for induction of expression even while corresponding DNA-based compositions did provide some level of expression.

[0009] Thus, there is a need for improved materials, formulations, production methods, and methods for delivery of CFTR mRNA for induction of CFTR expression, including in the mammalian lung, for the treatment of cystic fibrosis.

SUMMARY OF THE INVENTION

The present disclosure is based, in part, on the development of formulations of CFTR mRNA and non-naturally occurring CFTR mRNA and methods of administration thereof that can induce functional CFTR expression *in vivo*. The compositions, methods, and uses according to the disclosure can provide CFTR expression in the lung of a large mammal with a favorable safety profile suitable for effective treatment of cystic fibrosis.

[0010a(i)] In one aspect, there is provided a method of producing cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung of a mammal, the method comprising:

administering to the lung of the mammal by aerosolization a composition comprising an mRNA-loaded nanoparticle, wherein the mRNA is an in vitro transcribed mRNA and has a coding sequence at least 80% identical to SEQ ID NO: 3,

wherein the mRNA encodes a human CFTR protein comprising the amino acid sequence of SEQ ID NO:1.

[0010a(ii)] In one aspect, there is provided a pharmaceutical composition comprising an mRNA-loaded nanoparticle,

wherein the mRNA is an in vitro transcribed mRNA and has a coding sequence at least 80% identical to SEQ ID NO: 3, and

wherein the mRNA encodes a human cystic fibrosis transmembrane conductance regulator (CFTR) protein comprising the amino acid sequence of SEQ ID NO:1.

[0010a(iii)] In one aspect, there is provided the use of a composition comprising an mRNA-loaded nanoparticle, wherein the mRNA is an in vitro transcribed mRNA and has a coding sequence at least 80% identical to SEQ ID NO: 3, and

wherein the mRNA encodes a human cystic fibrosis transmembrane conductance regulator (CFTR) protein comprising the amino acid sequence of SEQ ID NO:1 in the preparation of a medicament for the treatment of cystic fibrosis.

[0010a(iv)] In one aspect, there is provided an in vitro transcribed mRNA comprising a coding

sequence at least 80% identical to SEQ ID NO: 3 for use in treating cystic fibrosis in a mammal.

[0010a] In one aspect, there is provided a use of an *in vitro* transcribed mRNA comprising a coding sequence at least 80% identical to SEQ ID NO: 3 in the manufacture of a medicament for treating cystic fibrosis in a mammal.

[0010b] In another aspect, there is provided a composition comprising an mRNA and a carrier, wherein the mRNA is an *in vitro* transcribed mRNA and has a coding sequence at least 80% identical to SEQ ID NO: 3.

[0010] Thus, in one embodiment, the present disclosure provides a method of *in vivo* production of CFTR, in particular, in the lung of a subject (e.g., a mammal) in need of delivery by delivering an mRNA encoding a CFTR protein. In some embodiments, the mRNA encoding a CFTR protein is delivered directly to the lung of the subject. As used herein, a "CFTR protein" encompasses any full length, fragment or portion of a CFTR protein which can be used to substitute for naturally-occurring CFTR protein activity and/or reduce the intensity, severity, and/or frequency of one or more symptoms associated with Cystic fibrosis. For example, a suitable CFTR protein according to the present invention may have an amino acid sequence identical to the wild-type human CFTR protein (SEQ ID NO:1). In some embodiments, a suitable CFTR protein according to the present invention may have an amino acid sequence at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the wild-type human CFTR protein (SEQ ID NO:1).

[0011] In one embodiment, the disclosure provides a method of inducing CFTR expression in epithelial cells in a lung of a mammal, the method comprising contacting the epithelial cells in the lung of the mammal with a composition, wherein: the composition is a pharmaceutical composition comprising an *in vitro* transcribed mRNA;the *in vitro* transcribed mRNA comprises a coding sequence which encodes SEQ ID NO: 1. In another embodiment, the

CLAIMS

1. Use of an *in vitro* transcribed mRNA comprising a coding sequence at least 80% identical to SEQ ID NO: 3 in the manufacture of a medicament for treating cystic fibrosis in a mammal.

2. The use according to claim 1, wherein the coding sequence is at least 90% identical to SEQ ID NO: 3.

3. The use according to claim 1, wherein the coding sequence is 100% identical to SEQ ID NO: 3.

4. The use according to any one of the preceding claims, wherein the mRNA comprises a 5' untranslated region (UTR), a 3' UTR, a signal peptide coding sequence, a cap structure and/or a tail structure.

5. The use according to claim 4, wherein the 5'-UTR comprises SEQ ID NO: 4 and/or the 3'-UTR comprises SEQ ID NO: 5.

6. The use according to claim 4 or 5, wherein the tail structure is a poly-A tail of at least 70, 100, 120, 150, 200, or 250 residues in length.

7. The use according to any one of claims 4-6, wherein the cap structure is a cap1 structure.

8. The use according to any one of the preceding claims, wherein the mRNA comprises one or more nonstandard nucleotide(s), optionally wherein the one or more nonstandard nucleotide(s) is/are chosen from 5-methyl-cytidine, pseudouridine, and 2-thio-uridine.

