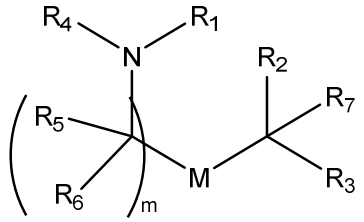
CLAIMS

1. A pharmaceutical composition comprising a lipid nanoparticle encapsulated mRNA that comprises an open reading frame (ORF) encoding a human Citrin polypeptide, wherein the ORF has at least 95% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 5 to 29, 129, 132, 135, and 138.

2. The pharmaceutical composition of claim 1, wherein the ORF has at least 98% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 5 to 29, 129, 132, 135, and 138.

3. The pharmaceutical composition of claim 1, wherein the ORF comprises the nucleic acid sequence of SEQ ID NO: 129, 132, 135, or 138.

4. A pharmaceutical composition comprising an mRNA comprising an open reading frame (ORF) encoding a Citrin polypeptide and a delivery agent comprising a compound having the formula (I)



(I),

or a salt or stereoisomer thereof, wherein

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR", -YR", and -R"M'R';

R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR", -YR", and -R*OR", or R_2 and R_3, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a carbocycle, heterocycle, -OR, $-O(CH_2)_nN(R)_2$, -C(O)OR, -OC(O)R, $-CX_3$, $-CX_2H$, $-CXH_2$, -CN, $-N(R)_2$,

$-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$, $-C(=NR_9)R$, $-C(O)N(R)OR$, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -S-S-, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2, C_{1-6} alkyl, -OR, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR", -YR", and H;

each R" is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

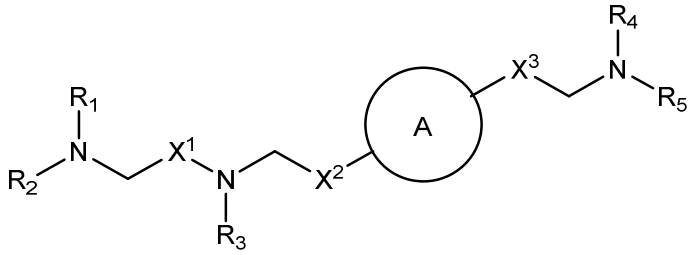
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13; and

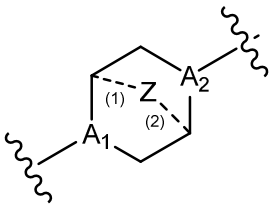
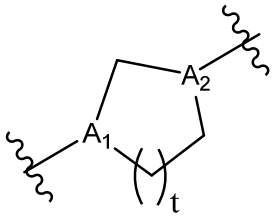
provided that when R_4 is $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, –CHQR, or $-CQ(R)_2$, then (i) Q is not $-N(R)_2$ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

5. A pharmaceutical composition comprising an mRNA comprising an open reading frame (ORF) encoding a Citrin polypeptide and a delivery agent, wherein the delivery agent comprises a compound having the formula (III)



(III),

or salts or stereoisomers thereof, wherein

ring A is  or  ;

t is 1 or 2;

A_1 and A_2 are each independently selected from CH or N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

each M is independently selected from the group consisting of -C(O)O-, -OC(O)-, -OC(O)O-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S- -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, an aryl group, and a heteroaryl group;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, $-CH_2-$, $-CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(O)O-, -OC(O)-, $-C(O)-CH_2-$, $-CH_2-C(O)-$, $-C(O)O-CH_2-$, $-OC(O)-CH_2-$, $-CH_2-C(O)O-$, $-CH_2-OC(O)-$, -CH(OH)-, -C(S)-, and -CH(SH)-;

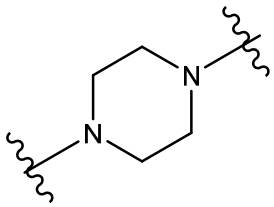
each Y is independently a C_{3-6} carbocycle;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each R is independently selected from the group consisting of C_{1-3} alkyl and a C_{3-6} carbocycle;

each R' is independently selected from the group consisting of C_{1-12} alkyl, C_{2-12} alkenyl, and H; and

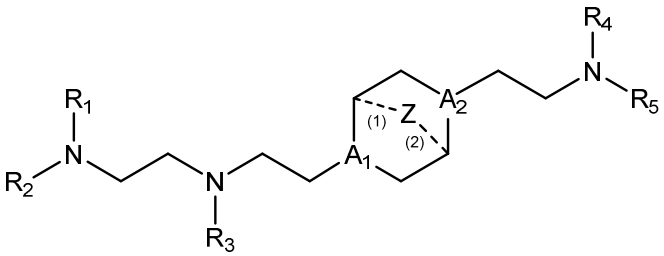
each R" is independently selected from the group consisting of C_{3-12} alkyl and C_{3-12} alkenyl,

wherein when ring A is , then

i) at least one of X^1, X^2, and X^3 is not -CH_2-; and/or

ii) at least one of R_1, R_2, R_3, R_4, and R_5 is -R"MR'.

