~~CLAIMS~~

~~1. A recombinant adeno-associated virus (rAAV) vector comprising (i) a capsid comprising a capsid protein of SEQ ID NO:12 and (ii) a nucleic acid comprising from 5' to 3': (a) an AAV2 terminal repeat (b) a promoter (c) a nucleotide sequence encoding a human cystic fibrosis transmembrane conductance regulator (CFTR) protein or a biologically active truncated CFTR protein lacking amino acids 708-759 of the human CFTR protein sequence (d) a polyadenylation sequence and (e) an AAV2 terminal repeat.~~

~~2. The rAAV virus according to claim 1, wherein the nucleotide sequence encoding human CFTR has the nucleotide sequence of SEQ ID NO:43 or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% or at least 99% identical thereto.~~

~~3. The rAAV virus according to claim 1 or 2, wherein the promoter is a constitutive promoter, optionally a truncated version of the CMV immediate/early enhancer/promoter.~~

~~4. The rAAV virus according to claim 1 or 2, wherein the promoter is a tissue specific promoter, preferably wherein the promoter directs preferential expression of the nucleic acid in a lung cell.~~

~~5. The rAAV virus according to claim 1 or 2, wherein the promoter is a CMV173 promoter and is operably linked to the nucleotide sequence encoding human CFTR.~~

~~6. The rAAV virus according to claim 5, wherein the CMV173 promoter has the sequence of SEQ ID NO:44.~~

~~7. The rAAV virus according to claim 1, wherein the nucleic acid comprises the nucleotide sequence of SEQ ID NO:45.~~

~~8. A method for treating cystic fibrosis or a lung disease associated therewith in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an infectious rAAV according to any one of claims 1-7, wherein the subject is preferably administered an amount of the rAAV effective to ameliorate~~

~~one or more characteristics of cystic fibrosis, optionally selected from upper airway inflammation, aberrant epithelia cytokine signaling and elevated IgE levels.~~

~~9. The method according to claim 8, wherein the rAAV is administered to the subject by pulmonary (bronchial) and/or nasal administration, preferably by inhalation, preferably is administered in a single dose.~~

~~10. The method according to claim 8 or 9, wherein the rAAV is administered to the subject at one or more doses of between 1 x 10^{13} to 5 x 10^{15} vector genomes (vg), more preferably between 1 x 10^{14} and 1 x 10^{15} vg.~~

~~11. An rAAV according to any one of claim 1-7 for use in the treatment of cystic fibrosis or a lung disease associated therewith.~~

~~12. An rAAV according to any one of claims 1-7 for use in the manufacture of a medicament for the treatment of cystic fibrosis or a lung disease associated therewith.~~

~~13. A pharmaceutical composition suitable for inhalation comprising an rAAV according to any one of claims 1-7 and a buffer comprising about 10 mM to about 50 mM citrate, about 70 mM to about 150 mM NaCl and optionally a surfactant, preferably a non-ionic surfactant such as Pluronic F-68, more preferably about 0.005% Pluronic F68, and having pH of about 6.0.~~

~~14. The pharmaceutical composition according to claim 13, comprising about 20 mM to about 50 mM citrate, about 85 mM to about 125 mM NaCl and about 0.005% Pluronic F68 and having a pH of about 6.0~~

~~15. The pharmaceutical composition according to claim 14, comprising about 20 mM citrate, about 125 mM NaCl and about 0.005% Pluronic F68 and having a pH of about 6.0.~~

~~16. The pharmaceutical composition according to any one of claims 13 to 15 comprising between from 10^{11} to 10^{14} vector genomes (vg) per ml.~~

~~17. The pharmaceutical composition according to claim 16, comprising from about 1 x 10^{13} to about 9 x 10^{13} vg/ml, preferably from about 2 x 10^{13} to 6 x 10^{13} vg/ml, more preferably about 2 x 10^{13} vg/ml.~~

18. The pharmaceutical composition according to any one of claims 13 to 17, wherein the pharmaceutical composition is in the form of a liquid/suspension suitable for aerosolized delivery or is formulated as an aerosol and/or is an inhaled dosage form.

19. A method for treating cystic fibrosis or a lung disease associated therewith in a subject in need thereof, comprising administering to the subject a pharmaceutical composition according to any one of claims 13 to 18.

