

through molecular cloning, antibody engineering, and even enzymatic methods. Antigen-binding fragments of antibodies have a considerable potential to overcome the disadvantages of conventional mAbs, such as poor penetration into solid tumors and Fc-mediated bystander activation of the immune system. Antibody fragments can be used on their own or linked to other molecules to generate numerous possibilities for bispecific, multi-specific, multimeric, or multifunctional molecules, and to achieve a variety of biological effects. Antibody fragments can offer several advantages over the use of conventional antibodies. For example, they can be produced easily, generally using microbial expression systems, which results in faster cultivation, higher yields, and lower production costs (Fernandes JC, Drug Discov Today. 2018 Dec; 23(12):1996-2002). Their small size allows access to challenging, cryptic epitopes, and tumour penetration, they have reduced immunogenicity, and the lack of Fc limits bystander activation of the immune system (Kholodenko *et al.* Curr Med Chem. 2019; 26(3):396-426). On the other hand, their smaller size results in faster renal excretion, which may require higher doses and/or more frequent dosing regimens *in vivo*.

Although LNPs have been shown to be advantageous for *in vivo* delivery, systemic delivery of RNA therapeutics other than liver hepatocytes remains highly challenging. The relatively large size of these LNPs reduces the therapeutic index for liver indications by several mechanisms: (1) larger LNPs are unable to efficiently bypass the fenestrae of the endothelial cells that line liver sinusoids, preventing access to target cells (hepatocytes); (2) larger LNPs are unable to be efficiently internalized by hepatocytes *via* clathrin-mediated endocytosis with several different receptors (*e.g.* asialoglycoprotein receptor (ASGPR), low-density lipoprotein (LDL) receptor); and (3) LNPs above a certain threshold size are prone to preferential uptake by cells of the reticuloendothelial system, which can provoke dose-limiting immune responses. Despite these advances, LNP-mediated delivery of larger, rigid polynucleotide cargos (*e.g.*, double stranded linear DNA, plasmid DNA, closed-ended double stranded DNA (ceDNA)) presents additional challenges relative to the smaller and/or flexible cargos (*e.g.*, siRNA). One such challenge involves the size of the resulting LNP when large, rigid cargo is encapsulated.

To fully realize the potential of LNP-targeted nucleic acid therapeutics, an efficient *in vivo* delivery system is needed.

SUMMARY

In an aspect of the present disclosure, there is provided a pharmaceutical composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises a cationic lipid, a sterol, a non-cationic lipid, and a PEG5000 PEGylated lipid, a therapeutic nucleic acid (TNA) comprising a non-viral vector, and at least one pharmaceutically acceptable excipient, wherein the LNP comprises a single-chain variable fragment (scFv) linked to the LNP, and wherein the scFv is directed against an antigen present on the surface of a cell.

In another aspect of the present disclosure, there is provided a use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer in a subject.

5 In a further aspect of the present disclosure, there is provided a use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for delivering a therapeutic nucleic acid (TNA) or increasing the concentration of the TNA to a tumor in a subject.

In another aspect of the present disclosure, there is provided a use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for delivering a therapeutic nucleic acid (TNA) or increasing the concentration of the TNA to the liver of a subject.

10 In a further aspect of the present disclosure, there is provided a pharmaceutical composition as disclosed herein for use in therapy.

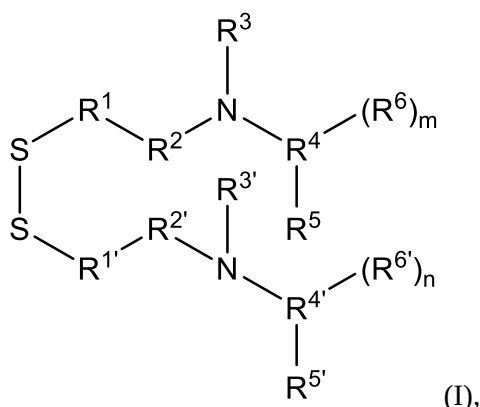
The present disclosure provides a pharmaceutical composition comprising a lipid nanoparticle (LNP), a therapeutic nucleic acid (TNA), and at least one pharmaceutically acceptable excipient, wherein the LNP comprises a single chain fragment variable (scFv) linked to the LNP, and wherein the scFv is directed against an antigen present on the surface of a cell (*e.g.*, a tumor cell). The LNP
15 compositions described herein advantageously provide efficient, covalent conjugation with minimal effects on particle size and stability. It is a finding of the present disclosure that maleimide

conjugation of scFv to LNP resulted in robust conjugation to the LNP along with other thiol based cross-linking methods and importantly, maintained LNP size and integrity.

According to a first example, the disclosure provides a pharmaceutical composition comprising a lipid nanoparticle (LNP), a therapeutic nucleic acid (TNA), and at least one pharmaceutically acceptable excipient, wherein the LNP comprises a single-chain variable fragment (scFv) linked to the LNP, and wherein the scFv is directed against an antigen present on the surface of a cell. In some examples, the scFv is covalently linked to the LNP. In some examples, the scFv is chemically conjugated to the LNP. In some examples, the scFv is chemically conjugated to the LNP via a non-cleavable linker. In some examples, the non-cleavable linker is a maleimide-containing linker. In some examples, the scFv is chemically conjugated to the LNP via a cleavable linker. In some examples, the cleavable linker is a pyridyldisulfide (PDS)-containing linker. In some examples, the scFv is linked to the LNP via transglutaminase-mediated conjugation. In some examples of any of the above aspects and examples, the antigen is a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA). In a further examples the antigen is human epidermal growth factor receptor 2 (HER2). In some examples of any of the above aspects and examples, the scFv is bivalent. In some examples of any of the above aspects and examples, the LNP is capable of being internalized into the cell. In some examples of any of the above aspects and examples, the scFv comprises an amino acid sequence of SEQ ID NO:2 or has a sequence similarity of at least 99% to the amino acid sequence set forth in SEQ ID NO:2. In some examples of any of the above aspects and examples, the scFv comprises an amino acid sequence of SEQ ID NO:3 or has a sequence similarity of at least 99% to the amino acid sequence set forth in SEQ ID NO:3. In some examples of any of the above aspects and examples, the LNP comprises a lipid selected from the group consisting of: a cationic lipid, a sterol or a derivative thereof, a non-cationic lipid, and a PEGylated lipid. In some examples of any of the above aspects and examples, the TNA is encapsulated in the LNP. In some examples of any of the above aspects and examples, the TNA is selected from the group consisting of minigenes, plasmids, minicircles, small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotides (ASO), ribozymes, closed-ended (ceDNA), ministring, doggybone™, protelomere closed ended DNA, or dumbbell linear DNA, dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), mRNA, tRNA, rRNA, DNA viral vectors, viral RNA vector, non-viral vector and any combination thereof. In some examples, the TNA is ceDNA. In some examples, the ceDNA is linear duplex DNA. In some examples, the TNA is mRNA. In some examples, the TNA is siRNA. In some examples, the TNA is a plasmid. In some examples of any of the above aspects and examples, the pharmaceutical composition is administered to a subject. In some examples, the subject is a human patient in need of treatment with LNP encapsulated with TNA. In some examples of any of the above aspects and examples, the composition is targeted to a cell expressing the cell-

surface antigen for which the scFv is directed. In some examples of any of the above aspects and examples, the composition is targeted to tumor cells. In some examples of any of the above aspects and examples, the composition is targeted to liver cells. In some examples of any of the above aspects and examples, the composition is targeted to hepatocytes in the liver.

