CLAIMS

1. A lipid nanoparticle (LNP) comprising a synthetic RNA viral genome encoding an oncolytic Coxsackievirus virus, wherein the Coxsackievirus is a CVA21 strain selected from ~~the Kuykendall strain, the~~ an EF strain and ~~the~~ an KY strain.

2. The LNP of claim 1, wherein the Coxsackievirus is the CVA21-KY strain, and wherein the CVA21-KY strain comprises a polynucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 5.

3. The LNP of claim 1, wherein the Coxsackievirus is the CVA21-EF strain, and wherein the CVA21-EF strain comprises a polynucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 9.

~~4. The LNP of claim 1, wherein the Coxsackievirus comprises a 5' UTR (IRES) sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 6 or 10.~~

~~5. The LNP of claim 1, wherein the Coxsackievirus comprises a P1 sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 7 or 11.~~

~~6. The LNP of claim 1, wherein the Coxsackievirus comprises a 3D sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 8 or 12.~~

~~7. The LNP of any one of claims 1-6, wherein the synthetic RNA viral genome does not comprise a polynucleotide sequence having more than 95%, more than 90%, more than 85%, or more than 80% sequence identity to SEQ ID NO: 1.~~

~~8. A lipid nanoparticle (LNP) comprising a synthetic RNA viral genome encoding an oncolytic Seneca Valley Virus (SVV), wherein the synthetic RNA viral genome comprises a polynucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 68.~~

9. The LNP of claim 8, wherein the synthetic RNA viral genome comprises a 5' UTR (IRES) sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to nucleic acids 1-670 of SEQ ID NO: 68.

10. The LNP of claim 8 or 9, wherein the synthetic RNA viral genome encodes a SVV VP2 protein comprising a S177A mutation.

11. The LNP of any one of claims 1-10, wherein delivery of the LNP to a cell results in production of viral particles by the cell, and wherein the viral particles are infectious and lytic.

12. The LNP of any one of claims 1-11, wherein the synthetic RNA viral genome further comprises a heterologous polynucleotide encoding an exogenous payload protein.

13. The LNP of any one of claims 1-11, further comprising a second recombinant RNA molecule encoding an exogenous payload protein.

14. The LNP of claim 12 or 13, wherein the exogenous payload protein comprises or consists of a MLKL 4HB domain, a Gasdermin D N-terminal fragment, a Gasdermin E N-terminal fragment, a HMGB1 Box B domain, a SMAC/Diablo, a Melittin, a L-amino-acid oxidase (LAAO), a disintegrin, a TRAIL (TNFSF10), a nitroreductase, a reovirus FAST protein, a leptin/FOSL2, an α-1,3-galactosyltransferase, or an adenosine deaminase 2 (ADA2).

15. The LNP of claim 14, wherein the nitroreductase is NfsB or NfsA.

16. The LNP of claim 14, wherein the reovirus FAST protein is ARV p14, BRV p15, or a p14-p15 hybrid.

17. The LNP of claim 12 or 13, wherein the exogenous payload protein is a fluorescent protein, an enzymatic protein, a cytokine, a chemokine, an antigen-binding molecule capable of binding to a cell surface receptor, or a ligand for a cell-surface receptor.

18. The LNP of claim 17, wherein:

a) the cytokine is selected from GM-CSF, IFNγ, IL-2, IL-7, IL-12, IL-18, IL-21, and IL-36γ;

b) the ligand for a cell-surface receptor is Flt3 ligand or TNFSF14; or

c) the chemokine is selected from CXCL10, CCL4, CCL21, and CCL5.

~~19. The LNP of claim 17, wherein the antigen-binding molecule is capable of binding to and inhibiting an immune checkpoint receptor.~~

~~20. The LNP of claim 19, wherein the immune checkpoint receptor is PD-1.~~

~~21. The LNP of claim 17, wherein the antigen-binding molecule is capable of binding to a tumor antigen.~~

~~22. The LNP of claim 21, wherein the antigen binding molecule is a bispecific T cell engager molecule (BiTE) or a bispecific light T cell engager molecule (LiTE).~~

~~23. The LNP of claim 21 or 22, wherein the tumor antigen is a viral antigen selected from HBV core (Hepatitis B core antigen), HBV pol, HbS Ag, HPV E6, HPV E7, Merkel cell polyoma large T antigen, and Epstein Barr virus antigen EBNA2 or BZLF1.~~

~~24. The LNP of claim 21 or 22, wherein the tumor antigen is DLL3 or EpCAM.~~

~~25. The LNP of any one of claims 1-24, wherein the synthetic RNA viral genome and/or the recombinant RNA molecule comprises a microRNA (miRNA) target sequence (miR-TS) cassette, wherein the miR-TS cassette comprises one or more miRNA target sequences.~~

~~26. The LNP of claim 25, wherein the one or more miRNAs are selected from miR-124, miR-1, miR-143, miR-128, miR-219, miR-219a, miR-122, miR-204, miR-217, miR-137, miR-142, and miR-126.~~

~~27. The LNP of claim 26, wherein the miR-TS cassette comprises:~~

~~a. one or more copies of a miR-124 target sequence, one or more copies of a miR-1 target sequence, and one or more copies of a miR-143 target sequence;~~

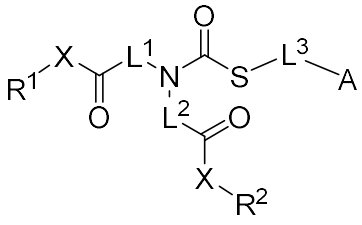
~~b. one or more copies of a miR-128 target sequence, one or more copies of a miR-219a target sequence, and one or more copies of a miR-122 target sequence;~~

~~c. one or more copies of a miR-128 target sequence, one or more copies of a miR-204 target sequence, and one or more copies of a miR-219 target sequence; or~~

~~d. one or more copies of a miR-217 target sequence, one or more copies of a miR-137 target sequence, and one or more copies of a miR-126 target sequence.~~

~~28.~~4. The LNP of any one of claims 1-~~27~~3, wherein the LNP comprises a cationic lipid, a helper lipid, a structural lipid, and a PEG-lipid.

~~29.~~5. The LNP of claim ~~28~~4, wherein the cationic lipid is a compound of Formula (I):



,

Formula (I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

A is $–N(CH_2R^{N1})(CH_2R^{N2})$ or a 4-7-membered heterocyclyl ring containing at least one N, wherein the 4-7-membered heterocyclyl ring is optionally substituted with 0-6 R^3;

each X is independently –O–, $–N(R^1)–$, or $–N(R^2)–$;

R^1 is selected from the group consisting of optionally substituted C_1-C_{31} aliphatic and steroidyl;

R^2 is selected from the group consisting of optionally substituted C_1-C_{31} aliphatic and steroidyl;

R^3 is optionally substituted C_1-C_6 aliphatic;

R^{N1} and R^{N2} are each independently hydrogen, hydroxy-C_1-C_6 alkyl, C_2-C_6 alkenyl, or a C_3-C_7 cycloalkyl;

L^1 is selected from the group consisting of an optionally substituted C_1-C_{20} alkylene chain and a bivalent optionally substituted C_2-C_{20} alkenylene chain;

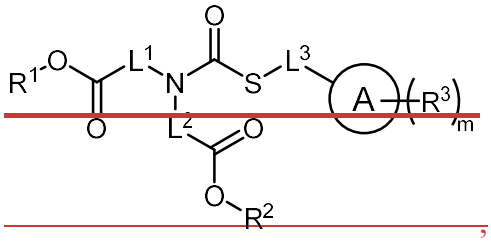
L^2 is selected from the group consisting of an optionally substituted C_1-C_{20} alkylene chain and a bivalent optionally substituted C_2-C_{20} alkenylene chain; and

L^3 is a bond, an optionally substituted C_1-C_6 alkylene chain, or a bivalent optionally substituted C_3-C_7 cycloalkylene; and

with the proviso that when A is $–N(CH_3)(CH_3)$ and X is O, L^3 is not an C_1-C_6 alkylene chain.