6. The pharmaceutical composition comprising an mRNA comprising an open reading frame (ORF) encoding a Citrin polypeptide and a delivery agent, wherein the delivery agent comprises a compound having the formula (IV)



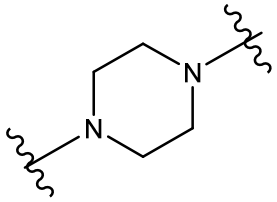
(IV),

or salts or stereoisomer thereof, wherein

A_1 and A_2 are each independently selected from CH or N and at least one of A_1 and A_2 is N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of C_{6-20} alkyl and C_{6-20} alkenyl;

wherein when ring A is  , then

i) R_1, R_2, R_3, R_4, and R_5 are the same, wherein R_1 is not C_{12} alkyl, C_{18} alkyl, or C_{18} alkenyl;

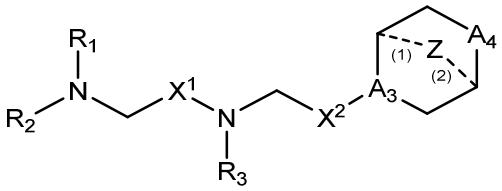
ii) only one of R_1, R_2, R_3, R_4, and R_5 is selected from C_{6-20} alkenyl;

iii) at least one of R_1, R_2, R_3, R_4, and R_5 have a different number of carbon atoms than at least one other of R_1, R_2, R_3, R_4, and R_5;

iv) R_1, R_2, and R_3 are selected from C_{6-20} alkenyl, and R_4 and R_5 are selected from C_{6-20} alkyl; or

v) R_1, R_2, and R_3 are selected from C_{6-20} alkyl, and R_4 and R_5 are selected from C_{6-20} alkenyl.

7. A pharmaceutical composition comprising an mRNA comprising an open reading frame (ORF) encoding a Citrin polypeptide and a delivery agent, wherein the delivery agent comprises a compound having the formula (V)



(V),

or salts or stereoisomers thereof, in which

A_3 is CH or N;

A_4 is CH_2 or NH; and at least one of A_3 and A_4 is N or NH;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, and R_3 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

each M is independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -$S(O)_2$-, an aryl group, and a heteroaryl group;

X^1 and X^2 are independently selected from the group consisting of -CH_2-, -$(CH_2)_2$-, -CHR-, -CHY-, -C(O)-, -C(O)O-, -OC(O)-, -C(O)-CH_2-, -CH_2-C(O)-, -C(O)O-CH_2-, -OC(O)-CH_2-, -CH_2-C(O)O-, -CH_2-OC(O)-, -CH(OH)-, -C(S) - and -CH(SH) -

each Y is independently a C_{3-6} carbocycle;

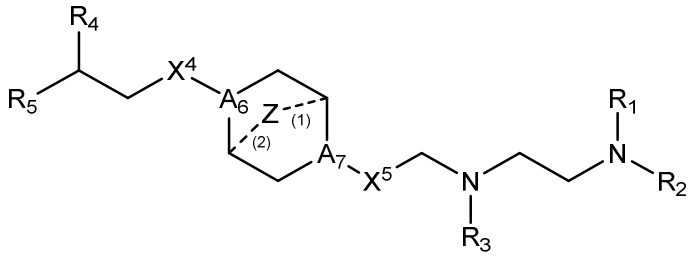
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each R is independently selected from the group consisting of C_{1-3} alkyl and a C_{3-6} carbocycle;

each R' is independently selected from the group consisting of C_{1-12} alkyl, C_{2-12} alkenyl, and H; and

each R" is independently selected from the group consisting of C_{3-12} alkyl and C_{3-12} alkenyl.

8. A pharmaceutical composition comprising an mRNA comprising an open reading frame (ORF) encoding a Citrin polypeptide and a delivery agent, wherein the delivery agent comprises a compound having the formula (VI):



(VI),

or salts or stereoisomers thereof, in which

A_6 and A_7 are each independently selected from CH or N, wherein at least one of A_6 and A_7 is N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

X^4 and X^5 are independently selected from the group consisting of -CH_2-, -$(CH_2)_2$-, -CHR-, -CHY-, -C(O)-, -C(O)O-, -OC(O)-, -C(O)-CH_2-, -CH_2-C(O)-, -C(O)O-CH_2-, -OC(O)-CH_2-, -CH_2-C(O)O-, -CH_2-OC(O)-, -CH(OH)-, -C(S)-, and -CH(SH)-;

R_1, R_2, R_3, R_4, and R_5 each are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

each M is independently selected from the group consisting of -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -$S(O)_2$-, an aryl group, and a heteroaryl group;

each Y is independently a C_{3-6} carbocycle;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

each R is independently selected from the group consisting of C_{1-3} alkyl and a C_{3-6} carbocycle;

each R' is independently selected from the group consisting of C_{1-12} alkyl, C_{2-12} alkenyl, and H; and each R" is independently selected from the group consisting of C_{3-12} alkyl and C_{3-12} alkenyl.

