

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 (cdDNA)) 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, wherein the scFv is chemically conjugated to the LNP to PEG5000 via a non-cleavable linker, wherein the non-cleavable linker is a maleimide linker.

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.

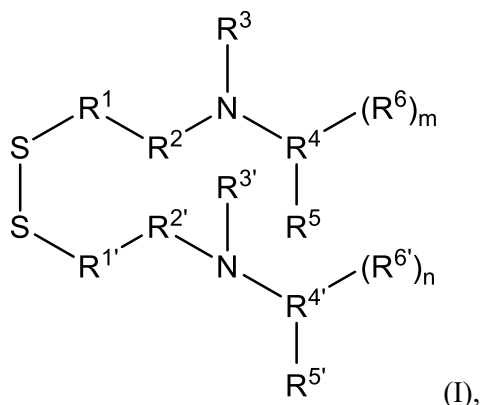
The present disclosure provides a pharmaceutical composition comprising a lipid nanoparticle (LNP), a therapeutic nucleic acid (TNA), and at least one pharmaceutically acceptable excipient, wherein the LNP comprises a single chain fragment variable (scFv) linked to the LNP, and wherein the scFv is directed 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

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, wherein the scFv is chemically conjugated to the LNP to PEG5000 via a non-cleavable linker, wherein the non-cleavable
10 linker is a maleimide linker.
2. The pharmaceutical composition of claim 1, wherein the antigen is a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA), optionally wherein the TAA or TSA is human epidermal growth factor receptor 2 (HER2).
15
3. The pharmaceutical composition of claim 1 or 2, wherein the scFv is bivalent.
4. The pharmaceutical composition of any one of claims 1-3, wherein the LNP is capable of being internalized into the cell.
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5. The pharmaceutical composition of any one of claims 1 to 4, wherein the TNA is encapsulated in the LNP.
6. The pharmaceutical composition of any one of claims 1-5, wherein the pharmaceutical
25 composition is to be administered to a subject.
7. The pharmaceutical composition of claim 6, wherein the subject is a human patient in need of treatment with the TNA encapsulated in the LNP.
- 30 8. The pharmaceutical composition of any one of claims 1-7, wherein the composition is to be targeted to a cell expressing the cell-surface antigen for which the scFv is directed.
9. The pharmaceutical composition of any one of claims 1-8, wherein:
the composition is to be targeted to tumor cells;
35 the composition is to be targeted to liver cells; or
the composition is to be targeted to hepatocytes in the liver.

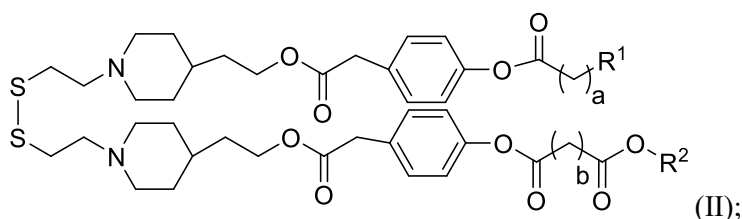
10. The pharmaceutical composition of claim 1, wherein:
the cationic lipid is represented by Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

- 5 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;
10 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;
 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
15 R^4 , 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}
20 alkenylene; and
m and n are each independently an integer selected from 1, 2, 3, 4, and 5.

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



or a pharmaceutically acceptable salt thereof, wherein:

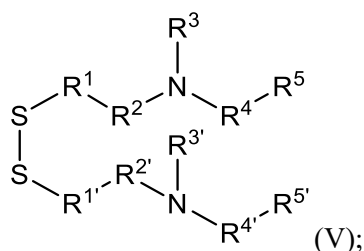
a is an integer ranging from 1 to 20;

b is an integer ranging from 2 to 10;

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

5 R² is (C₂-C₂₀)alkyl.

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



10 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;

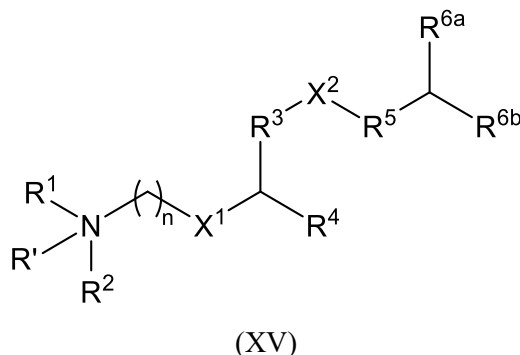
or alternatively, R² and R³ and/or R^{2'} and R^{3'} are taken together with their intervening N atom to form a 4- to 7-membered heterocyclyl;

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.