~~30. The LNP of claim 29, wherein the number of carbon atoms between the S of the thiolate and the closest N comprised in A is 2-4.~~

~~31. The LNP of claim 29 or 30, wherein the cationic lipid is a compound of Formula (I-a):~~



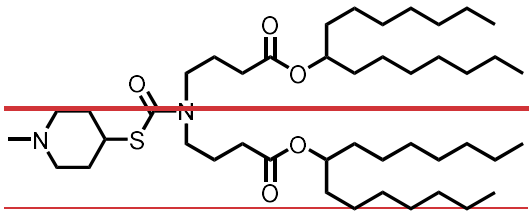
Formula (I-a)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

m is 0, 1, 2, 3, 4, 5, or 6.

32. The LNP of any one of claims 29-31, wherein A is an optionally substituted 5-6 membered heterocyclyl ring.

33. The LNP of claim 29, wherein the cationic lipid is



or a pharmaceutically acceptable salt or solvate thereof.

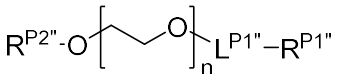
34. The LNP of claim 28, wherein the cationic lipid is selected from DLinDMA, DLin-KC2-DMA, DLin-MC3-DMA (MC3), COATSOME® SS-LC (former name: SS-18/4PE-13), COATSOME® SS-EC (former name: SS-33/4PE-15), COATSOME® SS-OC, COATSOME® SS-OP, Di((Z)-non-2-en-1-yl)9-((4-dimethylamino)butanoyl)oxy)heptadecanedioate (L-319), or N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP).

35. The LNP of any one of claims 28-34, wherein the helper lipid is selected from 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC); and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE).

36. The LNP of claim 28, wherein the cationic lipid is 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and wherein the helper lipid is 1,2-Dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE) or 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE).

~~37. The LNP of any one of claims 28-36, wherein the structural lipid is cholesterol.~~

~~38.~~6. The LNP of any one of claims ~~28-37~~4-5, wherein the PEG-lipid is a compound of Formula (A″):



Formula (A″)

or a pharmaceutically acceptable salt thereof, wherein:

n is an integer between 10 to 200, inclusive of all endpoints;

$L^{P1''}$ is a bond, $–[(CH_2)_{0-3}–C(O)O]_{1-3}–$, $–(CH_2)_{0-3}–C(O)O–(CH_2)_{1-3}–OC(O)–$, or $–C(O)N(H)–$;

$R^{P1''}$ is C_5-C_{25} alkyl or C_5-C_{25} alkenyl; and

$R^{P2''}$ is hydrogen or $–CH_3$,

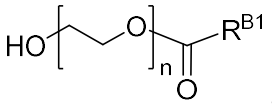
and wherein the LNP has a molar ratio of about 0.001% to about 5% PEG-lipid.

~~39. The LNP of claim 38, wherein $L^{P1''}$ is a bond, $CH_2C(O)O$, $CH_2CH_2C(O)O$, $CH_2C(O)OCH_2C(O)O$, $CH_2C(O)OCH_2CH_2OC(O)$, or $C(O)N(H)$.~~

~~40.~~7. The LNP of claim ~~38~~6, wherein $L^{P1''}$ is a bond.

~~41.~~8. The LNP of any one of claims ~~38-40~~6-7, wherein $R^{P2''}$ is hydrogen.

~~42.~~9. The LNP of any one of claims ~~28-37~~4-5, wherein the PEG-lipid is a compound of Formula (B):



Formula (B)

or a pharmaceutically acceptable salt thereof, wherein:

n is an integer between 10 to 200, inclusive of all endpoints; and

R^{B1} is C_5-C_{25} alkyl or C_5-C_{25} alkenyl, and

wherein the LNP has a molar ratio of about 0.001% to about 5% PEG-lipid.

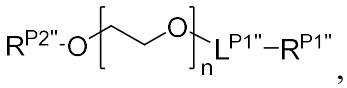
~~43. The LNP of any one of claims 28-37, wherein the PEG-lipid is selected from 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)] (DSPE-PEG); 1,2-dipalmitoyl-rac-glycerol methoxypolyethylene glycol (DPG-PEG); 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene (DSG-PEG); 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene (DSG-PEG); 1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene (DMG-PEG); and 1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene (DMG-PEG), or 1,2-~~

~~distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (DSPE-PEG-amine).~~

~~44. The LNP of any one of claims 28-37, wherein the PEG-lipid is selected from 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)-5000] (DSPE-PEG5K); 1,2-dipalmitoyl-rac-glycerol methoxypolyethylene glycol-2000 (DPG-PEG2K); 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene-5000 (DSG-PEG5K); 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene-2000 (DSG-PEG2K); 1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene-5000 (DMG-PEG5K); and 1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene-2000 (DMG-PEG2K).~~

~~45. The LNP of claim 28, wherein the cationic lipid comprises COATSOME® SS-OC, wherein the helper lipid comprises DSPC, the structural lipid comprises cholesterol (Chol) and wherein the PEG-lipid comprises DPG-PEG2000.~~

~~46.~~10. The LNP of claim ~~28~~4, wherein the cationic lipid comprises COATSOME® SS-OC, wherein the helper lipid comprises DSPC, the structural lipid comprises cholesterol (Chol) and wherein the PEG-lipid is a compound of Formula (A″):



,

Formula (A″)

or a pharmaceutically acceptable salt thereof, wherein:

n is an integer between 10 to 200, inclusive of all endpoints;

$L^{P1''}$ is a bond;

$R^{P1''}$ is C_5-C_{25} alkyl or C_5-C_{25} alkenyl; and

$R^{P2''}$ is hydrogen, and

wherein the LNP has a molar ratio of about 0.001% to about 5% PEG-lipid.

~~47.~~11. The LNP of any one of claims ~~28-37~~ 4-8 and ~~46~~10, wherein the PEG-lipid is selected from the group consisting of BRIJ™ S100, BRIJ™ S20, BRIJ™ O20 and BRIJ™ C20.

~~48.~~12. The LNP of ~~any one of claims 28-37 and 46~~claim 11, wherein the PEG-lipid is BRIJ™ S100.

49. The LNP of any one of claims 45-48, wherein the ratio of SS-OC:DSPC:Chol:PEG-lipid (as a percentage of total lipid content) is A:B:C:D, wherein A+B+C+D = 100%, and wherein

a. A = 40% - 60%, B = 10% - 25%, C = 20% - 30%, and D = 0.01% - 3%;

b. A = 45% - 50%, B = 20% - 25%, C = 25% - 30%, and D = 0.01% - 1%; or

c. A = about 49%, B = about 22%, C = about 28%, and D = about 0.5%

50. The LNP of any one of claims 45-48, wherein the ratio of SS-OC:DSPC:Chol:PEG-lipid (as a percentage of total lipid content) is A:B:C:D, wherein A+B+C+D = 100%, and wherein

a. A = 40% - 60%, B = 10% - 30%, C = 20% - 45%, and D = 0% - 3%;

b. A = 40% - 60%, B = 10% - 30%, C = 25% - 45%, and D = 0.01% - 3%;

c. A = 45% - 55%, B = 10% - 20%, C = 30% - 40%, and D = 1% - 2%;

d. A = 45% - 50%, B = 10% - 15%, C = 35% - 40%, and D = 1% - 2%; or

e. A = about 49%, B = about 11%, C = about 38%, and D = about 1.5%.

51. The LNP of any one of claims 45-48, wherein the ratio of SS-OC:DSPC:Chol:PEG-lipid (as a percentage of total lipid content) is about A:B:C:D, wherein A+B+C+D = 100%, and wherein

a. A = 45% - 65%, B = 5% - 20%, C = 20% - 45%, and D = 0% - 3%;

b. A = 50% - 60%, B = 5% - 15%, C = 30% - 45%, and D = 0.01% - 3%;

c. A = 55% - 60%, B = 5% - 15%, C = 30% - 40%, and D = 1% - 2%;

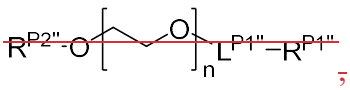
d. A = 55% - 60%, B = 5% - 10%, C = 30% - 35%, and D = 1% - 2%; or

e. A = about 58%, B = about 7%, C = about 33%, and D = about 1.5%.