9. The use according to any one of the preceding claims, wherein the composition is administered to the lung of the mammal by inhalation, intranasal administration, aerosolization, or nebulization.

10. The use according to any one of the preceding claims, wherein the mRNA comprises a coding sequence which encodes a human cystic fibrosis transmembrane conductance regulator (CFTR) protein of SEQ ID NO:1, optionally wherein the human CFTR protein is expressed in the epithelial cells of the lung.

11. The mRNA for use according to any one of the preceding claims, wherein the mRNA is administered with a carrier.

12. The use according to claim 11, wherein the carrier comprises an organic cation, such as a cationic lipid or a cationic organic polymer.

13. The use according to claim 12, wherein the cationic organic polymer is selected from the group consisting of polyethyleneimine (PEI), protamine, PEGylated protamine, poly-L-lysine (PLL), and PEGylated PLL.

14. The use according to claim 13, wherein the PEI is branched PEI with a molecular weight ranging from 10 kDa to 40 kDa.

15. The use according to any one of claims 11-14, wherein the carrier is a liposome.

16. A composition comprising an mRNA and a carrier, wherein the mRNA is an *in vitro* transcribed mRNA and has a coding sequence at least 80% identical to SEQ ID NO: 3.

17. The composition of claim 16, wherein the carrier comprises an organic cation, such as a cationic lipid or a cationic organic polymer.

18. The composition according to claim 17, wherein the cationic organic polymer is selected from the group consisting of polyethyleneimine (PEI), protamine, PEGylated protamine, poly-L-lysine (PLL), and PEGylated PLL.

19. The composition according to claim 18, wherein the PEI is branched PEI with a molecular weight ranging from 10 kDa to 40 kDa.

20. The composition of claim 16, wherein the carrier is a liposome.

CLAIMS

1. A method of producing cystic fibrosis transmembrane conductance regulator (CFTR) protein in a lung of a mammal, the method comprising:

administering to the lung of the mammal by aerosolization a composition comprising an mRNA-loaded nanoparticle, wherein the mRNA is an *in vitro* transcribed mRNA and has a coding sequence at least 80% identical to SEQ ID NO: 3,

wherein the mRNA encodes a human CFTR protein comprising the amino acid sequence of SEQ ID NO:1.

2. The method of claim 1, wherein the mRNA has the coding sequence at least 90% identical to SEQ ID NO: 3.

3. The method of claim 1, wherein the mRNA has the coding sequence 100% identical to SEQ ID NO: 3.

4. The method of claim 1, wherein the mRNA comprises a 5' untranslated region (UTR) and/or a 3' UTR.

5. The method of claim 4, wherein the 5'-UTR comprises SEQ ID NO: 4 and/or the 3'-UTR comprises SEQ ID NO: 5.

6. The method of claim 4, wherein the mRNA further comprises a poly-A tail.

7. The method of claim 6, wherein the poly-A tail is of at least 70, 100, 120, 150, 200, or 250 residues in length.

8. The method of claim 4, wherein the mRNA further comprises a 5' cap.

9. The method of claim 1, wherein the mRNA comprises at least one nonstandard nucleobase.

10. The method of claim 9, wherein the nonstandard nucleobase is chosen from one or more of 5-methyl-cytidine, pseudouridine, and 2-thio-uridine.

11. The method of claim 1, wherein the aerosolization is nebulization.

12. The method of claim 1, wherein the human CFTR protein is expressed in the epithelial cells of the lung.

13. The method of claim 1, wherein the composition further comprises a pharmaceutically acceptable carrier.

14. The method of claim 1, wherein the nanoparticle comprises one or more organic cations.

15. The method of claim 14, wherein the one or more organic cations are selected from the group consisting of polyethyleneimine (PEI), protamine, PEGylated protamine, poly-L-lysine (PLL), PEGylated PLL, and a cationic lipid.

16. The method of claim 1, wherein the nanoparticle comprises a polymer.

17. The method of claim 16, wherein the polymer comprises branched PEI with a molecular weight ranging from 10 kDa to 40 kDa.

18. The method of claim 1, wherein the nanoparticle is a liposome.

19. A pharmaceutical composition comprising an mRNA-loaded nanoparticle,

wherein the mRNA is an *in vitro* transcribed mRNA and has a coding sequence at least 80% identical to SEQ ID NO: 3, and

wherein the mRNA encodes a human cystic fibrosis transmembrane conductance regulator (CFTR) protein comprising the amino acid sequence of SEQ ID NO:1.

20. The pharmaceutical composition of claim 19, wherein the mRNA has the coding sequence at least 90% identical to SEQ ID NO: 3.

21. The pharmaceutical composition of claim 19, wherein the mRNA has the coding sequence 100% identical to SEQ ID NO: 3.

22. The pharmaceutical composition of claim 19, wherein the mRNA comprises a 5' untranslated region (UTR) and/or a 3' UTR.

23. The pharmaceutical composition of claim 22, wherein the 5'-UTR comprises SEQ ID NO: 4 and/or the 3'-UTR comprises SEQ ID NO: 5.