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



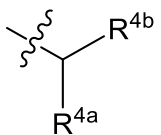
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or a pharmaceutically acceptable salt thereof, wherein:

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;

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;

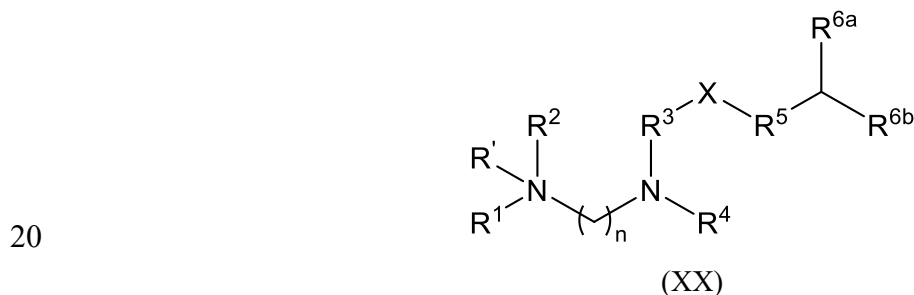
R⁵ is absent, C₁-C₈ alkylene, or C₂-C₈ alkenylene;

10 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^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.

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

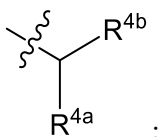


or a pharmaceutically acceptable salt thereof, wherein:

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;

25 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 C_1 - C_{16} unbranched alkyl or C_2 - C_{16} unbranched alkenyl;

R^5 is absent, C_1 - C_6 alkylene, or C_2 - C_6 alkenylene;

R^{6a} and R^{6b} are each independently C_7 - C_{14} alkyl or C_7 - C_{14} alkenyl;

- 5 X is $-\text{OC}(=\text{O})-$, $-\text{SC}(=\text{O})-$, $-\text{OC}(=\text{S})-$, $-\text{C}(=\text{O})\text{O}-$, $-\text{C}(=\text{O})\text{S}-$, $-\text{S}-\text{S}-$, $-\text{C}(\text{R}^a)=\text{N}-$, $-\text{N}=\text{C}(\text{R}^a)-$, $-\text{C}(\text{R}^a)=\text{NO}-$, $-\text{O}-\text{N}=\text{C}(\text{R}^a)-$, $-\text{C}(=\text{O})\text{NR}^a-$, $-\text{NR}^a\text{C}(=\text{O})-$, $-\text{NR}^a\text{C}(=\text{O})\text{NR}^a-$, $-\text{OC}(=\text{O})\text{O}-$, $-\text{OSi}(\text{R}^a)_2\text{O}-$, $-\text{C}(=\text{O})(\text{CR}^a_2)\text{C}(=\text{O})\text{O}-$, or $\text{OC}(=\text{O})(\text{CR}^a_2)\text{C}(=\text{O})-$; wherein:

R^a , for each occurrence, is independently hydrogen or C_1 - C_6 alkyl; and

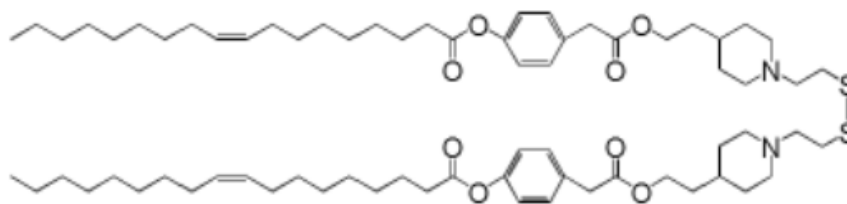
n is an integer selected from 1, 2, 3, 4, 5, and 6.