52. A lipid nanoparticle (LNP), comprising:

a. a synthetic RNA viral genome encoding a Seneca Valley virus (SVV); and

b. a cationic lipid, a helper lipid, a structural lipid, and a PEG-lipid, wherein the PEG-lipid is a compound of Formula (A″):



,

Formula (A″)

or a pharmaceutically acceptable salt thereof, wherein:

n is an integer between 10 to 200, inclusive of all endpoints;

$L^{P1''}$ is a bond, $[(CH_2)_{0-3}C(O)O]_{1-3}$, $(CH_2)_{0-3}C(O)O(CH_2)_{1-3}OC(O)$, or

~~C(O)N(H) ;~~

~~$R^{P1''}$ is C_5-C_{25} alkyl or C_5-C_{25} alkenyl; and~~

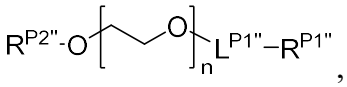
~~$R^{P2''}$ is hydrogen or $-CH_3$, and~~

~~wherein the LNP has a molar ratio of about 0.001% to about 5% PEG-lipid.~~

~~53.~~13. A lipid nanoparticle (LNP), comprising:

a. a synthetic RNA viral genome encoding ~~a Coxsackievirus~~an oncolytic virus; and

b. a cationic lipid, a helper lipid, a structural lipid, and a PEG-lipid, wherein the PEG-lipid is a compound of Formula (A″):



Formula (A″)

or a pharmaceutically acceptable salt thereof, wherein:

n is an integer between 10 to 200, inclusive of all endpoints;

$L^{P1''}$ is a bond, $-[(CH_2)_{0-3}-C(O)O]_{1-3}-$, $-(CH_2)_{0-3}-C(O)O-(CH_2)_{1-3}-OC(O)-$, or $-C(O)N(H)-$;

$R^{P1''}$ is C_5-C_{25} alkyl or C_5-C_{25} alkenyl; and

$R^{P2''}$ is hydrogen or $-CH_3$, and

wherein the LNP has a molar ratio of about 0.001% to about 5% PEG-lipid.

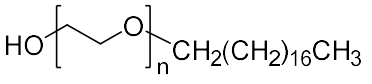
~~54. The LNP of claim 52 or 53, wherein R^{1} is C_{16}-C_{18} alkyl or C_{16}-C_{18} alkenyl.~~

~~55. The LNP of any one of claims 52-54, wherein $L^{P1''}$ is a bond, $CH_2C(O)O$, $CH_2CH_2C(O)O$, $CH_2C(O)OCH_2C(O)O$, $CH_2C(O)OCH_2CH_2OC(O)$, or $C(O)N(H)$.~~

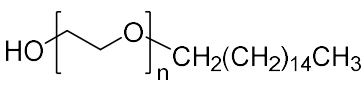
~~56.~~14. The LNP of ~~any one of claims 52-54~~claim 13, wherein $L^{P1''}$ is a bond.

~~57.~~15. The LNP of ~~any one of claims 52-56~~claim 13 or 14, wherein $R^{P2''}$ is hydrogen.

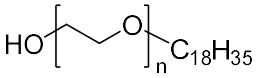
~~58.~~16. The LNP of claim ~~52 or 53~~13, wherein the PEG-lipid is a compound of Formula (A″-f1), Formula (A″-f2), or Formula (A″-f3):



Formula (A″-f1)



Formula (A″-f2)



Formula (A″-f3)

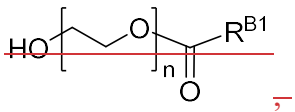
or a pharmaceutically acceptable salt thereof.

~~59. A lipid nanoparticle (LNP), comprising:~~

~~a. a synthetic RNA viral genome encoding a Seneca Valley virus (SVV); and~~

~~b. a cationic lipid, a helper lipid, a structural lipid, and a PEG-lipid, wherein the PEG-lipid is~~

~~a compound of Formula (B):~~



~~,~~

~~Formula (B)~~

~~or a pharmaceutically acceptable salt thereof, wherein:~~

~~n is an integer between 10 to 200, inclusive of all endpoints; and~~

~~R^{B1} is C_5-C_{25} alkyl or C_5-C_{25} alkenyl, and~~

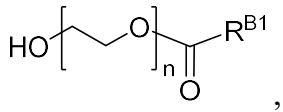
~~wherein the LNP has a molar ratio of about 0.001% to about 5% PEG-lipid.~~

~~60.~~17. A lipid nanoparticle (LNP), comprising:

a. a synthetic RNA viral genome encoding ~~a Coxsackievirus~~an oncolytic virus; and

b. a cationic lipid, a helper lipid, a structural lipid, and a PEG-lipid, wherein the PEG-lipid is

a compound of Formula (B):



,

Formula (B)

or a pharmaceutically acceptable salt thereof, wherein:

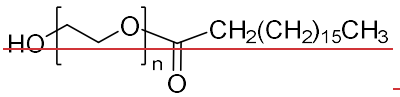
n is an integer between 10 to 200, inclusive of all endpoints; and

R^{B1} is C_5-C_{25} alkyl or C_5-C_{25} alkenyl, and

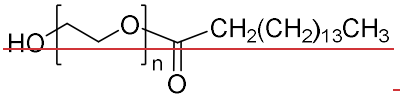
wherein the LNP has a molar ratio of about 0.001% to about 5% PEG-lipid.

~~61. The LNP of claim 59 or 60, wherein R^1 is C_{15}-C_{17} alkyl or C_{15}-C_{17} alkenyl.~~

~~62. The LNP of claim 59 or 60, wherein the PEG-lipid is a compound of Formula (B-a) or Formula (B-b):~~



Formula (B-a)



Formula (B-b)

or a pharmaceutically acceptable salt thereof.

63. The LNP of any one of claims 52-62, wherein n is on average about 20, about 40, about 50, or about 100.

64. The LNP of any one of claims 52-62, wherein n is on average about 100.

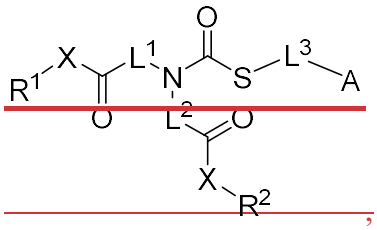
65. The LNP of any one of claims 52-64, wherein the PEG-lipid comprise a PEG moiety having an average molecular weight of of about 200 daltons to about 10,000 daltons, about 500 daltons to about 7,000 daltons, or about 800 daltons to about 6,000 daltons.

66. The LNP of any one of claims 52-65, wherein the PEG-lipid is selected from the group consisting of HO-PEG100-$CH_2(CH_2)_{16}CH_3$, HO-PEG20-$CH_2(CH_2)_{16}CH_3$, HO-PEG20-$CH_2(CH_2)_{14}CH_3$, HO-PEG20-$C_{18}H_{35}$, HO-PEG100-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG50-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG40-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG100-C(O)-$CH_2(CH_2)_{15}CH_3$, HO-PEG40-C(O)-$CH_2(CH_2)_{15}CH_3$, and HO-PEG50-C(O)-$CH_2(CH_2)_{15}CH_3$.

67.18. The LNP of any one of claims 52-6613-17, wherein the LNP induces a reduced immune response *in vivo* as compared to a control LNP lacking the PEG-lipid of Formula (A″) or Formula (B)and/or a ionizable lipid of Formula (I), optionally wherein a PEG-lipid in the control LNP is PEG2K-DPG or PEG2K-DMG.

68.19. The LNP of claim 6718, wherein the immune response is accelerated blood clearance (ABC) of the LNP and/or an anti-PEG IgM response.

