprising an AAV vector that comprises a codon optimized I-Ic nucleic sequence selected from any of SEQ ID NO: 385-412, or nucleic acid sequence having at least 80% sequence identity to SEQ ID NOS: 385-412, in which the composition is formulated to be administered as at least one total dose to be administered in a series of sub-doses, in which each sub-dose is to be administered over a period of time of about 1 minute to about 5 minutes, in which the at least one total dose is selected from a total dose-range of about 10^{13} vg to about 10^{15} vg.
19. An adeno-associated virus (AAV) vector comprising a nucleic acid sequence encoding a Phosphatase inhibitor (I-1) polypeptide operably linked to a promoter selected from any of:
- a cardiac-specific promoter selected from Table 2A or a variant thereof,
 - a muscle-specific promoter active in cardiac and skeletal muscle, or a variant thereof, or
 - any promoter when a cardiac tissue specific enhancer is present.
20. An AAV vector according to Claim 19, in which muscle-specific promoter active in cardiac and skeletal muscle is selected from Table 5A or Table 13A or a variant thereof.
21. A pharmaceutical composition comprising:
- (i) adeno-associated virus (AAV) vector comprising a nucleic acid sequence encoding a Phosphatase inhibitor (I-1) polypeptide operably linked to any one of:
 - (a) a cardiac-specific promoter selected from Table 2A or a variant thereof,

- (b) a muscle-specific promoter active in cardiac and skeletal muscle, or
 - (c) any promoter when a cardiac tissue specific enhancer is present, or a variant thereof; and
 - (ii) a pharmaceutically acceptable carrier,
- 5 in which the composition is formulated to be administered as at least one total dose to be administered in a series of sub-doses, in which each sub-dose is to be administered over a period of time of about 1 minute to about 5 minutes, in which the at least one total dose is selected from a total dose-range of about 10^{13} vg to about 10^{15} vg.
22. A cell comprising an AAV vector according to Claim 19 or 20.
- 10 23. Use of an AAV vector according to Claim 19 or 20 in the preparation of a medicament for expressing a phosphatase inhibitor (I-1) polypeptide in a subject with cardiomyopathy, in which at least one dose of the AAV vector is to be introduced into the subject,
- in which the subject with cardiomyopathy has a classification of heart failure,
- in which the at least one dose of the rAAV is selected from a total dose-range of about
- 15 10^{13} vg to about 10^{15} vg, in which the at least one total dose is to be administered in a series of sub-doses, in which each sub-dose is to be administered over a period of time of about 1 minute to about 5 minutes, and
- in which at least twelve months post-administration there is an improvement in the classification of heart failure.