CLAIMS

What is claimed is:

1. A lipid nanoparticle (LNP) for targeted delivery of one or more nucleic acids into a hematopoietic stem cell (HSC), wherein the LNP comprises:

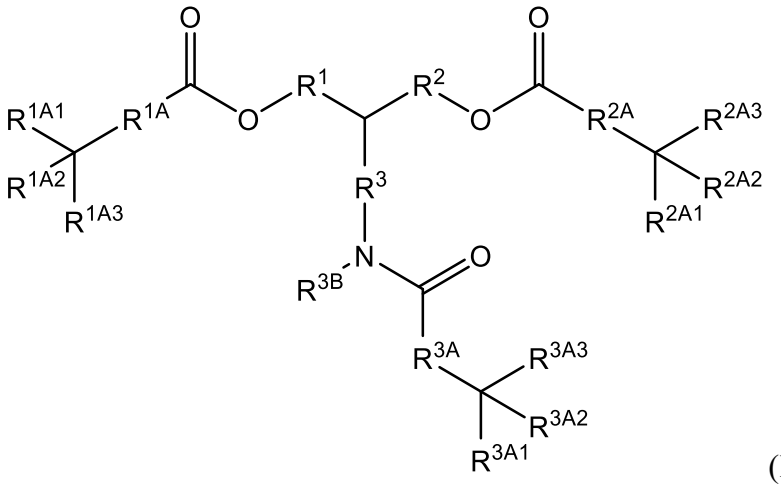
   (a) a lipid-antibody conjugate comprising the compound of Formula (I):

   [Lipid] – [optional linker] – [antibody] (I),

   wherein the antibody binds to CD105 and/or CD117;

   (b) an ionizable cationic lipid comprising:

   i. a compound of Formula (II'):



(II'),

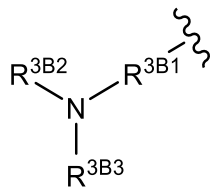
or a salt thereof, wherein:

R^1, R^2, and R^3 are each independently a bond or C_{1-3} alkylene;

R^{1A}, R^{2A}, and R^{3A} are each independently a bond or C_{1-10} alkylene;

R^{1A1}, R^{1A2}, R^{1A3}, R^{2A1}, R^{2A2}, R^{2A3}, R^{3A1}, R^{3A2}, and R^{3A3} are each independently H, C_{1-20} alkyl, C_{1-20} alkenyl, $-(CH_2)_{0-10}C(O)OR^{a1}$, or $-(CH_2)_{0-10}OC(O)R^{a2}$;

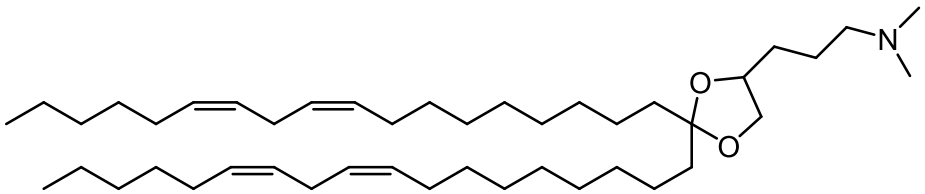
R^{a1} and R^{a2} are each independently C_{1-20} alkyl or C_{1-20} alkenyl;

R^{3B} is  ;

R^{3B1} is C_{1-6} alkylene; and

R^{3B2} and R^{3B3} are each independently H or C_{1-6} alkyl optionally substituted with one or more substituents each independently selected from the group consisting of -OH and -O-(C_{1-6} alkyl); or

ii. a compound having the following structure:



or a salt thereof; and

(c) one or more nucleic acids disposed in the LNP.

2. The LNP of claim 1, wherein the LNP comprises two lipid-antibody conjugates comprising the compound of Formula (I), wherein the first lipid-antibody conjugate and the second lipid-antibody conjugate are the same or different.

