CLAIMS

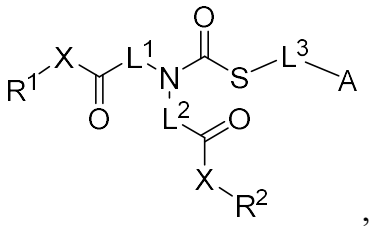
1. A lipid nanoparticle (LNP) comprising a synthetic RNA viral genome encoding an oncolytic Coxsackievirus virus, wherein the Coxsackievirus is a CVA21 strain selected from an EF strain and an KY strain.

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

5. The LNP of claim 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;

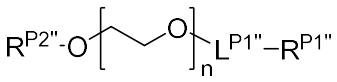
L^1 is selected from the group consisting of an optionally substituted C_1-C_{20} alkylene chain and a bivalent optionally substituted C_2-C_{20} alkenylene chain;

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.

6. The LNP of any one of claims 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, $–[(CH_2)_{0\text{-}3}–C(O)O]_{1\text{-}3}–$, $–(CH_2)_{0\text{-}3}–C(O)O–(CH_2)_{1\text{-}3}–OC(O)–$, or $–C(O)N(H)–$;

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

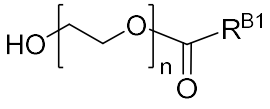
$R^{P2″}$ is hydrogen or $–CH_3$,

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

7. The LNP of claim 6, wherein $L^{P1″}$ is a bond.

8. The LNP of any one of claims 6-7, wherein $R^{P2″}$ is hydrogen.

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



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Formula (B)

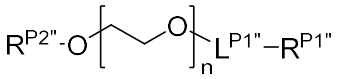
or a pharmaceutically acceptable salt thereof, wherein:

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.

10. The LNP of claim 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.

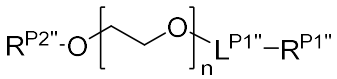
11. The LNP of any one of claims 4-8 and 10, wherein the PEG-lipid is selected from the group consisting of BRIJ™ S100, BRIJ™ S20, BRIJ™ O20 and BRIJ™ C20.

12. The LNP of claim 11, wherein the PEG-lipid is BRIJ™ S100.

13. A lipid nanoparticle (LNP), comprising:

a. a synthetic RNA viral genome encoding 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

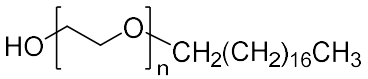
$R^{P2″}$ is hydrogen or $–CH_3$, and

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

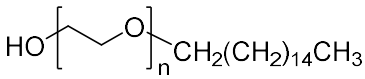
14. The LNP of claim 13, wherein $L^{P1″}$ is a bond.

15. The LNP of claim 13 or 14, wherein $R^{P2″}$ is hydrogen.

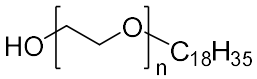
16. The LNP of claim 13, wherein the PEG-lipid is a compound of Formula (A″-f1), Formula (A″-f2), or Formula (A″-f3):



Formula (A″-f1)



Formula (A″-f2)



Formula (A″-f3)

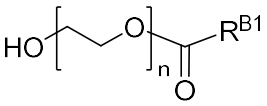
or a pharmaceutically acceptable salt thereof.

17. A lipid nanoparticle (LNP), comprising:

a. a synthetic RNA viral genome encoding 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.

18. The LNP of any one of claims 13-17, 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), optionally wherein a PEG-lipid in the control LNP is PEG2K-DPG or PEG2K-DMG.

19. The LNP of claim 18, wherein the immune response is accelerated blood clearance (ABC) of the LNP and/or an anti-PEG IgM response.

20. The LNP of any one of claims 13-19, wherein the cationic lipid is COATSOME® SS-OC, the structural lipid is cholesterol, the helper lipid is DSPC, and the PEG-lipid is BRIJ™ S100.

21. The LNP of any one of claims 13-20, wherein the oncolytic virus is a Coxsackievirus or a Seneca Valley virus (SVV).

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%.

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%.

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

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

26. The pharmaceutical composition of any one of claims 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).

27. The pharmaceutical composition of claim 26, wherein the composition is formulated for multiple administrations.

28. An *in vitro* method of killing a cancerous cell comprising exposing the cancerous cell to the LNP of any one of claims 1-23, or 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.

29. The LNP of any one of claims 1-23, or the pharmaceutical composition of any one of claims 24-27, for treating a cancer in a subject suffering from a cancer.

30. The LNP or the pharmaceutical composition of claim 29, wherein the particle or composition thereof is administered to the subject repeatedly.

31. The LNP or the pharmaceutical composition of any of claims 29-30, 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.

32. The LNP or the pharmaceutical composition of any of claims 29-30, 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.

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