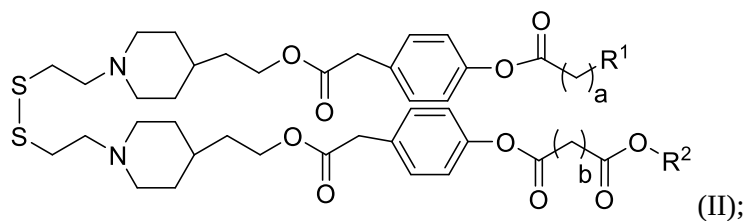
5 In some examples of any of the above aspects and examples, the cationic lipid is represented by Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

- 10 R^1 and $R^{1'}$ are each independently optionally substituted linear or branched C_{1-3} alkylene;
 R^2 and $R^{2'}$ are each independently optionally substituted linear or branched C_{1-6} alkylene;
 R^3 and $R^{3'}$ are each independently optionally substituted linear or branched C_{1-6} alkyl;
or alternatively, when R^2 is optionally substituted branched C_{1-6} alkylene, R^2 and R^3 , taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;
or alternatively, when $R^{2'}$ is optionally substituted branched C_{1-6} alkylene, $R^{2'}$ and $R^{3'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;
15 R^4 and $R^{4'}$ are each independently $-CR^a$, $-C(R^a)_2CR^a$, or $-[C(R^a)_2]_2CR^a$;
 R^a , for each occurrence, is independently H or C_{1-3} alkyl;
or alternatively, when R^4 is $-C(R^a)_2CR^a$, or $-[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, R^3 and R^4 , taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;
20 or alternatively, when $R^{4'}$ is $-C(R^a)_2CR^a$, or $-[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, $R^{3'}$ and $R^{4'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;
 R^5 and $R^{5'}$ are each independently hydrogen, C_{1-20} alkylene or C_{2-20} alkenylene;
 R^6 and $R^{6'}$, for each occurrence, are independently C_{1-20} alkylene, C_{3-20} cycloalkylene, or C_{2-20} alkenylene; and
25 m and n are each independently an integer selected from 1, 2, 3, 4, and 5.

In some examples of any of the above aspects and examples, the cationic lipid is represented by Formula (II):



or a pharmaceutically acceptable salt thereof, wherein:

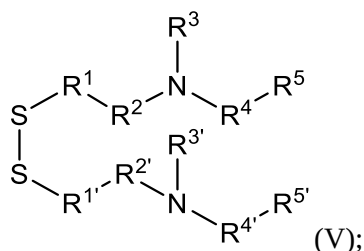
a is an integer ranging from 1 to 20;

b is an integer ranging from 2 to 10;

5 R¹ is absent or is selected from (C₂-C₂₀)alkenyl, -C(O)O(C₂-C₂₀)alkyl, and cyclopropyl substituted with (C₂-C₂₀)alkyl; and

R² is (C₂-C₂₀)alkyl.

In some examples of any of the above aspects and examples, the lipid is represented by the Formula (V):



or a pharmaceutically acceptable salt thereof, wherein:

R¹ and R^{1'} are each independently (C₁-C₆)alkylene optionally substituted with one or more groups selected from R^a;

R² and R^{2'} are each independently (C₁-C₂)alkylene;

15 R³ and R^{3'} are each independently (C₁-C₆)alkyl optionally substituted with one or more groups selected from R^b;

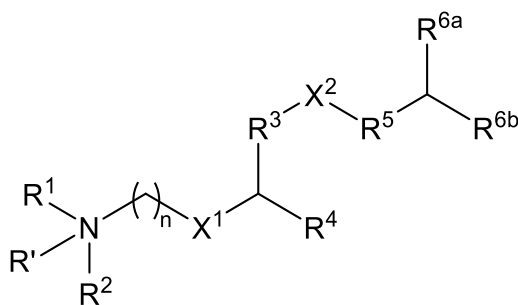
or alternatively, R² and R³ and/or R^{2'} and R^{3'} are taken together with their intervening N atom to form a 4- to 7-membered heterocyclyl;

R⁴ and R^{4'} are each a (C₂-C₆)alkylene interrupted by -C(O)O-;

20 R⁵ and R^{5'} are each independently a (C₂-C₃₀)alkyl or (C₂-C₃₀)alkenyl, each of which are optionally interrupted with -C(O)O- or (C₃-C₆)cycloalkyl; and

R^a and R^b are each halo or cyano.

In some examples of any of the above aspects and examples, the cationic lipid is represented by Formula (XV):



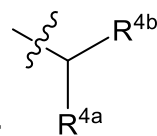
(XV)

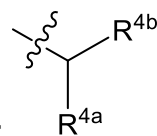
or a pharmaceutically acceptable salt thereof, wherein:

R' is absent, hydrogen, or C₁-C₆ alkyl; provided that when R' is hydrogen or C₁-C₆ alkyl, the nitrogen atom to which R', R¹, and R² are all attached is protonated;

R¹ and R² are each independently hydrogen, C₁-C₆ alkyl, or C₂-C₆ alkenyl;

R³ is C₁-C₁₂ alkylene or C₂-C₁₂ alkenylene;



R⁴ is C₁-C₁₆ unbranched alkyl, C₂-C₁₆ unbranched alkenyl, or ; wherein:

R^{4a} and R^{4b} are each independently C₁-C₁₆ unbranched alkyl or C₂-C₁₆ unbranched alkenyl;

R⁵ is absent, C₁-C₈ alkylene, or C₂-C₈ alkenylene;

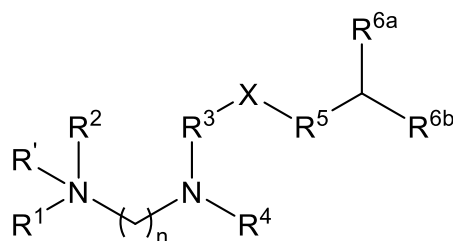
R^{6a} and R^{6b} are each independently C₇-C₁₆ alkyl or C₇-C₁₆ alkenyl; provided that the total number of carbon atoms in R^{6a} and R^{6b} as combined is greater than 15;

X¹ and X² are each independently -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, -S-S-, -C(R^a)=N-, -N=C(R^a)-, -C(R^a)=NO-, -O-N=C(R^a)-, -C(=O)NR^a-, -NR^aC(=O)-, -NR^aC(=O)NR^a-, -OC(=O)O-, -OSi(R^a)₂O-, -C(=O)(CR^a)C(=O)O-, or OC(=O)(CR^a)C(=O)-; wherein:

R^a, for each occurrence, is independently hydrogen or C₁-C₆ alkyl; and

n is an integer selected from 1, 2, 3, 4, 5, and 6.