3. The LNP of claim 1 or claim 2, wherein the antibody comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), wherein:

(a) the antibody binds to CD117, and wherein

(i) the VH comprises a CDR-H1 comprising the amino acid sequence of FTFSNYAMS (SEQ ID NO: 1), a CDR-H2 comprising the amino acid sequence of AISGSGGSTYYADSVKG (SEQ ID NO: 2), and a CDR-H3 comprising the amino acid sequence of AKGPPTYHTNYYYMDV (SEQ ID NO: 3), and

(ii) the VL comprises CDR-L1 comprising the amino acid sequence of RASQGISSWLA (SEQ ID NO: 4), a CDR-L2 comprising the amino acid sequence of AASSLQS (SEQ ID NO: 5), and a CDR-L3 comprising the amino acid sequence of QQTNSFPYT (SEQ ID NO: 6); or

(b) the antibody binds to CD117, and wherein

(i) the VH comprises a CDR-H1 comprising the amino acid sequence of FTFSDADMD (SEQ ID NO: 10), a CDR-H2 comprising the amino acid sequence of RTRNKAGSYTTEYAASVKG (SEQ ID NO: 11), and a CDR-

H3 comprising the amino acid sequence of AREPKYWIDFDL (SEQ ID NO: 12), and

(ii) the VL comprises CDR-L1 comprising the amino acid sequence of RASQSISSYLN (SEQ ID NO: 13), a CDR-L2 comprising the amino acid sequence of AASSLQS (SEQ ID NO: 14), and a CDR-L3 comprising the amino acid sequence of QQSYIAPYT (SEQ ID NO: 15); or

(c) the antibody binds to CD105, and wherein

(i) the VH comprises a CDR-H1 comprising the amino acid sequence of DAWMD (SEQ ID NO: 19), a CDR-H2 comprising the amino acid sequence of EIRSKASNHATYYAESVKG (SEQ ID NO: 20), and a CDR-H3 comprising the amino acid sequence of WRRFFDS (SEQ ID NO: 21), and

(ii) the VL comprises CDR-L1 comprising the amino acid sequence of RASSSVSYMH (SEQ ID NO: 22), a CDR-L2 comprising the amino acid sequence of ATSNLAS (SEQ ID NO: 23), and a CDR-L3 comprising the amino acid sequence of QQWSSNPLT (SEQ ID NO: 24).

4. The LNP of any one of claims 1-3, wherein the antibody binds to CD117 and comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), wherein

(a) the VH comprises a CDR-H1 comprising the amino acid sequence of FTFSDADMD (SEQ ID NO: 10), a CDR-H2 comprising the amino acid sequence of RTRNKAGSYTTEYAASVKG (SEQ ID NO: 11), and a CDR-H3 comprising the amino acid sequence of AREPKYWIDFDL (SEQ ID NO: 12), and

(b) the VL comprises CDR-L1 comprising the amino acid sequence of RASQSISSYLN (SEQ ID NO: 13), a CDR-L2 comprising the amino acid sequence of AASSLQS (SEQ ID NO: 14), and a CDR-L3 comprising the amino acid sequence of QQSYIAPYT (SEQ ID NO: 15).

5. The LNP of any one of claims 1-4, wherein the antibody comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), wherein

(a) the antibody binds to CD117, and wherein the VH comprises the amino acid sequence of SEQ ID NO: 7 and the VL comprises the amino acid sequence of SEQ ID NO: 8;

(b) the antibody binds to CD117, and wherein the VH comprises the amino acid sequence of SEQ ID NO: 16 and the VL comprises the amino acid sequence of SEQ ID NO: 17; or

(c) the antibody binds to CD105, and wherein the VH comprises the amino acid sequence of SEQ ID NO: 25 and the VL comprises the amino acid sequence of SEQ ID NO: 26.

6. The LNP of any one of claims 1-5, wherein the antibody comprises a Fab, F(ab')2, Fab'-SH, Fv, scFv fragment, or immunoglobulin single variable domain.

7. The LNP of claim 6, wherein the antibody is a Fab and wherein the Fab comprises a heavy chain domain and a light chain domain, and wherein

(a) the heavy chain and light chain domains of the Fab comprise the amino acid sequences set forth in SEQ ID NOs: 9 and 38, or have at least 85, 90, 95, 96, 97, 98, 99, or 99.5% sequence identity with the amino acid sequences set forth in SEQ ID NOs: 9 and 38;

(b) the heavy chain and light chain domains of the Fab comprise the amino acid sequences set forth in SEQ ID NOs: 18 and 39, or have at least 85, 90, 95, 96, 97, 98, 99, or 99.5% sequence identity with the amino acid sequences set forth in SEQ ID NOs: 18 and 39; or

(c) the heavy chain and light chain domains of the Fab comprise the amino acid sequences set forth in SEQ ID NOs: 27 and 40, or have at least 85, 90, 95, 96, 97, 98, 99, or 99.5% sequence identity with the amino acid sequences set forth in SEQ ID NOs: 27 and 40.