69. The LNP of any one of claims 52-68, wherein the cationic lipid is a compound of Formula (I):



Formula (I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

A is $-N(CH_2R^{N1})(CH_2R^{N2})$ or a 4-7-membered heterocyclyl ring containing at least one N, wherein the 4-7-membered heterocyclyl ring is optionally substituted with 0-6 R^3;

each X is independently $-O-$, $-N(R^1)-$, or $-N(R^2)-$;

R^1 is selected from the group consisting of optionally substituted C_1-C_{31} aliphatic and steroidyl;

R^2 is selected from the group consisting of optionally substituted C_1-C_{31} aliphatic and steroidyl;

R^3 is optionally substituted C_1-C_6 aliphatic;

R^{N1} and R^{N2} are each independently hydrogen, hydroxy-C_1-C_6 alkyl, C_2-C_6 alkenyl, or a C_3-C_7 cycloalkyl;

L^1 is selected from the group consisting of an optionally substituted C_1-C_{20} alkylene chain and a bivalent optionally substituted C_2-C_{20} alkenylene chain;

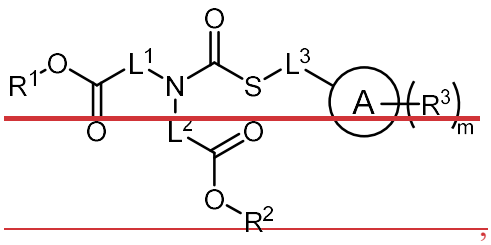
L^2 is selected from the group consisting of an optionally substituted C_1-C_{20} alkylene chain and a bivalent optionally substituted C_2-C_{20} alkenylene chain; and

L^3 is a bond, an optionally substituted C_1-C_6 alkylene chain, or a bivalent optionally substituted C_3-C_7 cycloalkylene; and

with the proviso that when A is $-N(CH_3)(CH_3)$ and X is O, L^3 is not an C_1-C_6 alkylene chain.

70. The LNP of claim 69, wherein the number of carbon atoms between the S of the thiolate and the closest N comprised in A is 2-4.

71. The LNP of claim 69 or 70, wherein the cationic lipid is a compound of Formula (I-a):



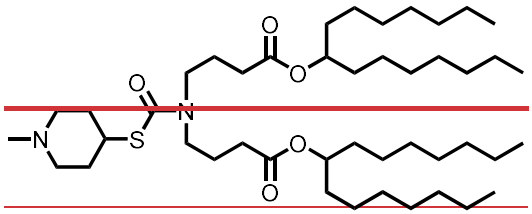
Formula (I-a)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

m is 0, 1, 2, 3, 4, 5, or 6.

72. The LNP of any one of claims 69-71, wherein A is an optionally substituted 5-6-membered heterocyclyl ring.

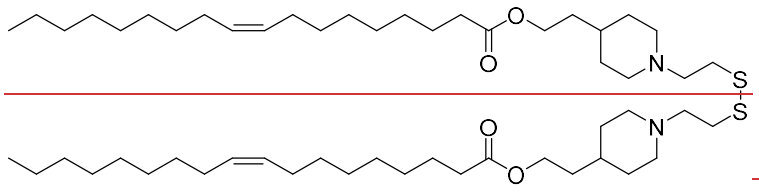
73. The LNP of claim 69, wherein the cationic lipid is



or a pharmaceutically acceptable salt or solvate thereof.

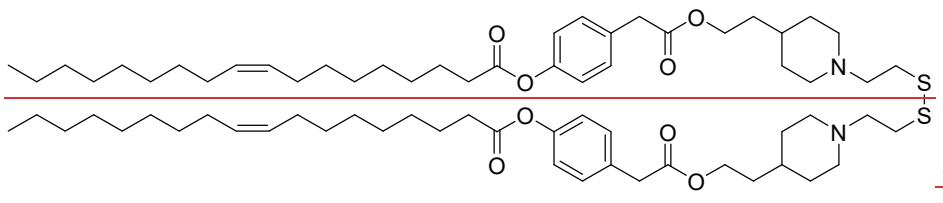
74. The LNP of any one of claims 52-68, wherein the cationic lipid is selected from DLinDMA, DLin-KC2-DMA, DLin-MC3-DMA (MC3), COATSOME® SS-LC (former name: SS-18/4PE-13), COATSOME® SS-EC (former name: SS-33/4PE-15), COATSOME® SS-OC, COATSOME® SS-OP, Di((Z)-non-2-en-1-yl)9-((4-dimethylamino)butanoyl)oxy)heptadecanedioate (L-319), N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), or a mixture thereof.

75. The LNP of any one of claims 52-68, wherein the cationic lipid is a compound of Formula (II-1a):



Formula (II-1a), or

a compound of Formula (II-2a):



~~Formula (II-2a).~~

~~76.~~20. The LNP of any one of claims ~~52-75~~13-19, wherein the cationic lipid is COATSOME® SS-OC~~a compound of Formula (II-1a)~~, the structural lipid is cholesterol, the helper lipid is DSPC, and the PEG-lipid is BRIJ™ S100.

~~77.~~21. The LNP of any one of claims 13-20, wherein the oncolytic virus is a Coxsackievirus or a Seneca Valley virus (SVV)~~52-75, wherein the cationic lipid is a compound of Formula (II-1a), the structural lipid is cholesterol, the helper lipid is DSPC, and the PEG-lipid is MYRJ™ S100, MYRJ™ S50, or MYRJ™ S40~~.

~~78.~~22. The LNP of claim 20, where the oncolytic virus is the Coxsackievirus, wherein the synthetic RNA viral genome comprises a polynucleotide sequence having at least 95%, or 100% sequence identity to SEQ ID NO: 5, wherein the LNP comprises COATSOME® SS-OC (SS-OC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol (Chol), and BRIJ™ S100, and wherein the ratio of SS-OC:DSPC:Chol:BRIJ™ S100 (as a percentage of total lipid content) is A:B:C:D, wherein A+B+C+D = 100%, and wherein A = 40% - 60%, B = 10% - 25%, C = 20% - 30%, and D = 0.01% - 3%~~any one of claims 52-77, wherein the LNP comprises a molar ratio of about 0.1% to about 2% PEG-lipid, such as about 0.2% to about 0.8 mol%, about 0.4% to about 0.6 mol%, about 0.7% to about 1.3%, or about 1.2% to about 1.8% PEG-lipid~~.

~~79.~~23. The LNP of claim 20, where the oncolytic virus is the Seneca Valley virus (SVV), wherein the synthetic RNA viral genome comprises a polynucleotide sequence having at least 95%, or 100% sequence identity to SEQ ID NO: 68, wherein the LNP comprises COATSOME® SS-OC (SS-OC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol (Chol), and BRIJ™ S100, and wherein the ratio of SS-OC:DSPC:Chol:BRIJ™ S100 (as a percentage of total lipid content) is A:B:C:D, wherein A+B+C+D = 100%, and wherein A = 40% - 60%, B = 10% - 25%, C = 20% - 30%, and D = 0.01% - 3%~~any one of~~

claims 52-78, wherein the LNP comprises a molar ratio of about 0.2% to about 0.8%, or about 0.5% PEG-lipid.

80. The LNP of any one of claims 52-78, wherein the LNP comprises a molar ratio of about 1.2% to about 1.8%, or about 1.5% PEG-lipid.

81. The LNP of any one of claims 52-80, wherein the LNP has a molar ratio of about 44% to about 54% cationic lipid, about 19% to about 25% helper lipid, about 24% to about 33% structural lipid, and about 0.2% to about 0.8% PEG-lipid.

82. The LNP of any one of claims 52-81, wherein the LNP comprises a compound of Formula (II-1a), cholesterol, DSPC, and a PEG-lipid selected from HO-PEG100-$CH_2(CH_2)_{16}CH_3$, HO-PEG20-$CH_2(CH_2)_{16}CH_3$, HO-PEG20-$CH_2(CH_2)_{14}CH_3$, HO-PEG20-$C_{18}H_{35}$, HO-PEG100-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG50-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG40-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG100-C(O)-$CH_2(CH_2)_{15}CH_3$, HO-PEG40-C(O)-$CH_2(CH_2)_{15}CH_3$, and HO-PEG50-C(O)-$CH_2(CH_2)_{15}CH_3$, wherein the molar ratio of compound of Formula (II-1a) : cholesterol : DSPC : PEG-lipid is 49 : 28.5 : 22 : 0.5.

83. The LNP of any one of claims 52-81, wherein the LNP comprises a compound of Formula (II-1a), cholesterol, DSPC, and a PEG-lipid selected from HO-PEG100-$CH_2(CH_2)_{16}CH_3$, HO-PEG20-$CH_2(CH_2)_{16}CH_3$, HO-PEG20-$CH_2(CH_2)_{14}CH_3$, HO-PEG20-$C_{18}H_{35}$, HO-PEG100-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG50-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG40-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG100-C(O)-$CH_2(CH_2)_{15}CH_3$, HO-PEG40-C(O)-$CH_2(CH_2)_{15}CH_3$, and HO-PEG50-C(O)-$CH_2(CH_2)_{15}CH_3$, wherein the molar ratio of compound of Formula (II-1a) : cholesterol : DSPC : PEG-lipid is 49 : 27.5 : 22 : 1.5.