In some examples of any of the above aspects and examples, the cationic lipid is represented by Formula (XX):



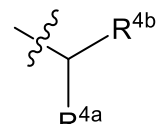
(XX)

or a pharmaceutically acceptable salt thereof, wherein:

R' is absent, hydrogen, or C₁-C₃ alkyl; provided that when R' is hydrogen or C₁-C₃ alkyl, the nitrogen atom to which R', R¹, and R² are all attached is protonated;

R¹ and R² are each independently hydrogen or C₁-C₃ alkyl;

R³ is C₃-C₁₀ alkylene or C₃-C₁₀ alkenylene;



5 R⁴ is C₁-C₁₆ unbranched alkyl, C₂-C₁₆ unbranched alkenyl, or ; wherein:

R^{4a} and R^{4b} are each independently C₁-C₁₆ unbranched alkyl or C₂-C₁₆ unbranched alkenyl;

R⁵ is absent, C₁-C₆ alkylene, or C₂-C₆ alkenylene;

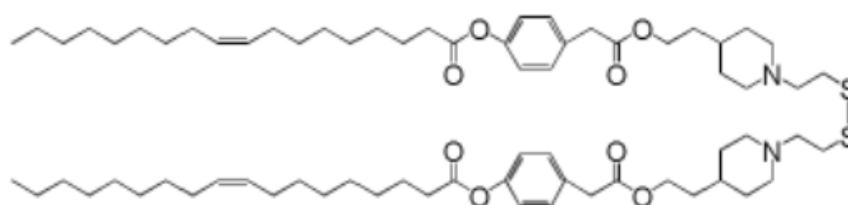
R^{6a} and R^{6b} are each independently C₇-C₁₄ alkyl or C₇-C₁₄ alkenyl;

10 X is -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, -S-S-, -C(R^a)=N-,
-N=C(R^a)-, -C(R^a)=NO-, -O-N=C(R^a)-, -C(=O)NR^a-, -NR^aC(=O)-, -NR^aC(=O)NR^a-,
-OC(=O)O-, -OSi(R^a)₂O-, -C(=O)(CR^a₂)C(=O)O-, or OC(=O)(CR^a₂)C(=O)-; wherein:

R^a, for each occurrence, is independently hydrogen or C₁-C₆ alkyl; and

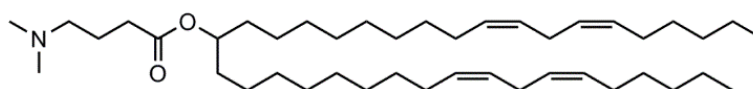
n is an integer selected from 1, 2, 3, 4, 5, and 6.

15 In some examples of any of the above aspects and examples, the cationic lipid is selected from any lipid in Table 2, Table 5, Table 6, Table 7, or Table 8. In some examples, the cationic lipid is a lipid having the structure:



or a pharmaceutically acceptable salt thereof. In some examples, the cationic lipid is MC3 (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-

25 DMA or MC3) having the following structure:



DLin-M-C3-DMA ("MC3")

or a pharmaceutically acceptable salt thereof.

In some examples of any of the above aspects and examples, the sterol or a derivative thereof is a cholesterol or a beta-sitosterol. In some examples, the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine (DSPE),
30 distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC),

dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG),
 dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE),
 palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE),
 dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal),
 5 dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE),
 distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-
 O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE,
 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), hydrogenated soy phosphatidylcholine
 (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM),
 10 dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG),
 distearoylphosphatidylglycerol (DSPG), dierythroylphosphatidylcholine (DEPC),
 palmitoyloleoylphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-
 dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-
 phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin,
 15 lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg
 sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate,
 lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof. In some examples,
 the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC),
 distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE). In some
 20 examples, the PEGylated lipid is selected from the group consisting of PEG-dilauryloxypropyl; PEG-
 dimyristyloxypropyl; PEG-dipalmitoyloxypropyl, PEG-distearoyloxypropyl; 1-(monomethoxy-
 polyethyleneglycol)-2,3-dimyristoylglycerol (DMG-PEG); PEG-dilaurylglycerol; PEG-
 dipalmitoylglycerol; PEG-disteryl glycerol; PEG-dilaurylglycamide; PEG-dimyristylglycamide; PEG-
 dipalmitoylglycamide; PEG-disteryl glycamide; (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-
 25 dioxaoctanyl] carbamoyl-[omega]-methyl-poly(ethylene glycol) (PEG-cholesterol); 3,4-
 ditetradecoxybenzyl-[omega]-methyl-poly(ethylene glycol) ether (PEG-DMB), and 1,2-dimyristoyl-
 sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol) (DSPE-PEG), and 1,2-
 distearoyl-sn-glycero-3-phosphoethanolamine-N-poly(ethylene glycol)-hydroxyl (DSPE-PEG-OH).
 In some examples, the PEGylated lipid is DMG-PEG, DSPE-PEG, DSPE-PEG-OH, or a combination
 30 thereof. In some examples of any of the above aspects and examples, the at least one PEGylated lipid
 is DMG-PEG2000, DSPE-PEG2000, DSPE-PEG2000-OH, DMG-PEG5000, DSPE-PEG5000,
 DSPE-PEG5000-OH, or a combination thereof. In some examples of any of the above aspects and
 examples, the scFv is chemically conjugated or covalently linked to a PEGylated lipid of the LNP to
 form a PEGylated lipid conjugate. In some examples of any of the above aspects and examples, the
 35 PEGylated lipid to which the scFv is chemically conjugated or covalently linked is DSPE-PEG. In
 some examples, the PEGylated lipid to which the scFv is chemically conjugated or covalently linked
 is DSPE-PEG2000. In some

examples, the PEGylated lipid to which the scFv is chemically conjugated or covalently linked is DSPE-PEG5000. In some examples of any of the above aspects and examples, the cationic lipid is present at a molar percentage of about 30% to about 80%. In some examples, the sterol is present at a molar percentage of about 20% to about 50%. In some examples of any of the above aspects and examples, the non-cationic lipid is present at a molar percentage of about 2% to about 20%. In some examples of any of the above aspects and examples, the at least one PEGylated lipid is present at a molar percentage of about 2.1% to about 10%.

In some examples of any of the above aspects and examples, the scFv are present at a total amount of about 0.02 µg/µg of TNA to about 0.1 µg/µg of TNA.

In some examples of any of the above aspects and examples, the pharmaceutical composition further comprises dexamethasone palmitate.

In some examples of any of the above aspects and examples, the LNP has a total lipid to TNA ratio of about 10:1 to about 40:1.

In some examples of any of the above aspects and examples, the LNP has a diameter ranging from about 40 nm to about 120 nm.

In some examples of any of the above aspects and examples, the nanoparticle has a diameter of less than about 100 nm.

In some examples of any of the above aspects and examples, the nanoparticle has a diameter of about 60 nm to about 80 nm.

In some examples of any of the above aspects and examples, the ceDNA comprises an expression cassette, and wherein the expression cassette comprises a promoter sequence and a transgene. In some examples, the expression cassette comprises a polyadenylation sequence. In some examples of any of the above aspects and examples, the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of the expression cassette. In some examples, the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR. In some examples, the expression cassette is connected to an ITR at 3' end (3' ITR). In some examples of any of the above aspects and examples, the expression cassette is connected to an ITR at 5' end (5' ITR).

In some examples of any of the above aspects and examples, the at least one ITR is an ITR derived from an AAV serotype, an ITR derived from an ITR of a goose virus, an ITR derived from a B19 virus ITR, or a wild-type ITR from a parvovirus. In some examples, said AAV serotype is selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12.

In some examples of any of the above aspects and examples, the at least one of the 5' ITR and the 3' ITR is a wild-type AAV ITR.

In some examples of any of the above aspects and examples, the at least one of the 5' ITR and the 3' ITR is a modified or mutant ITR.

In some examples of any of the above aspects and examples, the 5' ITR and the 3' ITR are symmetrical.