8. The LNP of claim 6, wherein the antibody is a Fab and wherein the Fab comprises a heavy chain domain and a light chain domain, and wherein the heavy chain and light chain domains of the Fab comprise the amino acid sequences set forth in SEQ ID NOs: 9 and 38, or have at least 85, 90, 95, 96, 97, 98, 99, or 99.5% sequence identity with the amino acid sequences set forth in SEQ ID NOs: 9 and 38.

9. The LNP of claim 6, wherein the antibody is a Fab and wherein the Fab comprises a heavy chain domain and a light chain domain, and wherein the heavy chain and light chain domains of the Fab comprise the amino acid sequences set forth in SEQ ID NOs: 18 and 39, or have at least 85, 90, 95, 96, 97, 98, 99, or 99.5% sequence identity with the amino acid sequences set forth in SEQ ID NOs: 18 and 39.

10. The LNP of claim 6, wherein the antibody is a Fab and wherein the Fab comprises a heavy chain domain and a light chain domain, and wherein the heavy chain and light chain domains of the Fab comprise the amino acid sequences set forth in SEQ ID NOs: 27 and 40, or have at least 85, 90, 95, 96, 97, 98, 99, or 99.5% sequence identity with the amino acid sequences set forth in SEQ ID NOs: 27 and 40.

11. The LNP of any one of claims 1-10, wherein the one or more nucleic acids is DNA or RNA.

12. The LNP of claim 11, wherein the RNA is an mRNA.

13. The LNP of any one of claims 1-12, wherein the one or more nucleic acids comprise an mRNA encoding a site-directed nuclease, a chemical base editor, a prime editor, or an epigenome editor.

14. The LNP of any one of claims 1-13, wherein the one or more nucleic acids comprise an mRNA encoding a site-directed nuclease, wherein the site-directed nuclease is a

    (i) CRISPR-associated (Cas) nuclease, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), or a megaTAL; or

    (ii) a Cas nuclease, ZFN, TALEN, or megaTAL comprising an amino acid sequence that confers binding to a target nucleotide sequence.

15. The LNP of any one of claims 1-14, wherein the one or more nucleic acids comprise

    (a) an mRNA encoding a CRISPR-associated (Cas) nuclease or a chemical base editor; and

    (b) a guide RNA (gRNA) comprising a nucleotide sequence that confers binding to a target nucleotide sequence.

16. The LNP of any one of claims 1-14, wherein the one or more nucleic acids comprise

    (a) an mRNA encoding a prime editor; and

(b) a prime editing guide RNA (pegRNA) comprising a nucleotide sequence that confers binding to a target nucleotide sequence.

17. The LNP of claim 15 or claim 16, wherein the Cas nuclease is a Type II or a Type V Cas enzyme, or a variant thereof or the Cas nuclease is a Cas9 enzyme, a Cas12 enzyme, a CasX enzyme, a Cas14 enzyme, or a variant thereof.

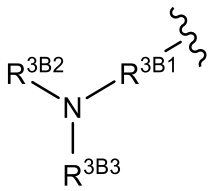
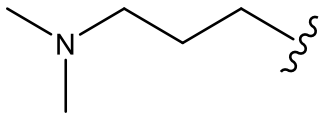
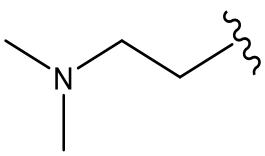
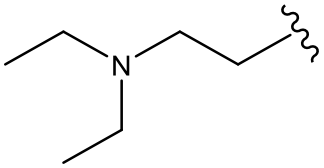
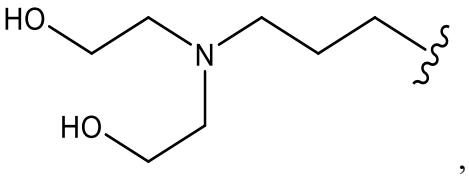
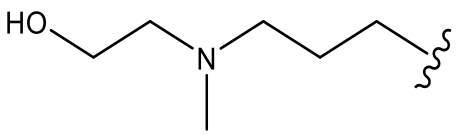
18. The LNP of any one of claims 15-17, wherein the gRNA or pegRNA comprises a sequence having at least 80% identity to at least 15 consecutive nucleotides of the target nucleotide sequence, optionally wherein the target nucleotide sequence is within the *BCL11A* erythroid enhancer.