24. The pharmaceutical composition of claim 22, wherein the mRNA further comprises a poly-A tail.

25. The pharmaceutical composition of claim 24, wherein the poly-A tail is of at least 70, 100, 120, 150, 200, or 250 residues in length.

26. The pharmaceutical composition of claim 22, wherein the mRNA further comprises a 5' cap.

27. The pharmaceutical composition of claim 19, wherein the mRNA comprises at least one nonstandard nucleobase.

28. The pharmaceutical composition of claim 27, wherein the nonstandard nucleobase is chosen from one or more of 5-methyl-cytidine, pseudouridine, and 2-thio-uridine.

29. The pharmaceutical composition of claim 19, wherein the composition is administered to the lung by aerosolization.

30. The pharmaceutical composition of claim 29, wherein the aerosolization is nebulization.

31. The pharmaceutical composition of claim 29, wherein the administration of the composition results in human CFTR protein expression in epithelial cells of the lung.

32. The pharmaceutical composition of claim 19, wherein the composition further comprises a pharmaceutically acceptable carrier.

33. The pharmaceutical composition of claim 19, wherein the nanoparticle comprises one or more organic cations.

34. The pharmaceutical composition of claim 33, wherein the one or more organic cations are selected from the group consisting of polyethyleneimine (PEI), protamine, PEGylated protamine, poly-L-lysine (PLL), PEGylated PLL, a cationic lipid and combinations thereof.

35. The pharmaceutical composition of claim 19, wherein the nanoparticle comprises a polymer.

36. The pharmaceutical composition of claim 35, wherein the polymer comprises branched PEI with a molecular weight ranging from 10 kDa to 40 kDa.

37. The pharmaceutical composition of claim 19, wherein the nanoparticle is a liposome.

38. Use of a composition comprising an mRNA-loaded nanoparticle, wherein the mRNA is an *in vitro* transcribed mRNA and has a coding sequence at least 80% identical to SEQ ID NO: 3, and

wherein the mRNA encodes a human cystic fibrosis transmembrane conductance regulator (CFTR) protein comprising the amino acid sequence of

SEQ ID NO:1 in the preparation of a medicament for the treatment of cystic fibrosis.

39. The use of claim 38, wherein the mRNA has the coding sequence at least 90% identical to SEQ ID NO: 3.

40. The use of claim 38, wherein the composition is administered via nebulization or aerosolization.

41. The use of claim 38, wherein the administering of the composition results in expression of human CFTR protein in the lung of the mammal.

42. The use of claim 41, wherein the expression of human CFTR protein is in epithelial cells of the lung.

43. An *in vitro* transcribed mRNA comprising a coding sequence at least 80% identical to SEQ ID NO: 3 for use in treating cystic fibrosis in a mammal.

44. The mRNA for use according to claim 43, wherein the coding sequence is at least 90% identical to SEQ ID NO: 3.

45. The mRNA for use according to claim 43, wherein the coding sequence is 100% identical to SEQ ID NO: 3.

46. The mRNA for use according to any one of the preceding claims, wherein the mRNA comprises a 5' untranslated region (UTR), a 3' UTR, a signal peptide coding sequence, a cap structure and/or a tail structure.

47. The mRNA for use according to claim 46, wherein the 5'-UTR comprises SEQ ID NO: 4 and/or the 3'-UTR comprises SEQ ID NO: 5.

48. The mRNA for use according to claim 46 or 47, wherein the tail structure is a poly-A tail of at least 70, 100, 120, 150, 200, or 250 residues in length.

49. The mRNA for use according to any one of claims 46-47, wherein the cap structure is a cap1 structure.

50. The mRNA for use according to any one of the preceding claims, wherein the mRNA comprises one or more nonstandard nucleotide(s), optionally wherein the one or more nonstandard nucleotide(s) is/are chosen from 5-methyl-cytidine, pseudouridine, and 2-thio-uridine.

51. The mRNA for use according to any one of the preceding claims, wherein the composition is administered to the lung of the mammal by inhalation, intranasal administration, aerosolization, or nebulization.

52. The mRNA for use according to any one of the preceding claims, wherein the mRNA comprises a coding sequence which encodes a human cystic fibrosis transmembrane conductance regulator (CFTR) protein of SEQ ID NO:1, optionally wherein the human CFTR protein is expressed in the epithelial cells of the lung.

53. The mRNA for use according to any one of the preceding claims, wherein the mRNA is administered with a carrier.

54. The mRNA for use according to claim 53, wherein the carrier comprises an organic cation, such as a cationic lipid or a cationic organic polymer.

55. The mRNA for use according to claim 54, wherein the cationic organic polymer is selected from the group consisting of polyethyleneimine (PEI), protamine, PEGylated protamine, poly-L-lysine (PLL), and PEGylated PLL.

56. The mRNA for use according to claim 55, wherein the PEI is branched PEI with a molecular weight ranging from 10 kDa to 40 kDa.

57. The mRNA for use according to any one of claims 53-56, wherein the carrier is a liposome.