In some examples of any of the above aspects and examples, the 5' ITR and the 3' ITR are asymmetrical.

5 In some examples of any of the above aspects and examples, the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.

In some examples of any of the above aspects and examples, the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette. In some examples of any of the above aspects and examples, the spacer sequence is at least 5 base pairs long in length.

10 In some examples of any of the above aspects and examples, the ceDNA has a nick or a gap.

In some examples of any of the above aspects and examples, the ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a ministring DNA, a dumbbell shaped linear duplex closed-ended DNA comprising two hairpin structures of ITRs in the 5' and 3' ends of an expression cassette, or a doggybone™ DNA.

15 In some aspects, the disclosure provides a method of treating a cancer in a subject, comprising administering to the subject an effective amount of the pharmaceutical composition of any one of the aspects and examples herein. In some examples, the subject is a human. .

In some examples, the disclosure provides a method of delivering a therapeutic nucleic acid (TNA) or increasing the concentration of the TNA to a tumor in a subject, comprising administering
20 to the subject an effective amount of the pharmaceutical composition of any one of the aspects and examples herein.

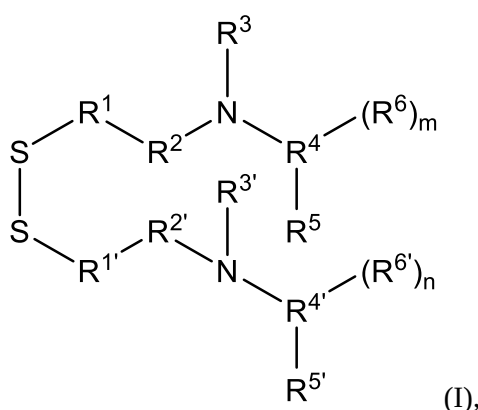
In some examples, the disclosure provides a method of delivering a therapeutic nucleic acid (TNA) or increasing the concentration of the TNA to the liver of a subject, comprising administering
25 to the subject an effective amount of the pharmaceutical composition of any one of the aspects and examples herein.

According to some examples, the LNP is internalized into the cell. According to some examples of the above aspects and examples, the LNP comprises a cationic lipid, a sterol or a derivative thereof, a non-cationic lipid, or a PEGylated lipid. According to some examples of the above aspects and examples, the TNA is encapsulated in the lipid. According to some examples of
30 the above aspects and examples, the TNA is selected from the group consisting of minigenes, plasmids, minicircles, small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotides (ASO), ribozymes, closed-ended (ceDNA), ministring, doggybone™, protelomere closed ended DNA, or dumbbell linear DNA, dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), mRNA, tRNA, rRNA, DNA viral
35 vectors, viral RNA vector, non-viral vector and any combination thereof. According to some examples, the TNA is ceDNA. According to some examples, the ceDNA is linear duplex

DNA. According to some examples, the TNA is mRNA. According to some examples, the TNA is siRNA. According to some examples, the TNA is a plasmid.

According to some examples, the LNP comprises a PEGylated lipid, wherein the PEGylated lipid is linked to the amino acid sequence encoding the scFv (the scFv polypeptide). According to some examples of the above aspects and examples, the pharmaceutical composition is administered to a subject. According to some examples of the above aspects and examples, the subject is a human patient in need of treatment with LNP encapsulated with TNA. According to some examples of the above aspects and examples, the composition is targeted to a cell or tissue expressing the target antigen via binding of the scFv in the LNP to the antigen target. According to some examples of the above aspects and examples, the composition is targeted to tumor cells. According to some examples, the tumor is a solid tumor. According to some examples, the tumor is a hematological tumor. According to some examples of the above aspects and examples, the composition is targeted to liver cells.

According to some examples of the above aspects and examples, the cationic lipid is represented by Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

R¹ and R^{1'} are each independently optionally substituted linear or branched C₁₋₃ alkylene;

R² and R^{2'} are each independently optionally substituted linear or branched C₁₋₆ alkylene;

R³ and R^{3'} are each independently optionally substituted linear or branched C₁₋₆ alkyl;

or alternatively, when R² is optionally substituted branched C₁₋₆ alkylene, R² and R³, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

or alternatively, when R^{2'} is optionally substituted branched C₁₋₆ alkylene, R^{2'} and R^{3'}, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

R⁴ and R^{4'} are each independently -CR^a, -C(R^a)₂CR^a, or -[C(R^a)₂]₂CR^a;

R^a, for each occurrence, is independently H or C₁₋₃ alkyl;

or alternatively, when R⁴ is -C(R^a)₂CR^a, or -[C(R^a)₂]₂CR^a and when R^a is C₁₋₃ alkyl, R³ and R⁴, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

or alternatively, when $R^{4'}$ is $-C(R^a)_2CR^a$, or $-[C(R^a)_2]_2CR^a$ and when R^a is C_{1-3} alkyl, $R^{3'}$ and $R^{4'}$, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

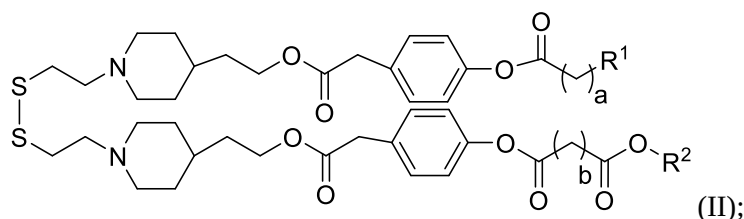
R^5 and $R^{5'}$ are each independently hydrogen, C_{1-20} alkylene or C_{2-20} alkenylene;

R^6 and $R^{6'}$, for each occurrence, are independently C_{1-20} alkylene, C_{3-20} cycloalkylene, or C_{2-20}

5 alkenylene; and

m and n are each independently an integer selected from 1, 2, 3, 4, and 5.

According to some examples of the above aspects and examples, the cationic lipid is represented by Formula (II):



10 or a pharmaceutically acceptable salt thereof, wherein:

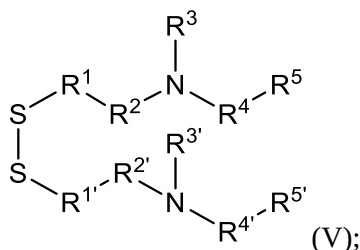
a is an integer ranging from 1 to 20;

b is an integer ranging from 2 to 10;

R^1 is absent or is selected from (C_2-C_{20}) alkenyl, $-C(O)O(C_2-C_{20})$ alkyl, and cyclopropyl substituted with (C_2-C_{20}) alkyl; and

15 R^2 is (C_2-C_{20}) alkyl.

According to some examples of the above aspects and examples, the lipid is represented by the Formula (V):



or a pharmaceutically acceptable salt thereof, wherein:

20 R^1 and $R^{1'}$ are each independently (C_1-C_6) alkylene optionally substituted with one or more groups selected from R^a ;

R^2 and $R^{2'}$ are each independently (C_1-C_2) alkylene;

R^3 and $R^{3'}$ are each independently (C_1-C_6) alkyl optionally substituted with one or more groups selected from R^b ;

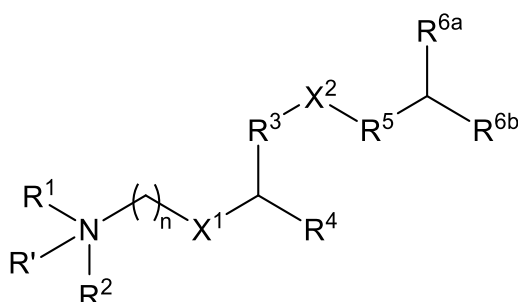
25 or alternatively, R^2 and R^3 and/or $R^{2'}$ and $R^{3'}$ are taken together with their intervening N atom to form a 4- to 7-membered heterocyclyl;

R^4 and $R^{4'}$ are each a (C_2-C_6) alkylene interrupted by $-C(O)O-$;

R^5 and $R^{5'}$ are each independently a (C_2-C_{30}) alkyl or (C_2-C_{30}) alkenyl, each of which are optionally interrupted with $-C(O)O-$ or (C_3-C_6) cycloalkyl; and

R^a and R^b are each halo or cyano.