84. The LNP of any one of claims 52-81, wherein the LNP comprises a compound of Formula (II-1a), cholesterol, DSPC, and a PEG-lipid selected from HO-PEG100-$CH_2(CH_2)_{16}CH_3$, HO-PEG20-$CH_2(CH_2)_{16}CH_3$, HO-PEG20-$CH_2(CH_2)_{14}CH_3$, HO-PEG20-$C_{18}H_{35}$, HO-PEG100-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG50-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG40-C(O)-$CH_2(CH_2)_{13}CH_3$, HO-PEG100-C(O)-$CH_2(CH_2)_{15}CH_3$, HO-PEG40-C(O)-$CH_2(CH_2)_{15}CH_3$, and HO-PEG50-C(O)-$CH_2(CH_2)_{15}CH_3$, wherein the molar ratio of compound of Formula (II-1a) : cholesterol : DSPC : PEG-lipid is 49 : 38.5 : 11 : 1.5.

~~85. The LNP of any one of claims 52-84, wherein the LNP has a lipid-nitrogen-to-phosphate (N:P) ratio of about 1 to about 25.~~

~~86. The LNP of any one of claims 52-85, wherein the LNP has a N:P ratio of about 14.~~

~~87. The LNP of any one of claims 1-86, wherein hyaluronan is conjugated to the surface of the LNP.~~

~~88.~~24. A pharmaceutical composition comprising a plurality of lipid nanoparticles according to any one of claims 1-~~87~~23.

~~89. The pharmaceutical composition of claim 88, wherein the plurality of LNPs have an average diameter of about 50 nm to about 500 nm, about 150 nm to about 500 nm, about 200 nm to about 500 nm, about 300 nm to about 500 nm, about 350 nm to about 500 nm, about 400 nm to about 500 nm, about 425 nm to about 500 nm, about 450 nm to about 500 nm, or about 475 nm to about 500 nm.~~

~~90.~~25. The pharmaceutical composition of claim ~~88~~24, wherein the plurality of LNPs have an average diameter of about 50 nm to about 120 nm.

~~91. The pharmaceutical composition of claim 88, wherein the plurality of LNPs have an average diameter of about 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, 100 nm, 110 nm, or about 120 nm.~~

~~92. The pharmaceutical composition of claim 88, wherein the plurality of LNPs have an average diameter of about 100 nm.~~

~~93. The pharmaceutical composition of any one of claims 88-92, wherein the plurality of LNPs have an average zeta potential of between about 40 mV to about -40 mV, about 20 mV to about -20 mV, about 10 mV to about -10 mV, about 5 mV to about -5 mV, or about 20 mV to about -40 mV.~~

~~94. The pharmaceutical composition of any one of claims 88-92, wherein the plurality of LNPs have an average zeta potential of less than about 5 mV, less than about 0 mV, less than about -5 mV, less than about -10 mV, less than about -20 mV, less than about -30 mV, less than about -35 mV, or less than about -40 mV.~~

~~95. The pharmaceutical composition of any one of claims 88-92, wherein the plurality of LNPs have an average zeta-potential of between about -50 mV to about -20 mV, about -40 mV to about -20 mV, about -30 mV to about -10 mV, about -20 mV to about 0 mV, about -15 mV to about 5 mV, or about -10 mV to about 10 mV.~~

~~96. The pharmaceutical composition of claim 94 or 95, wherein the plurality of LNPs have an average zeta-potential of about -30 mV, about -31 mV, about -32 mV, about -33 mV, about -34 mV, about -35 mV, about -36 mV, about -37 mV, about -38 mV, about -39 mV, or about -40 mV.~~

~~97. The pharmaceutical composition of any one of claims 88-96, wherein administering the pharmaceutical composition to a subject delivers the recombinant RNA polynucleotide to a target cell of the subject, and wherein the recombinant RNA polynucleotide produces an infectious oncolytic virus capable of lysing the target cell of the subject.~~

~~98. The pharmaceutical composition of claim 97, wherein the target cell is a cancerous cell.~~

~~99. The pharmaceutical composition of any one of claims 88-98, wherein the composition is formulated for intravenous and/or intratumoral delivery.~~

~~100.~~26. The pharmaceutical composition of any one of claims ~~88-99~~24-25, wherein the composition has a duration of therapeutic effect *in vivo* greater than that of a composition lacking the PEG-lipid of Formula (A″) and/or a ionizable lipid of Formula (I).

~~101. The pharmaceutical composition of claim 99 or 100, wherein the composition has a duration of therapeutic effect *in vivo* of about 1 hour or longer, about 2 hours or longer, about 3 hours or longer, about 4 hours or longer, about 5 hours or longer, about 6 hours or longer, about 7 hours or longer, about 8 hours or longer, about 9 hours or longer, about 10 hours or longer, about 12 hours or longer, about 14 hours or longer, about 16 hours or longer, about 18 hours or longer, about 20 hours or longer, about 25 hours or longer, about 30 hours or longer, about 35 hours or longer, about 40 hours or longer, about 45 hours or longer, or about 50 hours or longer.~~

~~102. The pharmaceutical composition of claim 99 or 100, wherein the composition has a half-life and/or an AUC *in vivo* greater than or equal to that of a pre-determined threshold value.~~

~~103. The pharmaceutical composition of any one of claims 88-102, wherein the encapsulation efficiency of the synthetic RNA viral genome by the LNP is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%.~~

~~104. The pharmaceutical composition of any one of claims 88 to 103, wherein the composition has a total lipid concentration of about 10 mM, about 20 mM, about 30 mM, about 40 mM, or about 50 mM.~~

~~105. The pharmaceutical composition of any one of claims 88-104, wherein the composition is formulated at a pH of about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, or about 6.~~

~~106.~~27. The pharmaceutical composition of ~~any one of claims 88 to 105~~claim 26, wherein the composition is formulated for multiple administrations.

~~107. The pharmaceutical composition of claim 106, wherein a subsequent administration is administered at least 3 days, at least 5 days, at least 7 days, at least 9 days, at least 11 days, at least 14 days, or at least 21 days after a first administration.~~

~~108. The pharmaceutical composition of any one of claims 88 to 107, further comprising a pharmaceutically acceptable carrier.~~

~~109. A recombinant RNA molecule comprising a synthetic RNA viral genome encoding an oncolytic Coxsackievirus virus, wherein the Coxsackievirus is a CVA21 strain selected from the Kuykendall strain, the EF strain and the KY strain.~~

~~110. The recombinant RNA molecule of claim 109, wherein the Coxsackievirus is the CVA21-KY strain, and wherein the CVA21-KY strain comprises a polynucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity according to SEQ ID NO: 5.~~

~~111. The recombinant RNA molecule of claim 109, wherein the Coxsackievirus is the CVA21-EF strain, and wherein the CVA21-EF strain comprises a polynucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity according to SEQ ID NO: 9.~~

~~112. The recombinant RNA molecule of claim 109, wherein the Coxsackievirus comprises a 5' UTR (IRES) sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity according to SEQ ID NO: 6 or 10.~~

~~113. The recombinant RNA molecule of claim 109, wherein the Coxsackievirus comprises a P1 sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity according to SEQ ID NO: 7 or 11.~~

~~114. The recombinant RNA molecule of claim 109, wherein the Coxsackievirus comprises a 3D sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity according to SEQ ID NO: 8 or 12.~~

~~115. The recombinant RNA molecule of any one of claims 109-114, wherein the synthetic RNA viral genome does not comprise a polynucleotide sequence having more than 95%, more than 90%, more than 85%, or more than 80% sequence identity according to SEQ ID NO: 1.~~

~~116. The recombinant RNA molecule of any one of claims 109-115, wherein the recombinant RNA molecule does not comprise an RNA viral genome having 100% sequence identity to that of a wildtype Coxsackievirus virus.~~

~~117. A recombinant RNA molecule comprising a synthetic RNA viral genome encoding a Seneca Valley virus (SVV), wherein the SVV comprises is a chimeric SVV, and wherein the synthetic RNA viral genome comprises a polynucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 68.~~