20. A method of delivering a heterologous nucleic acid to a lung cell comprising contacting the lung cell with an rAAV virion comprising (i) a capid comprising a capsid protein of SEQ ID NO:12 and (ii) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product, whereby the gene product is expressed in the lung cell.

21. The method according to claim 20, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a human cystic fibrosis transmembrane conductance regulator (CFTR) protein or a biologically active truncated CFTR protein lacking amino acids 708-759 of the human CFTR protein sequence.

22. The method according to claim 21, wherein the heterologous nucleic acid comprises the sequence of SEQ ID NO:45 or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% or at least 99% identical thereto.

23. The method according to any one of claims 20 to 22, wherein the lung cell is an airway epithelial cell.

24. The method according to claim 23, wherein the epithelial cell is an alveolar epithelium cell, a bronchial (primary, secondary or tertiary) epithelial cell or a tracheal epithelial cell.

25. The method according to claim 24, wherein the lung cell is a lung alveolar epithelial type 1 (AECI) or type 2 (AECII) cell.

26. The method according to any one of claims 20 to 22, wherein the lung cell is a smooth muscle or endothelial cell.

27. A method for treating a pulmonary disease, comprising administering to a subject in need thereof, a therapeutically effective amount of a recombinant AAV (rAAV) comprising (i) a capsid comprising a capsid protein comprising the amino acid sequence set forth as SEQ ID NO:12 and (ii) a heterolous nucleic acid comprising a nucleotide sequence encoding one or more gene products operably linked to one or more promoters, or administering to the subject a pharmaceutical composition comprising the rAAV.

28. The method according to claim 27, wherein the pulmonary disease is selected from chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), pulmonary arterial hypertension, pulmonary hypertension, lung cancer (primary, secondary and metastatic), surfactant deficiency, viral and/or bacterial infection, cystic fibrosis, acute bronchitis, pneumonia (including viral, bacterial, and fungal pneumonia), respiratory tract infections (including pharyngitis, croup, aspergillus, coocidiomycosis, hantavirus pulmonary syndrome, and histoplasmosis), chemical and hypersensitivity pneumonitis, tuberculosis and other mycobacterial infections (including but not limited to mycobacterium avium), sarcoidosis, respiratory syncytial virus, pulmonary edema, acute respiratory distress syndrome (ARDS), pneumoconiosis (including black lung disease, asbestosis, and silicosis), interstitial lung disease (including sarcoidosis and autoimmune disease), pulmonary embolism, pleual effusion, pleuritis, mesothelioma, pneumothorax, actue bronchitis, bronchiolitis (including bronchiolitis obliterans), sudden infant death syndrome, sleep apnoea, bronchiectasis, bronchopulmonary dysplasia, cryptogenic organizing pneumonia, E-cigarette or vaping use associated lung injury (EVALI), Middle Eastern Respiratory Syndrome (MERS), primary ciliary dyskinesia, Severe Acute Respiratory Syndrome (SARS), alpha-1-antitrypsin deficiency, asthma, interstitital lung disease, and COVID-19 (Coronavirus Disease 2019).

29. The method according to claim 28, wherein the pulmonary disease is chronic obstructive pulmonary disease (COPD) or idiopathic pulmonary fibrosis (IPF).

30. The method according to claim 29, wherein the pulmonary disease is COPD, and wherein the rAAV optionally comprises a heterologous nucleic acid encoding a gene product targeting one or more genes selected from alpha-1-

~~antitrypsin, alpha-1-antichymotrypsin, alpha-1-macroglobulin, matrix metalloproteinase 1 (MMP1), matrix metalloproteinase 12 (MMP12), microsomal epoxide hydrolyase, CYP1A1, Glutathione S-transferase, heme oxygenase-1, TGF-beta-1, TNF-alpha, IL-1 complex, IL-8, IL-13, human leukocyte antigen (HLA-B7 and Bw16), vitamin D binding protein, and beta-2-adrenergic receptor.~~

~~31. The method according to claim 29, wherein the pulmonary disease is IPF, and wherein the rAAV optionally comprises a heterologous nucleic acid encoding a gene product targeting one or more genes selected from SFTPA1 (surfactant A1) and Caveolin-1.~~