According to some examples of the above aspects and examples, the cationic lipid is represented by Formula (XV):



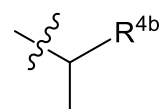
5 (XV)

or a pharmaceutically acceptable salt thereof, wherein:

R' is absent, hydrogen, or C₁-C₆ alkyl; provided that when R' is hydrogen or C₁-C₆ alkyl, the nitrogen atom to which R', R¹, and R² are all attached is protonated;

R¹ and R² are each independently hydrogen, C₁-C₆ alkyl, or C₂-C₆ alkenyl;

10 R³ is C₁-C₁₂ alkylene or C₂-C₁₂ alkenylene;



R⁴ is C₁-C₁₆ unbranched alkyl, C₂-C₁₆ unbranched alkenyl, or ; wherein:

R^{4a} and R^{4b} are each independently C₁-C₁₆ unbranched alkyl or C₂-C₁₆ unbranched alkenyl;

R⁵ is absent, C₁-C₈ alkylene, or C₂-C₈ alkenylene;

15 R^{6a} and R^{6b} are each independently C₇-C₁₆ alkyl or C₇-C₁₆ alkenyl; provided that the total number of carbon atoms in R^{6a} and R^{6b} as combined is greater than 15;

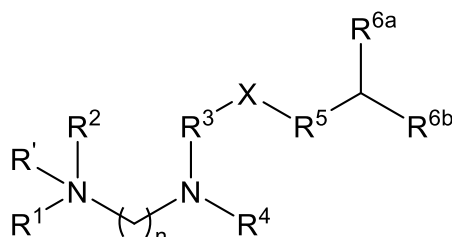
X¹ and X² are each independently -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, -S-S-, -C(R^a)=N-, -N=C(R^a)-, -C(R^a)=NO-, -O-N=C(R^a)-, -C(=O)NR^a-, -NR^aC(=O)-, -NR^aC(=O)NR^a-, -OC(=O)O-, -OSi(R^a)₂O-, -C(=O)(CR^a)C(=O)O-, or

20 OC(=O)(CR^a)C(=O)-; wherein:

R^a, for each occurrence, is independently hydrogen or C₁-C₆ alkyl; and

n is an integer selected from 1, 2, 3, 4, 5, and 6.

According to some examples of the above aspects and examples, the cationic lipid is represented by Formula (XX):



25

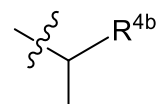
(XX)

or a pharmaceutically acceptable salt thereof, wherein:

R' is absent, hydrogen, or C₁-C₃ alkyl; provided that when R' is hydrogen or C₁-C₃ alkyl, the nitrogen atom to which R', R¹, and R² are all attached is protonated;

5 R¹ and R² are each independently hydrogen or C₁-C₃ alkyl;

R³ is C₃-C₁₀ alkylene or C₃-C₁₀ alkenylene;



R⁴ is C₁-C₁₆ unbranched alkyl, C₂-C₁₆ unbranched alkenyl, or ; wherein:

R^{4a} and R^{4b} are each independently C₁-C₁₆ unbranched alkyl or C₂-C₁₆ unbranched alkenyl;

10 R⁵ is absent, C₁-C₆ alkylene, or C₂-C₆ alkenylene;

R^{6a} and R^{6b} are each independently C₇-C₁₄ alkyl or C₇-C₁₄ alkenyl;

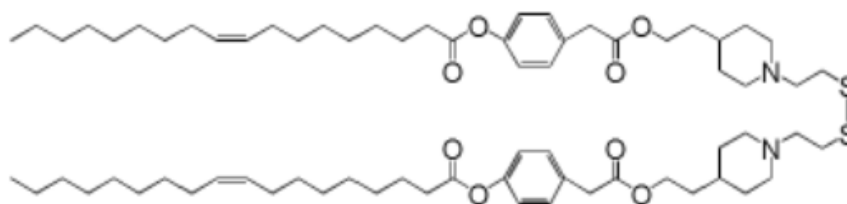
X is -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, -S-S-, -C(R^a)=N-,
-N=C(R^a)-, -C(R^a)=NO-, -O-N=C(R^a)-, -C(=O)NR^a-, -NR^aC(=O)-, -NR^aC(=O)NR^a-,
-OC(=O)O-, -OSi(R^a)₂O-, -C(=O)(CR^a₂)C(=O)O-, or OC(=O)(CR^a₂)C(=O)-; wherein:

15 R^a, for each occurrence, is independently hydrogen or C₁-C₆ alkyl; and

n is an integer selected from 1, 2, 3, 4, 5, and 6.

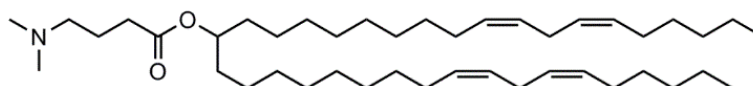
According to some examples of the above aspects and examples, the cationic lipid is selected from any lipid in Table 2, Table 5, Table 6, Table 7, or Table 8.

20 According to some examples of the above aspects and examples, the cationic lipid is a lipid having the structure:



25 or a pharmaceutically acceptable salt thereof.

According to some examples of the above aspects and examples, the cationic lipid is MC3 (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-DMA or MC3) having the following structure:



30 DLin-M-C3-DMA ("MC3")

or a pharmaceutically acceptable salt thereof.

According to some examples of the above aspects and examples, the sterol or a derivative thereof is a cholesterol or beta-sitosterol. According to some examples, the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine (DSPE), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC),

5 dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE),

10 distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG),

15 distearoylphosphatidylglycerol (DSPG), dierythroylphosphatidylcholine (DEPC), palmitoyloleoylphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg

20 sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof. According to some examples, the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE). According to some examples, the PEGylated lipid is selected from the group consisting of PEG-

25 dilauryloxypropyl; PEG-dimyristyloxypropyl; PEG-dipalmitoyloxypropyl, PEG-distearoyloxypropyl; 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (DMG-PEG); PEG-dilaurylglycerol; PEG-dipalmitoylglycerol; PEG-disteryl glycerol; PEG-dilaurylglycamide; PEG-dimyristylglycamide; PEG-dipalmitoylglycamide; PEG-disteryl glycamide; (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl] carbamoyl-[omega]-methyl-poly(ethylene glycol) (PEG-cholesterol); 3,4-

30 ditetradecoxybenzyl-[omega]-methyl-poly(ethylene glycol) ether (PEG-DMB), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol) (DSPE-PEG), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-poly(ethylene glycol)-hydroxyl (DSPE-PEG-OH). According to some examples, the PEGylated lipid is DMG-PEG, DSPE-PEG, DSPE-PEG-OH, or a combination thereof. According to some examples, the at least one PEGylated lipid is DMG-

35 PEG2000, DSPE-PEG2000, DSPE-PEG2000-OH, or a combination thereof.

According to some examples of the above aspects and examples, the scFv is chemically conjugated or covalently linked to a PEGylated lipid of the LNP to form a PEGylated lipid conjugate. According to some examples, the PEGylated lipid to which the scFv is chemically conjugated or covalently linked is DSPE-PEG. According to some examples of the above aspects and examples, the scFv is covalently linked to the LNP via a non-cleavable linker. According to some examples, the non-cleavable linker is a maleimide-containing linker.