9. A pharmaceutical composition comprising an mRNA comprising an open reading frame (ORF) encoding a Citrin polypeptide and a delivery agent, wherein the delivery agent comprises a compound selected from the group consisting of Compounds 1-342 or Compound 419-428, salts and stereoisomers thereof, and any combination thereof.

10. The pharmaceutical composition of claim 9, wherein the delivery agent is a lipid nanoparticle comprising Compound 18, Compound 428, a salt or a stereoisomer thereof, or any combination thereof.

11. The pharmaceutical composition of claim 9, wherein the delivery agent is a lipid nanoparticle comprising Compound 18, DSPC, Cholesterol, and Compound 428.

12. The pharmaceutical composition of any one of claims 4 to 11, wherein the ORF has at least 95% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 5 to 29, 129, 132, 135, and 138.

13. The pharmaceutical composition of any one of claims 4 to 11, wherein the ORF comprises the nucleic acid sequence of SEQ ID NO: 129, 132, 135, or 138.

14. The pharmaceutical composition of any one of claims 1 to 13, wherein the mRNA comprises a 3' UTR comprising a nucleic acid sequence at least 90% identical to a 3' UTR sequence selected from the group consisting of SEQ ID NOs: 48-72, 80, 81, 102-105, 108-117, 124, 125, 147-157, or any combination thereof.

15. The pharmaceutical composition of claim 14, wherein the 3' UTR comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 48-72, 80, 81, 102-105, 108-117, 124, 125, 147-157, and any combination thereof.

16. The pharmaceutical composition of any one of claims 1 to 15, wherein the mRNA comprises a 5' UTR comprising a nucleic acid sequence at least 90% identical to a 5' UTR sequence selected from the group consisting of SEQ ID NO: 30-47, 79, 120-122, 126-128 or any combination thereof.

17. The pharmaceutical composition of claim 16, wherein the 5' UTR comprises a sequence selected from the group consisting of SEQ ID NO: 30-47, 79, 120-122, 126-128, and any combination thereof.

18. The pharmaceutical composition of any one of the preceding claims, wherein the Citrin polypeptide is a wild type, variant, or mutant having a Citrin activity.

19. The pharmaceutical composition of claim 18, wherein the Citrin polypeptide is a wild type Citrin isoform 1 or 2 protein.

20. The pharmaceutical composition of claim 18, wherein the Citrin polypeptide comprises a wild type polypeptide sequence selected from SEQ ID NO: 1 and 3.

21. Use of the pharmaceutical composition of any one of the preceding claims in the manufacture of a medicament for expressing a Citrin polypeptide in a human subject having citrullinemia type 2 (CTLN2), wherein the medicament is to be administered as a single dose or as a plurality of single unit doses to the subject.

22. Use of a pharmaceutical composition comprising an mRNA that comprises an open reading frame (ORF) encoding a human Citrin polypeptide in the manufacture of a medicament for expressing a Citrin polypeptide in a human subject having citrullinemia type 2 (CTLN2), wherein the medicament is to be administered as a single dose or as a plurality of single unit doses to the subject.

23. Use of the pharmaceutical composition of any one of claims 1 to 20 in the manufacture of a medicament for treating, preventing or delaying the onset and/or progression of citrullinemia type 2 (CTLN2) signs or symptoms in a human subject in need thereof, wherein the administration treats, prevents or delays the onset and/or progression of one or more of the signs or symptoms of CTLN2 in the subject.

24. Use of a pharmaceutical composition comprising an mRNA that comprises an open reading frame (ORF) encoding a human Citrin polypeptide in the manufacture of a medicament for treating, preventing or delaying the onset and/or progression of citrullinemia type 2 (CTLN2) signs or symptoms in a human subject in need thereof, wherein the administration treats, prevents or delays the onset and/or progression of one or more of the signs or symptoms of CTLN2 in the subject.

25. Use of the pharmaceutical composition of any one of claims 1 to 20 in the manufacture of a medicament for the treatment of citrullinemia type 2 (CTLN2), wherein a single intravenous dose of the medicament is to be administered.