~~32. The method according to claim 28, wherein the pulmonary disease is alpha-1-antitrypsin deficiency and wherein the rAAV comprises a heterologous nucleic acid encoding a functional alpha-1-antitrypsin.~~

~~33. The method according to any one of claims 27 to 32 wherein the rAAV or pharmaceutical composition is administered by pulmonary, endobronchial, intranasal, intratracheal, and/or intrabronchial administration.~~

~~34. The method according to any one of claims 27 to 33, wherein at least one dose of about 10^{12} to 10^{15} vector genomes (vg) of the rAAV is administered to the subject.~~

~~35. The method according to claim 34, wherein the subject is administered about 1 x 10^{13} to about 5 x 10^{15} vg, preferably about 1 x 10^{14} vg to about 1 x 10^{15} vg.~~

~~36. A nucleic acid encoding a biologically active human CFTR protein lacking amino acids 708-759 and codon optimized for expression in humans, the nucleic acid comprising the nucleotide sequence set forth as SEQ ID NO:43 or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% or at least 99% identical thereto.~~

~~37. The nucleic acid of claim 36 comprising or consisting of the nucleotide sequence set forth as SEQ ID NO:43.~~

~~38. An expression cassette comprising the nucleic acid according to claim 36 or 37 and an expression control sequence operably linked and heterologous to the nucleic acid sequence.~~

~~39. The expression cassette of claim 38, wherein the expression control sequence comprises a CMV173 promoter having the nucleotide sequence set forth as SEQ ID NO:44.~~

~~40. The expression cassette of claim 38 or 39, comprising from 5' to 3': (a) an AAV2 terminal repeat (b) an CMV173 promoter (c) codon optimized gene of SEQ ID NO:43 (d) an AAV2 terminal repeat.~~

~~41. The expression cassette of claim 40, further comprising an SV40 polyadenylation sequence between (c) and (d).~~

Claims

1. A method of delivering a heterologous nucleic acid to a lung cell in a subject comprising administering to the subject a composition comprising a recombinant adeno-associated virus (rAAV) vector, said rAAV vector comprising (i) a capsid comprising a capsid protein comprising the amino acid sequence as set forth as SEQ ID NO:12 or an amino acid sequence at least 90% identical to SEQ ID NO: 12 and comprising a Thr at amino acid 469 and an Ala at amino acid 598 based on the amino acid numbering set forth in SEQ ID NO: 12 and (ii) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product, whereby the step of administering comprises pulmonary, endobronchial, intranasal, intratracheal, and/or intrabronchial administration.

2 The method according to claim 1, wherein the lung cell is selected from an airway epithelial cell, a smooth muscle cell, and an endothelial cell.

3. The method according to claim 2, wherein the airway epithelial cell is a basal cell, a goblet cell or a cilia cell.

4. The method according to claim 3, wherein the airway epithelial cell is a lung alveolar epithelial type 1 (AECI), a lung alveolar type 2 (AECII) cell, a bronchial epithelial cell or a tracheal epithelial cell.

5. The method according to any one of claims 1-4, wherein the composition is formulated as an aerosol.

6. The method according to claim 5, wherein the method comprises administering the composition by a nebulizer.

7. The method according to any one of claims 1-6, wherein the composition comprises 10^{11} to 10^{14} vector genomes (vg) of the rAAV per ml.

8. The method according to any one of claims 1-7, wherein the nucleotide sequence encoding the gene product is operably linked to a promoter.

9. The method according to claim 8, wherein the promoter is a constitutive promoter.

10. The method according to claim 8, wherein the promoter is a tissue-specific promoter.

11. The method according to any one of claims 1-10, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a gene product selected from: cystic fibrosis transmembrane conductance regulator (CFTR protein) or a biologically active fragment thereof, SFTPA1 (surfactant A1), Caveolin-1, alpha-1-antitrypsin, alpha-1-antichymotrypsin, alpha-1-macroglobulin, matrix metalloproteinase 1 (MMP1), matrix metalloproteinase 12 (MMP12), microsomal epoxide hydrolyase, CYP1A1, Glutathione S-transferase, heme oxygenase-1, TGF-beta-1, TNF-alpha, IL-1 complex, IL-8, IL-13, human leukocyte antigen, vitamin D binding protein, beta-2-adrenergic receptor.