According to some examples of the above aspects and examples, the scFv is covalently linked to the LNP via a cleavable linker.

According to some examples of the above aspects and examples, the scFv is covalently linked to the LNP via a pyridyldisulfide (PDS)-containing linker.

According to some examples of the above aspects and examples, the cationic lipid is present at a molar percentage of about 30% to about 80%. According to some examples, the sterol is present at a molar percentage of about 20% to about 50%. According to some examples of the above aspects and examples, the non-cationic lipid is present at a molar percentage of about 2% to about 20%.

According to some examples of the above aspects and examples, the at least one PEGylated lipid is present at a molar percentage of about 2.1% to about 10%. According to some examples of the above aspects and examples, scFv polypeptide is present at a total amount of about 0.02 µg/µg of TNA to about 0.1 µg/µg of TNA.

According to some examples of the above aspects and examples, the pharmaceutical composition further comprises dexamethasone palmitate. According to some examples of the above aspects and examples, the LNP has a total lipid to TNA ratio of about 10:1 to about 40:1. According to some examples of the above aspects and examples, the LNP has a diameter ranging from about 40 nm to about 120 nm. According to some examples of the above aspects and examples, the nanoparticle has a diameter of less than about 100 nm. According to some examples of the above aspects and examples, the nanoparticle has a diameter of about 60 nm to about 80 nm. According to some examples of the above aspects and examples, the ceDNA comprises an expression cassette, and wherein the expression cassette comprises a promoter sequence and a transgene. According to some examples, the expression cassette comprises a polyadenylation sequence.

According to some examples of the above aspects and examples, the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of the expression cassette. According to some examples, the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR. According to some examples, the expression cassette is connected to an ITR at 3' end (3' ITR). According to some examples of the above aspects and examples, the expression cassette is connected to an ITR at 5' end (5' ITR). According to some examples of the above aspects and examples, the at least one ITR is an ITR derived from an AAV serotype, derived from an ITR of goose virus, derived from a B19 virus

ITR, a wild-type ITR from a parvovirus. According to some examples of the above aspects and examples, the AAV serotype is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12. According to some examples of the above aspects and examples, the at least one of the 5' ITR and the 3' ITR is a wild-type AAV ITR. According to some examples of the above aspects and examples, the at least one of the 5' ITR and the 3' ITR is a modified or mutant ITR. According to some examples of the above aspects and examples, the 5' ITR and the 3' ITR are symmetrical. According to some examples of the above aspects and examples, the 5' ITR and the 3' ITR are asymmetrical. According to some examples of the above aspects and examples, the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette. According to some examples of the above aspects and examples, the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette. According to some examples of the above aspects and examples, the spacer sequence is at least 5 base pairs long in length. According to some examples of the above aspects and examples, the ceDNA has a nick or a gap. According to some examples of the above aspects and examples, the ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a ministring DNA, a dumbbell shaped linear duplex closed-ended DNA comprising two hairpin structures of ITRs in the 5' and 3' ends of an expression cassette, or a doggybone™ DNA.

According to another example, the disclosure features a method of treating *e.g.*

According to another example, the disclosure provides a method of delivering a therapeutic nucleic acid (TNA) or increasing the concentration of the TNA to a tumor of a subject, comprising administering to the subject an effective amount of the pharmaceutical composition of any one of the aspects and examples herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present disclosure, briefly summarized above and discussed in greater detail below, can be understood by reference to the illustrative embodiments of the disclosure depicted in the appended drawings. However, the appended drawings illustrate only typical embodiments of the disclosure and are therefore not to be considered limiting of scope, for the disclosure may admit to other equally effective embodiments.

FIGS. 1A-1F show that trastuzumab-derived α -HER2 scFv exhibited clear HER2-specific membrane targeting and internalization *in vitro*. Alexa-fluor 488- (AF488) labeled anti-HER2 scFv was used to show HER2 receptor engagement in SkBR3 (**FIG. 1A**) and SkOV3 (**FIG. 1B**) Her2-expressing (HER2+) cell lines, but not in MCF7 cells (**FIG. 1C**), which do not express Her2 receptor (HER2-). A second immunofluorescent label (pHrhodo) was used to demonstrate ligand internalization. As shown in **FIGS. 1D-1F**, SkBR3 and SkOV3 cells that express the HER2 receptor showed ligand internalization (**FIG. 1D** and **FIG. 1E**), while the MCF7 HER2- cell line did not (**FIG. 1F**).

CLAIMS

What is Claimed is:

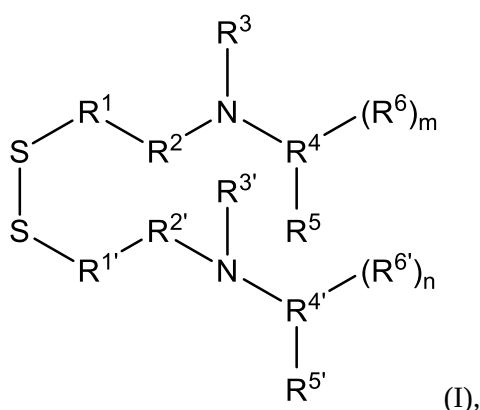
1. A pharmaceutical composition comprising a lipid nanoparticle (LNP), wherein the LNP
5 comprises a cationic lipid, a sterol, a non-cationic lipid, and a PEG5000 PEGylated lipid, a therapeutic nucleic acid (TNA) comprising a non-viral vector, and at least one pharmaceutically acceptable excipient, wherein the LNP comprises a single-chain variable fragment (scFv) linked to the LNP, and wherein the scFv is directed against an antigen present on the surface of a cell.
- 10 2. The pharmaceutical composition of claim 1, wherein the scFv is covalently linked to the LNP or the scFv is chemically conjugated to the LNP to PEG5000.
3. The pharmaceutical composition of claim 2, wherein the scFv is chemically conjugated to the LNP via a non-cleavable linker; or the scFv is chemically conjugated to the LNP via a cleavable
15 linker.
4. The pharmaceutical composition of claim 3, wherein the non-cleavable linker is a maleimide-containing linker.
- 20 5. The pharmaceutical composition of claim 1, wherein the scFv is linked to the LNP via transglutaminase-mediated conjugation.
6. The pharmaceutical composition of any one of claims 1-5, wherein the antigen is a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA), optionally wherein the antigen is human
25 epidermal growth factor receptor 2 (HER2).
7. The pharmaceutical composition of any one of claims 1-6, wherein the scFv is bivalent.
8. The pharmaceutical composition of any one of claims 1-7, wherein the LNP is capable of
30 being internalized into the cell.
9. The pharmaceutical composition of any one of claims 1 to 8, wherein the TNA is encapsulated in the LNP.
- 35 10. The pharmaceutical composition of any one of claims 1-9, wherein the pharmaceutical composition is to be administered to a subject.