19. The LNP of any one of claims 1-18, wherein the one or more nucleic acids further comprise a donor template nucleic acid comprising a sequence having at least 80% identity to at least 15 consecutive nucleotides of the target nucleotide sequence, optionally wherein the target nucleotide sequence is within the *BCL11A* erythroid enhancer.

20. The LNP of any one of claims 1-19, wherein the ionizable cationic lipid comprises a compound of Formula (II').

21. The LNP of claim 20, wherein R^{3B2} and R^{3B3} are each independently methyl or ethyl, each optionally substituted with one or more -OH.

22. The LNP of claim 21, wherein R^{3B2} and R^{3B3} are each unsubstituted methyl.

23. The LNP of claim 21, wherein  is , , , ,  or .

24. The LNP of any one of claims 20-23, wherein R^1 and R^2 are each methylene and R^3 is a bond.

25. The LNP of any one of claims 20-24, wherein R^{1A}, R^{2A}, and R^{3A} are each independently a bond or -$(CH_2)_{1-10}$-.

26. The LNP of any one of claims 20-25, wherein R^{1A} and R^{2A} are each independently a bond, -$(CH_2)_2$-, -$(CH_2)_4$-, -$(CH_2)_6$-, -$(CH_2)_7$-, or -$(CH_2)_8$-; and R^{3A} is a bond, -CH_2-, -$(CH_2)_2$-, or -$(CH_2)_7$-.

27. The LNP of any one of claims 20-26, wherein:

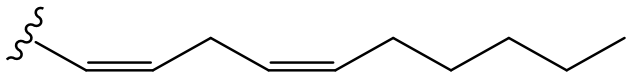
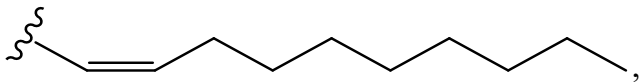
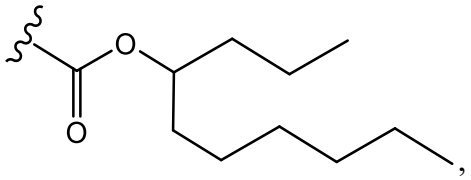
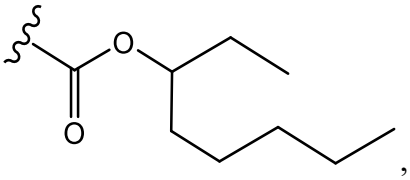
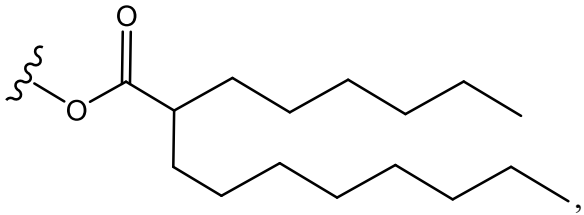
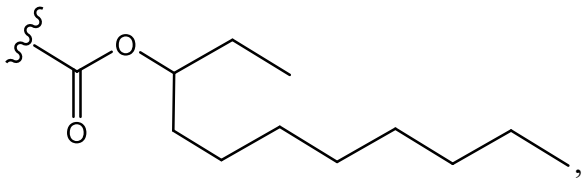
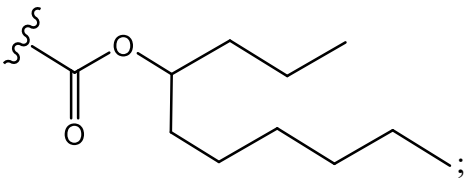
(i) R^{1A1} and R^{2A1} are each independently -CH=CH-(C_{1-15} alkyl), -CH=CH-CH_2-CH=CH-(C_{1-10} alkyl), -$(CH_2)_{0-4}$C(O)OCH(C_{1-10} alkyl)(C_{1-15} alkyl), or -$(CH_2)_{0-4}$OC(O)CH(C_{1-10} alkyl)(C_{1-15} alkyl); and R^{1A2}, R^{1A3}, R^{2A2}, and R^{2A3} are each H;

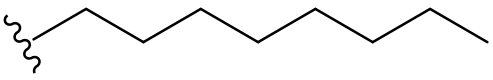
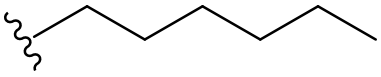
(ii) R^{1A1} and R^{2A1} are each C_{1-15} alkyl; and R^{1A2} and R^{2A2} are each C_{1-15} alkyl; and R^{1A3} and R^{2A3} are each H;