~~118. The recombinant RNA molecule of any one of claims 109-117, further comprising a microRNA (miRNA) target sequence (miR-TS) cassette inserted into the polynucleotide sequence encoding the oncolytic virus, wherein the miR-TS cassette comprises one or more miRNA target sequences, and wherein expression of one or more of the corresponding miRNAs in a cell inhibits replication of the encoded virus in the cell.~~

~~119. The recombinant RNA molecule of claim 118, wherein the one or more miRNAs are selected from miR-124, miR-1, miR-143, miR-128, miR-219, miR-219a, miR-122, miR-204, miR-217, miR-137, miR-142, and miR-126.~~

~~120. The recombinant RNA molecule of claim 119, wherein the miR-TS cassette comprises:~~

a. one or more copies of a miR-124 target sequence, one or more copies of a miR-1 target sequence, and one or more copies of a miR-143 target sequence;
b. one or more copies of a miR-128 target sequence, one or more copies of a miR-219a target sequence, and one or more copies of a miR-122 target sequence;
c. one or more copies of a miR-128 target sequence, one or more copies of a miR-204 target sequence, and one or more copies of a miR-219 target sequence; or
d. one or more copies of a miR-217 target sequence, one or more copies of a miR-137 target sequence, and one or more copies of a miR-126 target sequence.

121. The recombinant RNA molecule of any one of claims 109-120, wherein the recombinant RNA molecule is capable of producing a replication-competent oncolytic virus when introduced into a cell by a non-viral delivery vehicle.

122. The recombinant RNA molecule of claim 121, wherein the cell is a mammalian cell.

123. The recombinant RNA molecule of claim 122, wherein the cell is a mammalian cell present in a mammalian subject.

124. The recombinant RNA molecule of any one of claims 118-123, wherein the one or more miR-TS cassettes is incorporated into the 5' untranslated region (UTR) or 3' UTR of one or more viral genes.

125. The recombinant RNA molecule of any one of claims 118-123, wherein the one or more miR-TS cassettes is incorporated into the open reading frame (ORF), the 5' untranslated region (UTR), or the 3' UTR of one or more viral genes.

126. The recombinant RNA molecule of any of claims 109-125, wherein the recombinant RNA molecule is inserted into a nucleic acid vector.

127. The recombinant RNA molecule of claim 126, wherein the nucleic acid vector is a replicon.

128. The recombinant RNA molecule of claims 109-127, wherein the synthetic RNA viral genome further comprises a heterologous polynucleotide encoding an exogenous payload protein.

~~129. The recombinant RNA molecule of claim 128, wherein the exogenous payload protein comprises or consists of a MLKL 4HB domain, a Gasdermin D N-terminal fragment, a Gasdermin E N-terminal fragment, a HMGB1 Box B domain, a SMAC/Diablo, a Melittin, a L-amino acid oxidase (LAAO), a disintegrin, a TRAIL (TNFSF10), a nitroreductase, a reovirus FAST protein, a leptin/FOSL2, an α-1,3-galactosyltransferase, or an adenosine deaminase 2 (ADA2).~~

~~130. The LNP of claim 129, wherein the nitroreductase is NfsB or NfsA.~~

~~131. The LNP of claim 129, wherein the reovirus FAST protein is ARV p14, BRV p15, or a p14-p15 hybrid.~~

~~132. The recombinant RNA molecule of claim 128, wherein the exogenous payload protein is a fluorescent protein, an enzymatic protein, a cytokine, a chemokine, an antigen-binding molecule capable of binding to a cell surface receptor, or a ligand capable of binding to a cell surface receptor.~~

~~133. The recombinant RNA molecule of claim 132, wherein~~

~~a) the cytokine is selected from GM-CSF, IFNγ, IL-2, IL-7, IL-12, IL-18, IL-21, and IL-36γ;~~

~~b) the ligand for a cell-surface receptor is Flt3 ligand or TNFSF14;~~

~~c) the chemokine is selected from CXCL10, CCL4, CCL21, and CCL5.~~

~~134. The recombinant RNA molecule of claim 132, wherein the antigen-binding molecule is capable of binding to and inhibiting an immune checkpoint receptor.~~

~~135. The recombinant RNA molecule of claim 134, wherein the immune checkpoint receptor is PD-1.~~

~~136. The recombinant RNA molecule of claim 132, wherein the antigen-binding molecule is capable of binding to a tumor antigen.~~

~~137. The recombinant RNA molecule of claim 136, wherein the antigen binding molecule is a bispecific T cell engager molecule (BiTE) or a bispecific light T cell engager molecule (LiTE).~~

~~138. The recombinant RNA molecule of claim 136 or 137, wherein the tumor antigen is a viral antigen selected from HBV-core (Hepatitis B core antigen), HBV-pol, HbS-Ag, HPV E6, HPV E7, Merkel cell polyoma large T antigen, and Epstein Barr virus antigen EBNA2 or BZLF1.~~

~~139. The recombinant RNA molecule of claim 136 or 137, wherein the tumor antigen is DLL3 or EpCAM.~~

~~140. A recombinant DNA template comprising from 5' to 3', a promoter sequence, a 5' junctional cleavage sequence, a polynucleotide sequence encoding an RNA molecule comprising a synthetic RNA viral genome, a poly-A tail, and a 3' junctional cleavage sequence.~~

~~141. A recombinant DNA molecule comprising from 5' to 3', a promoter sequence, a 5' junctional cleavage sequence, a polynucleotide sequence encoding an RNA molecule comprising a synthetic RNA viral genome, a poly-A tail, and a 3' junctional cleavage sequence, wherein the RNA molecule is selected from any one of claims 109-139.~~

~~142. The recombinant DNA molecule of claim 140 or 141, comprising a leader sequence between the promoter sequence and the 5' junctional cleavage sequence.~~

~~143. A recombinant DNA molecule comprising from 5' to 3', a promoter sequence, a leader sequence, a 5' junctional cleavage sequence, a polynucleotide sequence encoding a recombinant RNA molecule comprising a synthetic RNA viral genome, a poly-A tail, and a 3' junctional cleavage sequence.~~

~~144. The recombinant DNA molecule of claim 142 or 143, wherein the leader sequence is less than 100 bp in length.~~

~~145. The recombinant DNA molecule of any one of claims 140-144, wherein the promoter sequence is a T7 promoter sequence.~~

~~146. The recombinant DNA molecule of any one of claims 140-145, wherein the poly-A tail is about 50-90 bp in length or about 65-75 bp in length.~~

~~147. The recombinant DNA molecule of claim 145, wherein the poly-A tail is about 70 bp in length.~~

~~148. The recombinant DNA molecule of any one of claims 140-145, wherein the poly-A tail is about 10-50 bp, or 25-35 bp in length.~~

~~149. The recombinant DNA molecule of any one of claims 140-148, wherein the 5' junctional cleavage sequence comprises or consists of a ribozyme sequence and the 3' junctional cleavage sequence comprises or consists of a ribozyme sequence.~~

~~150. The recombinant DNA molecule of claim 149, wherein the 5' ribozyme sequence is a hammerhead ribozyme sequence and wherein the 3' ribozyme sequence is a hepatitis delta virus ribozyme sequence.~~

~~151. The recombinant DNA molecule of any one of claims 140-148, wherein the 5' junctional cleavage sequence comprises or consists of an RNAseH primer binding sequence and the 3' junctional cleavage sequence comprises or consists of a restriction enzyme recognition sequence.~~

~~152. The recombinant DNA molecule of any one of claims 140-148, wherein the 5' junctional cleavage sequence comprises or consists of a ribozyme sequence and the 3' junctional cleavage sequence comprises or consists of a restriction enzyme recognition sequence.~~

~~153. The recombinant DNA molecule of claim 152, wherein the 5' ribozyme sequence comprises or consists of a hammerhead ribozyme sequence, a Pistol ribozyme sequence, or a modified Pistol ribozyme sequence.~~

~~154. The recombinant DNA molecule of any one of claims 140-153, wherein the 3' junctional cleavage sequence comprises or consists of a Type IIS restriction enzyme recognition sequence.~~