12. The method according to claim 11, wherein the gene product is a human cystic fibrosis transmembrane conductance regulator (CFTR) protein or a biologically active truncated CFTR protein lacking amino acids 708-759 of the human CFTR protein sequence.

13. The method according to claim 12, wherein the gene product is a biologically truncated CFTR protein lacking amino acids 708-759 of the human CFTR protein sequence.

14. The method according to claim 13, wherein the heterologous nucleic acid comprises the nucleotide sequence set forth in SEQ ID NO:43 or a sequence at least 80% identical thereto.

15. A pharmaceutical composition comprising an rAAV vector, said rAAV vector comprising (i) a capsid comprising a capsid protein of SEQ ID NO:12 or an amino acid sequence at least 90% identical to SEQ ID NO:12 and comprising a Thr at amino acid 469 and an Ala at amino acid 598 based on the amino acid numbering set forth in SEQ ID NO: 12 and (ii) a heterologous nucleic acid comprising a nucleotide sequence encoding a CFTR or a biologically active truncated CFTR protein lacking amino acids 708-759 of the human CFTR protein sequence, said nucleotide sequence operably linked to an expression control sequence.

16. The pharmaceutical composition according to claim 15, wherein the nucleotide sequence encoding the CFTR protein is at least 80% identical to the nucleotide sequence set forth as SEQ ID NO:43.

17. The pharmaceutical composition according to claim 16, wherein the nucleotide sequence encoding the CFTR comprises the nucleotide sequence set forth as SEQ ID NO:43.

18. The pharmaceutical composition according to any one of claims 15-17, wherein the expression control sequence comprises a ubiquitous promoter, preferably wherein the ubiquitous promoter is a CMV173.

19. The pharmaceutical composition according to any one of claims 15-18, wherein the composition is formulated for inhalation or is suitable for inhalation.

20. The pharmaceutical composition according to any one of claims 15 to 19, comprising about 20 mM to about 50 mM citrate, about 85 mM to about 125 mM NaCl and about 0.005% Pluronic F68 and having a pH of about 6.0.

21. The pharmaceutical composition according to claim 20, comprising about 20 mM citrate, about 125 mM NaCl and about 0.005% Pluronic F68 and having a pH of about 6.0.

22. The pharmaceutical composition according to any one of claims 15-21 for use in therapy.

23. The use of a pharmaceutical composition according to any one of claims 15-21, in the manufacture of a medicament for the treatment of cystic fibrosis, wherein the pharmaceutical composition is for pulmonary, endobronchial, intranasal, intratracheal, and/or intrabronchial administration.

24. Use of a composition comprising a recombinant adeno-associated virus (rAAV) vector in the manufacture of a medicament for the treatment of lung disorder, wherein a heterologous nucleic acid is to be delivered to a lung cell in a subject, wherein said rAAV vector comprises (i) a capsid comprising a capsid protein comprising the amino acid sequence set forth as SEQ ID NO:12 or an amino acid sequence at least 90% identical to SEQ ID NO:12 and comprising a Thr at amino acid 469 and an Ala at amino acid 598 based on the amino acid numbering set forth in SEQ ID NO: 12 and (ii) a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product, wherein the composition is to be administered by pulmonary, endobronchial, intranasal, intratracheal, and/or intrabronchial administration.

25. The use according claim 24, wherein the composition is to be used in the treatment of cystic fibrosis, pulmonary disease, or a lung disease associated therewith.

26. The use according to claim 25, wherein the composition is to be used in the treatment of cystic fibrosis.

27. The method according to claim 1, wherein the capsid protein comprises an amino acid sequence at least 96% identical to SEQ ID NO: 12.

28. The method according to claim 1, wherein the capsid protein comprises an amino acid sequence at least 97% identical to SEQ ID NO: 12.

29. The pharmaceutical composition according to claim 15, wherein the capsid protein comprises an amino acid sequence at least 98% identical to SEQ ID NO: 12.

30. The pharmaceutical composition according to claim 15, wherein the capsid protein comprises an amino acid sequence at least 99% identical to SEQ ID NO: 12.