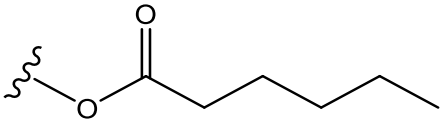
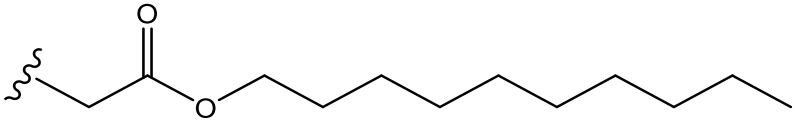
(iii) R^{1A1} and R^{2A1} are each -$(CH_2)_{0-4}$OC(O)CH_2(C_{1-15} alkyl); R^{1A2} and R^{2A2} are each -$(CH_2)_{0-4}$C(O)OCH_2(C_{1-15} alkyl); and R^{1A3} and R^{2A3} are each H; or

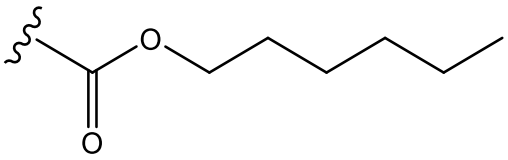
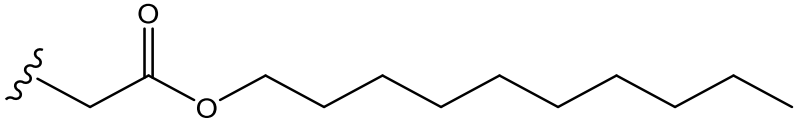
(iv) R^{1A1} and R^{2A1} are each -C(O)OCH_2(C_{1-15} alkyl); R^{1A2} and R^{2A2} are each -$(CH_2)_{0-4}$C(O)OCH_2(C_{1-15} alkyl); and R^{1A3} and R^{2A3} are each H.

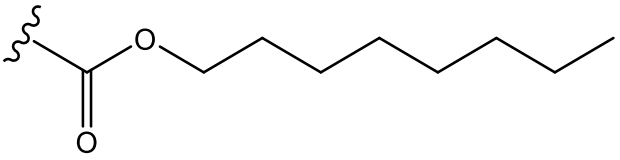
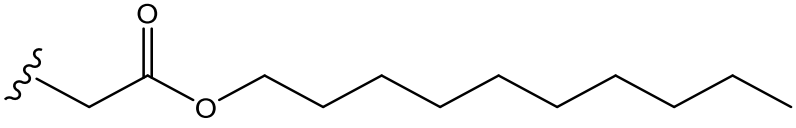
28. The LNP of any one of claims 20-26, wherein

(i) R^{1A1} and R^{2A1} are each , , , , , , or ;

(ii) R^{1A1} and R^{2A1} are each ; R^{1A2} and R^{2A2} are each ; and R^{1A3} and R^{2A3} are each H;

(iii) R^{1A1} and R^{2A1} are each ; R^{2A1} and R^{2A2} are each ; and R^{1A3} and R^{2A3} are each H;

(iv) R^{1A1} and R^{2A1} are each ; R^{1A2} and R^{2A2} are each ; and R^{1A3} and R^{2A3} are each H; or

(v) R^{1A1} and R^{2A1} are each ; R^{2A1} and R^{2A2} are each ; and R^{1A3} and R^{2A3} are each H.

29. The LNP of any one of claims 20-26, wherein:

(i) R^{3A1} and R^{3A2} are each independently C_{1-15} alkyl; and R^{3A3} is H;

(ii) R^{3A1} is C_{1-15} alkyl; and R^{3A2} and R^{3A3} are each H;

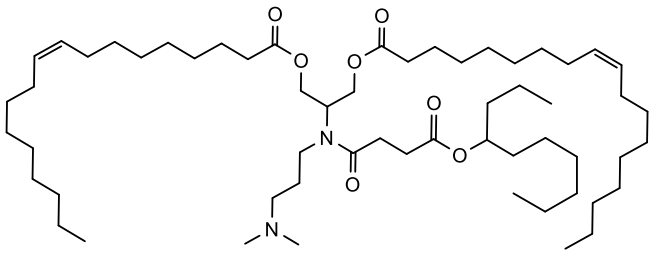
(iii) R^{3A1} is -C(O)OCH(C_{1-5} alkyl)(C_{1-10} alkyl); and R^{3A2} and R^{3A3} are each H;

(iv) R^{3A1} is -$(CH_2)_{0-4}$OC(O)CH_2(C_{1-10} alkyl); R^{3A2} is -$(CH_2)_{0-4}$(O)OCH_2(C_{1-10} alkyl); and R^{3A3} is H;

(v) R^{3A1} is $-(CH_2)_{0-4}C(O)OCH_2(C_{1-10}$ alkyl); R^{3A2} is $-(CH_2)_{0-4}C(O)OCH_2(C_{1-10}$ alkyl); and R^{3A3} is H; or

(vi) R^{3A1}, R^{3A2}, and R^{3A3} are H.