11. The pharmaceutical composition of claim 10, wherein the subject is a human patient in need of treatment with LNP encapsulated with TNA.

12. The pharmaceutical composition of any one of claims 1-11, wherein the composition is
5 targeted to a cell expressing the cell-surface antigen for which the scFv is directed.

13. The pharmaceutical composition of any one of claims 1-12, wherein:
the composition is targeted to tumor cells;
the composition is targeted to liver cells; or
10 the composition is targeted to hepatocytes in the liver.

14. The pharmaceutical composition of claim 1, wherein:
the cationic lipid is represented by Formula (I):



15 or a pharmaceutically acceptable salt thereof, wherein:

R¹ and R^{1'} are each independently optionally substituted linear or branched C₁₋₃ alkylene;

R² and R^{2'} are each independently optionally substituted linear or branched C₁₋₆ alkylene;

R³ and R^{3'} are each independently optionally substituted linear or branched C₁₋₆ alkyl;

or alternatively, when R² is optionally substituted branched C₁₋₆ alkylene, R² and R³, taken
20 together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

or alternatively, when R^{2'} is optionally substituted branched C₁₋₆ alkylene, R^{2'} and R^{3'}, taken
together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

R⁴ and R^{4'} are each independently -CR^a, -C(R^a)₂CR^a, or -[C(R^a)₂]₂CR^a;

R^a, for each occurrence, is independently H or C₁₋₃ alkyl;

or alternatively, when R⁴ is -C(R^a)₂CR^a, or -[C(R^a)₂]₂CR^a and when R^a is C₁₋₃ alkyl, R³ and
25 R⁴, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

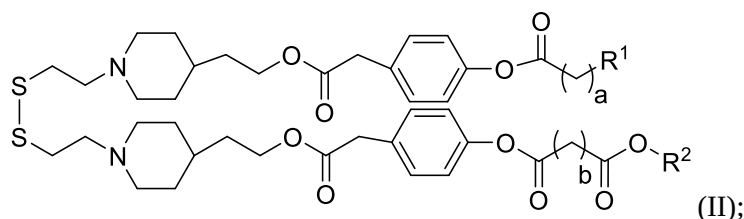
or alternatively, when R^{4'} is -C(R^a)₂CR^a, or -[C(R^a)₂]₂CR^a and when R^a is C₁₋₃ alkyl, R^{3'} and
R^{4'}, taken together with their intervening N atom, form a 4- to 8-membered heterocyclyl;

R⁵ and R^{5'} are each independently hydrogen, C₁₋₂₀ alkylene or C₂₋₂₀ alkenylene;

R^6 and $R^{6'}$, for each occurrence, are independently C_{1-20} alkylene, C_{3-20} cycloalkylene, or C_{2-20} alkenylene; and

m and n are each independently an integer selected from 1, 2, 3, 4, and 5.

- 5 15. The pharmaceutical composition of claim 1, wherein the cationic lipid is represented by Formula (II):



or a pharmaceutically acceptable salt thereof, wherein:

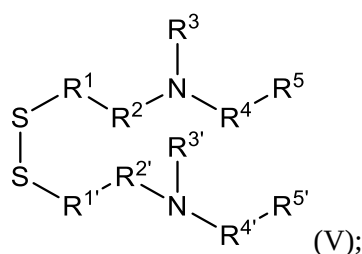
a is an integer ranging from 1 to 20;

- 10 b is an integer ranging from 2 to 10;

R^1 is absent or is selected from (C_2-C_{20}) alkenyl, $-C(O)O(C_2-C_{20})$ alkyl, and cyclopropyl substituted with (C_2-C_{20}) alkyl; and

R^2 is (C_2-C_{20}) alkyl.

- 15 16. The pharmaceutical composition of claim 1, wherein the lipid is represented by the Formula (V):



or a pharmaceutically acceptable salt thereof, wherein:

- 20 R^1 and $R^{1'}$ are each independently (C_1-C_6) alkylene optionally substituted with one or more groups selected from R^a ;

R^2 and $R^{2'}$ are each independently (C_1-C_2) alkylene;

R^3 and $R^{3'}$ are each independently (C_1-C_6) alkyl optionally substituted with one or more groups selected from R^b ;

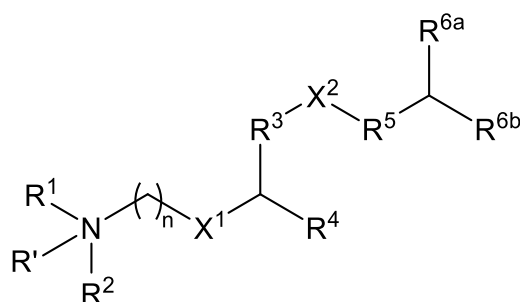
- 25 or alternatively, R^2 and R^3 and/or $R^{2'}$ and $R^{3'}$ are taken together with their intervening N atom to form a 4- to 7-membered heterocyclyl;

R^4 and $R^{4'}$ are each a (C_2-C_6) alkylene interrupted by $-C(O)O-$;

R^5 and $R^{5'}$ are each independently a (C_2-C_{30}) alkyl or (C_2-C_{30}) alkenyl, each of which are optionally interrupted with $-C(O)O-$ or (C_3-C_6) cycloalkyl; and

R^a and R^b are each halo or cyano.

17. The pharmaceutical composition of claim 1, wherein the cationic lipid is represented by Formula (XV):



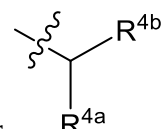
(XV)

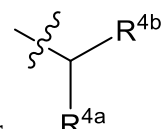
or a pharmaceutically acceptable salt thereof, wherein:

R' is absent, hydrogen, or C₁-C₆ alkyl; provided that when R' is hydrogen or C₁-C₆ alkyl, the nitrogen atom to which R', R¹, and R² are all attached is protonated;

R¹ and R² are each independently hydrogen, C₁-C₆ alkyl, or C₂-C₆ alkenyl;

R³ is C₁-C₁₂ alkenylene or C₂-C₁₂ alkenylene;



R⁴ is C₁-C₁₆ unbranched alkyl, C₂-C₁₆ unbranched alkenyl, or ; wherein:

R^{4a} and R^{4b} are each independently C₁-C₁₆ unbranched alkyl or C₂-C₁₆ unbranched alkenyl;

R⁵ is absent, C₁-C₈ alkenylene, or C₂-C₈ alkenylene;

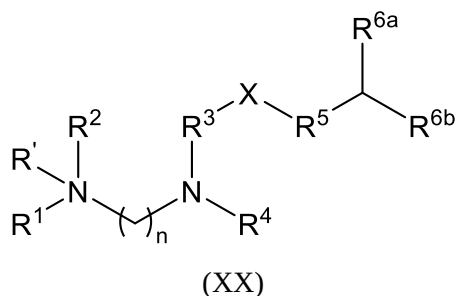
R^{6a} and R^{6b} are each independently C₇-C₁₆ alkyl or C₇-C₁₆ alkenyl; provided that the total number of carbon atoms in R^{6a} and R^{6b} as combined is greater than 15;

X¹ and X² are each independently -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, -S-S-, -C(R^a)=N-, -N=C(R^a)-, -C(R^a)=NO-, -O-N=C(R^a)-, -C(=O)NR^a-, -NR^aC(=O)-, -NR^aC(=O)NR^a-, -OC(=O)O-, -OSi(R^a)₂O-, -C(=O)(CR^a)₂C(=O)O-, or OC(=O)(CR^a)₂C(=O)-; wherein:

R^a, for each occurrence, is independently hydrogen or C₁-C₆ alkyl; and

n is an integer selected from 1, 2, 3, 4, 5, and 6.