~~155. The recombinant DNA molecule of any one of claims 140-154, wherein the RNA molecule encodes the RNA viral genome of a Coxsackievirus (CVA).~~

~~156. The recombinant DNA molecule of claim 155, wherein the Coxsackievirus is a CVA21 strain.~~

~~157. The recombinant DNA molecule of any one of claims 155-156, wherein the leader sequence comprises or consists of a polynucleotide sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% sequence identity according to SEQ ID NO: 14 or 15.~~

~~158. The recombinant DNA molecule of any one of claims 155-157, wherein the 5' junctional cleavage sequence comprises or consists of a Pistol ribozyme sequence having at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 18, and wherein the P2 motif of the 5' ribozyme sequence has the polynucleotide sequence of "TTTT".~~

~~159. The recombinant DNA molecule of any one of claims 155-157, wherein the 5' junctional cleavage sequence comprises or consists of a Pistol ribozyme sequence having at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 17, and wherein the P2 motif of the 5' ribozyme sequence has the polynucleotide sequence of "TTTA".~~

~~160. The recombinant DNA molecule of any one of claims 155-159, wherein the 3' junctional cleavage sequence comprises or consists of a BsmBI recognition sequence.~~

~~161. The recombinant DNA molecule of any one of claims 155-159, wherein the 3' junctional cleavage sequence comprises or consists of a BsaI recognition sequence.~~

~~162. The recombinant DNA molecule of claim 156, wherein the promoter sequence is a T7 promoter sequence, wherein the leader sequence consists of a polynucleotide sequence according to SEQ ID NO: 15, wherein the 5' junctional cleavage sequence comprises or consists of a Pistol ribozyme sequence according to SEQ ID NO: 18, wherein the poly-A tail is about 70 bp in length, and wherein the 3' junctional cleavage sequence comprises or consists of a BsmBI recognition sequence.~~

~~163. The recombinant DNA molecule of claim 156, wherein the promoter sequence is a T7 promoter sequence, wherein the leader sequence consists of a polynucleotide sequence according to SEQ ID NO: 15, wherein the 5' junctional cleavage sequence comprises or consists of a Pistol ribozyme sequence according to SEQ ID NO: 18, wherein the poly-A tail is about 70 bp in length, and wherein the 3' junctional cleavage sequence comprises or consists of a BsaI recognition sequence.~~

~~164. The recombinant DNA molecule of any one of claims 140-154, wherein the RNA molecule encodes the RNA viral genome of a Seneca Valley virus (SVV).~~

~~165. The recombinant DNA molecule of claim 164, wherein the leader sequence comprises or consists of a polynucleotide sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% sequence identity according to any one of SEQ ID NO: 53-63.~~

~~166. The recombinant DNA molecule of claim 164, wherein the leader sequence comprises or consists of a polynucleotide sequence having at least 70%, at least 80%, at least 90%, at least 95%, or 100% sequence identity according to SEQ ID NO: 58.~~

~~167. The recombinant DNA molecule of any one of claims 164 to 166, wherein the 5' ribozyme sequence is a Pistol ribozyme sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to SEQ ID NO: 64 or 65, and wherein the P2 motif of the 5' ribozyme sequence has the polynucleotide sequence of "TCAA" or "TTAA".~~

~~168. The recombinant DNA molecule of any one of claims 164 to 167, wherein the RNA viral genome comprises a 5' UTR (IRES) sequence having at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity to nucleic acids 1-670 of SEQ ID NO: 68.~~

~~169. The recombinant DNA molecule of any one of claims 164 to 168, wherein the 3' junctional cleavage sequence comprises or consists of a SapI recognition sequence.~~

~~170. The recombinant DNA molecule of claim 164, wherein the promoter sequence is a T7 promoter sequence, wherein the leader sequence consists of a polynucleotide sequence according to SEQ ID NO: 53, wherein the 5' junctional cleavage sequence comprises or consists of a Pistol ribozyme sequence according to SEQ ID NO: 64, wherein the poly-A tail is about 70 bp in length, and wherein the 3' junctional cleavage sequence comprises or consists of a SapI recognition sequence.~~

~~171. The recombinant DNA molecule of claim 164, wherein the promoter sequence is a T7 promoter sequence, wherein the leader sequence consists of a polynucleotide sequence according to SEQ ID NO: 58, wherein the 5' junctional cleavage sequence comprises or consists of a Pistol ribozyme sequence according to SEQ ID NO: 64, wherein the poly-A tail is about 70 bp in length, and wherein the 3' junctional cleavage sequence comprises or consists of a SapI recognition sequence.~~

~~172. The recombinant DNA molecule of any one of claims 140-171, wherein the recombinant DNA molecule does not comprise additional nucleic acid within the region spanning the promoter sequence and the 3' junctional cleavage sequence.~~

~~173. A method of producing a recombinant RNA molecule, comprising in vitro transcription of the DNA molecule of any one of claims 140-172 and purification of the resulting recombinant RNA molecule.~~

~~174. The method of claim 173, wherein the recombinant RNA molecule comprises 5' and 3' ends that are native to the oncolytic virus encoded by the synthetic RNA viral genome.~~

~~175. A composition comprising an effective amount of the recombinant RNA molecule of any one of claims 109-139, and a carrier suitable for administration to a mammalian subject.~~

~~176. A particle comprising the recombinant RNA molecule of any one of claims 109-139.~~

~~177. The particle of claim 176, wherein the particle is biodegradable.~~

~~178. The particle of claim 177, wherein the particle is selected from the group consisting of a nanoparticle, an exosome, a liposome, and a lipoplex.~~

~~179. The particle of claim 178, wherein the exosome is a modified exosome derived from an intact exosome or an empty exosome.~~

~~180. A pharmaceutical composition comprising a plurality of particles according to any one of claims 176-179.~~

~~181. The pharmaceutical composition of claim 180, wherein the plurality of particles have an average size of about 50 nm to about 500 nm, about 150 nm to about 500 nm, about 200 nm to about 500 nm, about 300 nm to about 500 nm, about 350 nm to about 500 nm, about 400 nm to about 500 nm, about 425 nm to about 500 nm, about 450 nm to about 500 nm, or about 475 nm to about 500 nm.~~

~~182. The pharmaceutical composition of claim 180 wherein the plurality of particles have an average size of about 50 nm to about 120 nm.~~

~~183. The pharmaceutical composition of claim 180 wherein the plurality of particles have an average size of about 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, 100 nm, 110 nm, or about 120 nm.~~

~~184. The pharmaceutical composition of claim 180 wherein the plurality of particles have an average size of about 100 nm.~~

~~185. The pharmaceutical composition of any one of claims 180-184, wherein the plurality of particles have an average zeta-potential of between about 40 mV to about -40 mV, about 20 mV to about -20 mV, about 10 mV to about -10 mV, about 5 mV to about -5 mV, or about 20 mV to about -40 mV.~~

~~186. The pharmaceutical composition of any one of claims 180-184, wherein the plurality of particles have an average zeta-potential of less than about 5 mV, less than about 0 mV, less than about -5 mV, less than about -10 mV, less than about -20 mV, less than about -30 mV, less than about -35 mV, or less than about -40 mV.~~

~~187. The pharmaceutical composition of any one of claims 180-186, wherein the plurality of particles have an average zeta-potential of between about -50 mV to about -20 mV, about -40 mV to about -20 mV, about -30 mV to about -10 mV, about -20 mV to about 0 mV, about -15 mV to about 5 mV, or about -10 mV to about 10 mV.~~

~~188. The pharmaceutical composition of any one of claims 180-186, wherein the plurality of particles have an average zeta-potential of about -30 mV, about -31 mV, about -32 mV, about -33 mV, about -34 mV, about -35 mV, about -36 mV, about -37 mV, about -38 mV, about -39 mV, or about -40 mV.~~

~~189. The pharmaceutical composition of any one of claims 180-188, wherein delivery of the composition to a subject delivers the encapsulated recombinant RNA molecule to a target cell, and wherein the encapsulated recombinant RNA molecule produces an infectious virus capable of lysing the target cell.~~

~~190. An inorganic particle comprising the recombinant RNA molecule of any one of claims 109-139.~~