26. Use of a pharmaceutical composition comprising an mRNA that comprises an open reading frame (ORF) encoding a human Citrin polypeptide in the manufacture of a medicament for the treatment of citrullinemia type 2 (CTLN2), wherein a single intravenous dose of the medicament is to be administered.

27. Use of the pharmaceutical composition of any one of claims 1 to 20 in the manufacture of a medicament for reducing an ammonia and/or triglyceride blood plasma level in a human subject having citrullinemia type 2 (CTLN2).

28. Use of a pharmaceutical composition comprising an mRNA that comprises an open reading frame (ORF) encoding a human Citrin polypeptide in the manufacture of a medicament for reducing an ammonia and/or triglyceride blood plasma level in a human subject having citrullinemia type 2 (CTLN2).

29. The use of any one of claims 21 to 28, wherein

(i) the ammonia blood and/or plasma level is reduced at least 2-fold, at least 3-fold, at least 5-fold, at least 10-fold, at least 15 fold, at least 20-fold or at least 50-fold compared to a reference ammonia blood and/or plasma level in a subject having CTLN2, for at least 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, or at least 120 hours post-administration, and/or

(ii) the triglyceride blood and/or plasma level is reduced at least 1.5-fold, 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, or at least 20-fold compared to a reference triglyceride blood and/or plasma level in a subject having CTLN2, for at least 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, or at least 120 hours post-administration.

30. Use of the pharmaceutical composition of any one of claims 1 to 20 in the manufacture of a medicament for increasing the level of Citrin activity in a human subject having citrullinemia type 2 (CTLN2).

31. Use of a pharmaceutical composition comprising an mRNA that comprises an open reading frame (ORF) encoding a human Citrin polypeptide in the manufacture of a

medicament for increasing the level of Citrin activity in a human subject having citrullinemia type 2 (CTLN2).

32. The use of any one of claims 21 to 31, wherein the level of Citrin activity is increased at least 1.5-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, or at least 50-fold as compared to a reference Citrin activity level in a subject having CTLN2 for at least 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, or at least 120 hours post-administration.

33. The use of any one of claims 21 to 32, wherein 12 hours after the medicament is administered to the subject, the Citrin activity in the subject is increased at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 600% compared to the subject's baseline Citrin activity.

34. The use of claim 33, wherein the Citrin activity is increased in the liver and/or kidneys of the subject.

35. The use of claim 33 or 34, wherein the increased Citrin activity persists for greater than 24, 36, 48, 60, 72, or 96 hours.

36. The use of any one of claims 21 to 35, wherein the medicament is to be administered to the subject having citrullinemia type 2 (CTLN2).

37. The use of any one of the claims 21 to 36, wherein 24 hours after the medicament is administered to the subject the level of ammonia in the subject is reduced by at least 100%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, or at least 30% compared to the subject's baseline ammonia.

38. The use of claim 37, wherein the level of ammonia is reduced in the plasma of the subject.

39. The use of claim 37 or 38, wherein after the administration to the subject the level of ammonia in the subject is reduced compared to the baseline level in the subject for at least one day, at least two days, at least three days, at least four days, at least five days, at least one week, at least two weeks, at least three weeks, or at least one month.

40. The use of any one of the claims 21-39, wherein 24 hours after the medicament is administered to the subject the level of triglycerides in the subject is reduced by at least 100%, at least 80%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, or at least 10% compared to the subject's baseline triglycerides.

41. The use of claim 40, wherein the level of triglycerides is reduced in the plasma and/or liver of the subject.

42. The use of claim 40 or 41, wherein after the administration to the subject the level of triglycerides in the subject is reduced compared to the baseline level in the subject for at least one day, at least two days, at least three days, at least four days, at least five days, at least one week, at least two weeks, at least three weeks, or at least one month.

43. The use of any one of claims 21-42, wherein the subject is symptomatic for CTLN2.

44. The use of any one of claims 21-42, wherein the subject is presymptomatic for CTLN2.

45. The use of any one of claims 21-44, wherein the level of the Citrin polypeptide activity level is sufficient to prevent the onset of CTLN2 signs and/or symptoms.

46. The use of any one of claims 21-45, wherein the medicament is to be administered as a single dose of less than 1.5 mg/kg, less than 1.25 mg/kg, less than 1 mg/kg, or less than 0.75 mg/kg.

47. The use of any one of claims 21-46, wherein the medicament is to be administered to the subject is about once a week, about once every two weeks, or about once a month.

48. The use of any one of claims 21-47, wherein the medicament is to be administered intravenously.

49. The use of any one of claims 21-48, wherein the pharmaceutical composition comprises a lipid nanoparticle.