18. The pharmaceutical composition of claim 1, wherein the cationic lipid is represented by Formula (XX):

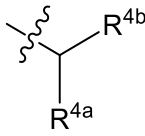


or a pharmaceutically acceptable salt thereof, wherein:

R' is absent, hydrogen, or C₁-C₃ alkyl; provided that when R' is hydrogen or C₁-C₃ alkyl, the nitrogen atom to which R', R¹, and R² are all attached is protonated;

R¹ and R² are each independently hydrogen or C₁-C₃ alkyl;

R³ is C₃-C₁₀ alkylene or C₃-C₁₀ alkenylene;

R⁴ is C₁-C₁₆ unbranched alkyl, C₂-C₁₆ unbranched alkenyl, or ; wherein:

R^{4a} and R^{4b} are each independently C₁-C₁₆ unbranched alkyl or C₂-C₁₆ unbranched alkenyl;

R⁵ is absent, C₁-C₆ alkylene, or C₂-C₆ alkenylene;

R^{6a} and R^{6b} are each independently C₇-C₁₄ alkyl or C₇-C₁₄ alkenyl;

X is -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, -S-S-, -C(R^a)=N-, -N=C(R^a)-, -C(R^a)=NO-, -O-N=C(R^a)-, -C(=O)NR^a-, -NR^aC(=O)-, -NR^aC(=O)NR^a-, -OC(=O)O-, -OSi(R^a)₂O-, -C(=O)(CR^a)C(=O)O-, or OC(=O)(CR^a)C(=O)-; wherein:

R^a, for each occurrence, is independently hydrogen or C₁-C₆ alkyl; and

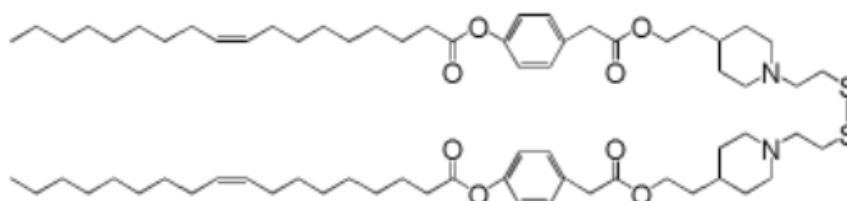
n is an integer selected from 1, 2, 3, 4, 5, and 6.

19. The pharmaceutical composition of claim 1, wherein

the cationic lipid is selected from a lipid comprising any lipid set forth in Table 2, Table 5, Table 6, Table 7, and Table 8.

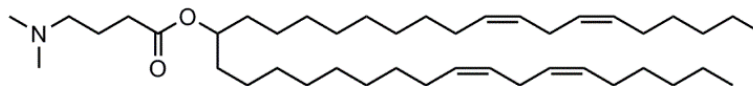
20. The pharmaceutical composition of claim 1, wherein

the cationic lipid is a lipid having the structure:



or a pharmaceutically acceptable salt thereof.

21. The pharmaceutical composition of claim 1, wherein the cationic lipid is MC3 (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-DMA or MC3) having the following structure:



DLin-M-C3-DMA ("MC3")

or a pharmaceutically acceptable salt thereof.

22. The pharmaceutical composition of any one of claims 14 to 21, wherein the sterol or a derivative thereof is a cholesterol or a beta-sitosterol.

23. The pharmaceutical composition of claim 1, wherein the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine (DSPE), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierythroylphosphatidylcholine (DEPC), palmitoyl-oleoylphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof.

24. The pharmaceutical composition of claim 23, wherein the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

25. The pharmaceutical composition of claim 1, wherein the PEG5000 PEGylated lipid is selected from the group consisting of PEG-dilauryloxypropyl; PEG-dimyristyloxypropyl; PEG-dipalmitoyloxypropyl, PEG-distearoyloxypropyl; 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (DMG-PEG); PEG-dilaurylglycerol; PEG-dipalmitoylglycerol; PEG-disteryl-glycerol; PEG-dilaurylglycamide; PEG-dimyristylglycamide; PEG-dipalmitoylglycamide; PEG-disteryl-glycamide; (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl] carbamoyl-[omega]-methyl-poly(ethylene glycol) (PEG-cholesterol); 3,4-ditetradecoxybenzyl-[omega]-methyl-poly(ethylene glycol) ether (PEG-DMB), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol) (DSPE-PEG), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-poly(ethylene glycol)-hydroxyl (DSPE-PEG-OH).

26. The pharmaceutical composition of claim 25, wherein the PEGylated lipid is DMG-PEG5000, DSPE-PEG5000, DSPE-PEG5000-OH, or a combination thereof.

27. The pharmaceutical composition of any one of claims 1 or 25-26, wherein the scFv is chemically conjugated or covalently linked to a PEGylated lipid of the LNP to form a PEGylated lipid conjugate.

28. The pharmaceutical composition of claim 27, wherein the PEGylated lipid to which the scFv is chemically conjugated or covalently linked is DSPE-PEG5000.

29. The pharmaceutical composition of any one of claims 14-21, wherein the cationic lipid is present at a molar percentage of about 30% to about 80%.

30. The pharmaceutical composition of claim 22, wherein the sterol is present at a molar percentage of about 20% to about 50%.

31. The pharmaceutical composition of any one of claims 23 to 24, wherein the non-cationic lipid is present at a molar percentage of about 2% to about 20%.

32. The pharmaceutical composition of any one of claims 25 to 28, wherein the at least one PEGylated lipid is present at a molar percentage of about 2.1% to about 10%.

33. The pharmaceutical composition of any one of claims 1 to 32, wherein the scFv are present at a total amount of about 0.02 µg/µg of TNA to about 0.1 µg/µg of TNA.
34. The pharmaceutical composition of any one of claims 1 to 33, further comprising
5 dexamethasone palmitate.
35. The pharmaceutical composition of any one of claims 1 to 34, wherein:
the LNP has a total lipid to TNA ratio of about 10:1 to about 40:1;
the LNP has a diameter ranging from about 40 nm to about 120 nm;
10 the LNP has a diameter of about 60 nm to about 80 nm; and/or
the LNP has a diameter of less than about 100 nm.
36. The pharmaceutical composition of any one of claims 1 to 35, wherein the non-viral vector
comprises an expression cassette, and wherein the expression cassette comprises a promoter sequence
15 and a transgene.
37. The pharmaceutical composition of claim 36, wherein the expression cassette comprises a
polyadenylation sequence.
- 20 38. Use of a pharmaceutical composition of any one of claims 1 to 37 in the manufacture of a
medicament for treating cancer in a subject.
39. The use according to claim 38, wherein the subject is a human.
- 25 40. Use of a pharmaceutical composition of any one of claims 1-37 in the manufacture of a
medicament for delivering a therapeutic nucleic acid (TNA) or increasing the concentration of the
TNA to a tumor in a subject.
- 30 41. Use of a pharmaceutical composition of any one of claims 1-37 in the manufacture of a
medicament for delivering a therapeutic nucleic acid (TNA) or increasing the concentration of the
TNA to the liver of a subject.
42. A pharmaceutical composition of any one of claims 1-37 for use in therapy.