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15. The pharmaceutical composition of claim 1, wherein the cationic lipid is selected from any lipid set forth in Table 2, Table 5, Table 6, Table 7, and Table 8.

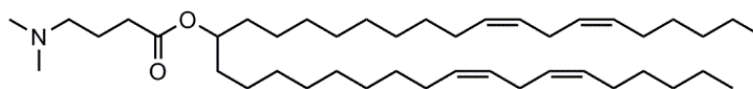
- 15 16. The pharmaceutical composition of claim 1, wherein the cationic lipid is a lipid having the structure:

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or a pharmaceutically acceptable salt thereof.

17. 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.

- 30 18. The pharmaceutical composition of any one of claims 1 to 17, wherein the sterol is a cholesterol or a beta-sitosterol.

19. 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),
5 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), distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-
10 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), dierycoylphosphatidylcholine (DEPC),
15 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 sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebroside, dicetylphosphate,
20 lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof.

20. The pharmaceutical composition of claim 19, wherein the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

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21. The pharmaceutical composition of claim 1, wherein the PEG5000 PEGylated lipid is selected from the group consisting of PEG-dilauryloxypropyl; PEG-dimyristyloxypropyl; PEG-dipalmitoxypropyl, PEG-distearoxypropyl; 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (DMG-PEG); PEG-dilaurylglycerol; PEG-dipalmitoylglycerol; PEG-disteryl-
30 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-
35 3-phosphoethanolamine-N-poly(ethylene glycol)-hydroxyl (DSPE-PEG-OH).

22. The pharmaceutical composition of claim 21, wherein the PEG5000 PEGylated lipid is DMG-PEG5000, DSPE-PEG5000, DSPE-PEG5000-OH, or a combination thereof.
- 5 23. The pharmaceutical composition of any one of claims 1 or 21-22, wherein the scFv is chemically conjugated to a PEGylated lipid of the LNP to form a PEGylated lipid conjugate.
24. The pharmaceutical composition of claim 23, wherein the PEGylated lipid to which the scFv is chemically conjugated is DSPE-PEG5000.
- 10 25. The pharmaceutical composition of any one of claims 10-17, wherein the cationic lipid is present at a molar percentage of 30% to 80%.
26. The pharmaceutical composition of claim 18, wherein the sterol is present at a molar percentage of 20% to 50%.
- 15 27. The pharmaceutical composition of any one of claims 19 to 20, wherein the non-cationic lipid is present at a molar percentage of 2% to 20%.
28. The pharmaceutical composition of any one of claims 21 to 24, wherein the at least one PEGylated lipid is present at a molar percentage of 2.1% to 10%.
- 20 29. The pharmaceutical composition of any one of claims 1 to 28, wherein the scFv are present at a total amount of 0.02 $\mu\text{g}/\mu\text{g}$ of TNA to 0.1 $\mu\text{g}/\mu\text{g}$ of TNA.
- 25 30. The pharmaceutical composition of any one of claims 1 to 29, further comprising dexamethasone palmitate.
31. The pharmaceutical composition of any one of claims 1 to 30, wherein:
the LNP has a total lipid to TNA ratio of 10:1 to 40:1;
30 the LNP has a diameter ranging from 40 nm to 120 nm;
the LNP has a diameter of 60 nm to 80 nm; and/or
the LNP has a diameter of less than 100 nm.
32. The pharmaceutical composition of any one of claims 1 to 31, wherein the non-viral vector
35 comprises an expression cassette, and wherein the expression cassette comprises a promoter sequence and a transgene.

33. The pharmaceutical composition of claim 32, wherein the expression cassette comprises a polyadenylation sequence.

5 34. Use of a pharmaceutical composition of any one of claims 1 to 33 in the manufacture of a medicament for treating cancer in a subject.

35. The use according to claim 34, wherein the subject is a human.

10 36. Use of a pharmaceutical composition of any one of claims 1-33 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.

15 37. Use of a pharmaceutical composition of any one of claims 1-33 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.

38. A pharmaceutical composition of any one of claims 1-33 for use in therapy.