~~191. The inorganic particle of claim 190, wherein the inorganic particle is selected from the group consisting of a gold nanoparticle (GNP), gold nanorod (GNR), magnetic nanoparticle (MNP), magnetic nanotube (MNT), carbon nanohorn (CNH), carbon fullerene, carbon nanotube (CNT), calcium phosphate nanoparticle (CPNP), mesoporous silica nanoparticle (MSN), silica nanotube (SNT), or a starlike hollow silica nanoparticle (SHNP).~~

~~192. A composition comprising the inorganic particle of any one of claims 190-191, wherein the average diameter of the particles is less than about 500 nm, is between about 50 nm and 500 nm, is between about 250 nm and about 500 nm, or is about 350 nm.~~

~~193. The LNP of any one of claims 1-87, the particle of any one of claims 176-179, or the inorganic particle of any one of claims 190-191, further comprising a second recombinant RNA molecule encoding a payload molecule.~~

~~194. The LNP, particle, or inorganic particle of claim 193, wherein the second recombinant RNA molecule is a replicon.~~

~~195. A pharmaceutical composition comprising the LNP of any one of claims 1-87, the particle of any one of claims 176-179, or the inorganic particle of any one of claims 190-191, wherein the composition is formulated for intravenous and/or intratumoral delivery.~~

~~196. The pharmaceutical composition of claim 195, wherein the target cell of the LNP, the particle, or the inorganic particle is a cancerous cell.~~

~~197.~~28. ~~A~~ An *in vitro* method of killing a cancerous cell comprising exposing the cancerous cell to the ~~particle~~ LNP of any one of claims 1-~~87, 176-179, or 190-191, the recombinant RNA molecule of any one of claims 109-139~~23, or ~~compositions thereof~~the pharmaceutical composition of any one of claims 24-27, under conditions sufficient for the intracellular delivery of the particle to said cancerous cell, wherein the replication-competent virus produced by the encapsulated polynucleotide results in killing of the cancerous cell.

~~198. The method of claim 197, wherein the replication-competent virus is not produced in non-cancerous cells.~~

~~199. The method of claim 197 or 198, wherein the method is performed *in vivo*, *in vitro*, or *ex vivo*.~~

200.29. A method of treating a cancer in a subject comprising administering to a subject suffering from the cancer an effective amount of the The particle LNP of any one of claims 1-87, 176-179, or 190-191, the recombinant RNA molecule of any one of claims 109-139, or compositions thereof23, or the pharmaceutical composition of any one of claims 24-27, for treating a cancer in a subject suffering from a cancer.

201. The method of claim 200, wherein the particle or composition thereof is administered intravenously, intranasally, intratumorally, intraperitoneally, or as an inhalant.

202. The method of claim 200, wherein the particle or composition thereof is administered intratumorally and/or intravenously.

203.30. The method LNP or the pharmaceutical composition of any one of claims 200-202claim 29, wherein the particle or composition thereof is administered to the subject repeatedly.

204. The method of any one of claims 200-203, wherein the subject is a mouse, a rat, a rabbit, a cat, a dog, a horse, a non-human primate, or a human.

205.31. The method LNP or the pharmaceutical composition of any of claims 200-20429-30, wherein the cancer is lung cancer, breast cancer, colon cancer, or pancreatic cancer, and wherein the synthetic RNA viral genome comprises a polynucleotide sequence derived from the KY strain.

206.32. The method LNP or the pharmaceutical composition of any of claims 200-20429-30, wherein the cancer is bladder cancer, renal cell carcinoma, ovarian cancer, gastric cancer or liver cancer, and wherein the synthetic RNA viral genome comprises a polynucleotide sequence derived from the EF strain.

207. The method of any one of claims 197-204, wherein the cancer is selected from lung cancer, breast cancer, ovarian cancer, cervical cancer, prostate cancer, testicular cancer, colorectal cancer, colon cancer, pancreatic cancer, liver cancer, renal cell carcinoma, gastric cancer, head and neck cancer, thyroid cancer, malignant glioma, glioblastoma, melanoma, B-cell chronic lymphocytic leukemia, multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), Merkel cell carcinoma, diffuse large B-cell lymphoma

~~(DLBCL), sarcoma, a neuroblastoma, a neuroendocrine cancer, a rhabdomyosarcoma, a medulloblastoma, a bladder cancer, and marginal zone lymphoma (MZL).~~

~~208. The method of any of claims 197-204, wherein the cancer is selected from the groups consisting of lung cancer, breast cancer, colon cancer, pancreatic cancer, bladder cancer, renal cell carcinoma, ovarian cancer, gastric cancer and liver cancer.~~

~~209. The method of any of claims 197-204, wherein the cancer is renal cell carcinoma, lung cancer, or liver cancer.~~

~~210. The method of claim 205, 207, or 208, wherein the lung cancer is small cell lung cancer or non-small cell lung cancer (e.g., squamous cell lung cancer or lung adenocarcinoma).~~

~~211. The method of any of claims 206, 207, and 208, wherein the liver cancer is hepatocellular carcinoma (HCC) (e.g., Hepatitis B virus associated HCC).~~

~~212. The method of claim 207, wherein the prostate cancer is treatment-emergent neuroendocrine prostate cancer.~~

~~213. The method of any one of claims 197-204, wherein the cancer is lung cancer, liver cancer, prostate cancer (e.g., CRPC-NE), bladder cancer, pancreatic cancer, colon cancer, gastric cancer, breast cancer, neuroblastoma, renal cell carcinoma, ovarian cancer, rhabdomyosarcoma, medulloblastoma, neuroendocrine cancer, Merkel cell carcinoma, or melanoma.~~

~~214. The method of any one of claims 197-204, wherein the cancer is small cell lung cancer (SCLC) or neuroblastoma.~~

~~215. A method of treating a cancer in a subject in need thereof comprising administering an effective amount of a CVA21-EF virus to the subject.~~

~~216. A method of treating a cancer in a subject in need thereof comprising administering an effective amount of a CVA21-KY virus to the subject~~

~~217. A method of treating a cancer in a subject in need thereof comprising administering an effective amount of a CVA21-Kuykendall virus to the subject.~~

~~218. The method of any one of claims 215-217, wherein the virus is administered intratumorally and/or intravenously.~~

~~219. The method of any one of claims 197-218, further comprising administering an immune checkpoint inhibitor to the subject.~~

~~220. The method of claim 219, wherein the immune checkpoint inhibitor is an inhibitor of PD-1.~~

~~221. The method of any one of claims 197-218, further comprising administering an engineered immune cell comprising an engineered antigen receptor.~~

~~222. A method of treating a cancer in a subject in need thereof, comprising administering a therapeutically effective amount of an oncolytic Coxsackievirus, wherein the Coxsackievirus is a CVA21 strain, or a polynucleotide encoding the CVA21 to the subject, wherein the cancer is classified as sensitive to CVA21 infection based on the expression of ICAM-1 and/or the percentage of ICAM-1 positive cancer cells.~~

~~223. A method of treating a cancer in a subject in need thereof, comprising:~~

~~(a) determining the expression level of ICAM1 and/or the percentage of ICAM-1 positive cancer cells in the cancer;~~

~~(b) classifying the cancer as sensitive to Coxsackievirus 21 (CVA21) infection based on the expression of ICAM-1 and/or the percentage of ICAM-1 positive cancer cells determined in (a); and~~

~~(c) administering a therapeutically effective amount of CVA21 or a polynucleotide encoding the CVA21 to the subject if the cancer is classified as sensitive to CVA21 infection in step (b).~~

~~224. A method of selecting a subject suffering from a cancer for treatment with a Coxsackievirus 21 (CVA21) or a polynucleotide encoding the CVA21, comprising:~~

~~(a) determining the expression level of ICAM-1 and/or the percentage of ICAM-1 positive cancer cells in the cancer;~~

~~(b) classifying the cancer as sensitive to CVA21 infection based on the expression level of ICAM-1 and/or the percentage of ICAM1 positive cancer cells as determined in (a);~~

~~(c) selecting the subject for treatment with the CVA21 or the polynucleotide encoding the CVA21 if the cancer is classified as sensitive to CVA21 infection in (b); and~~

~~(d) administering the CVA21 or the polynucleotide encoding the CVA21 to the selected subjects~~

~~225. The method of any one of claims 222-224, wherein the CVA21 strain is CVA21-KY~~.