30. The LNP of any one of claims 20-29, wherein the ionizable cationic lipid is



Lipid 15

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31. The LNP of any one of claims 1-30, wherein the antibody is covalently coupled to a lipid in the LNP via a polyethylene glycol (PEG) containing linker.

32. The LNP of any one of claims 1-31, wherein the lipid antibody conjugate comprises [DSPE]-[PEG(2000)-maleimide]-[anti-hCD117 Fab], wherein the [DSPE]-[PEG(2000)-maleimide] is conjugated to the anti-hCD117 Fab via covalent coupling between the maleimide group and a C-terminal cysteine in the heavy chain (HC) of the anti-hCD117 Fab.

33. The LNP of any one of claims 1-32, wherein the LNP further comprises one or more of a structural lipid (e.g., a sterol), a neutral phospholipid, and a free PEG-lipid.

34. The LNP of claim 33, wherein (i) the sterol is present in the lipid blend in a range of 20-70 (e.g., 30-50) mole percent; (ii) the sterol is cholesterol; (iii) the neutral phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and sphingomyelin; (iv) the neutral phospholipid is present in the lipid blend in a range of 5-15 mole percent; (v) the free PEG-lipid is selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols, for example a PEG-lipid may be PEG-dioleoylglycerol (PEG-DOG), PEG-dimyristoyl-glycerol (PEG-DMG), PEG-dipalmitoylglycerol (PEG-DPG), PEG-dilinoleoyl-glycero-phosphatidyl ethanolamine

(PEG-DLPE), PEG-dimyrstoyl-phosphatidylethanolamine (PEG-DMPE), PEG-dipalmitoyl-phosphatidylethanolamine (PEG-DPPE), PEG-distearoylglycerol (PEG-DSG), PEG-diacylglycerol (PEG-DAG, e.g., PEG-DMG, PEG-DPG, and PEG-DSG), PEG-ceramide, PEG-distearoyl-glycero-phosphoglycerol (PEG-DSPG), PEG-dioleoyl-glycero-phosphoethanolamine (PEG-DOPE), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) lipid; (vi) the free PEG-lipid comprises a diacylphosphatidylethanolamine comprising Dipalmitoyl (C16) chain or Distearoyl (C18) chain, and optionally the free PEG-lipid comprises PEG-DPG and PEG-DMG; (vii) the free PEG-lipid is present in the lipid blend in a range of 1-4 mole percent; (viii) the free PEG-lipid comprises the same or a different lipid as the lipid in the lipid-immune cell targeting group conjugate; or any combination of (i) to (viii).

35. The LNP of claim 33 or 34, wherein the (i) LNP has a mean diameter in the range of 50-200 nm; (ii) the LNP has a polydispersity index in a range from 0.05 to 1; or (iii) the LNP has a zeta potential of from about +10 mV to about + 30 mV at pH 5; or any combination of (i) to (iii).

36. The LNP of any of claims 33-35, wherein the LNP comprises:

    (a) the ionizable cationic lipid;

    (b) the lipid-antibody conjugate comprising the compound of the following formula:

    [Lipid] - [optional linker] - [antibody],

    wherein the antibody binds to CD105 and/or CD117;

    (c) a sterol or other structural lipid;

    (d) a neutral phospholipid;

    (e) a free Polyethylene glycol (PEG) lipid; and

    (f) the one or more nucleic acids.

37. A method of targeting the delivery of a nucleic acid to a hematopoietic stem cell (HSC), optionally *ex vivo* in a subject, the method comprising to be administered to the subject, the LNP of any one of claims 1-36, wherein the LNP comprises the nucleic acid.

38. The method of claim 37, wherein the method further comprises to be administered to the subject, an HSC mobilization agent, and wherein the LNP is to be administered to the subject intravenously, optionally wherein the HSC mobilization agent is to be administered to the subject before, during, or before and during administration of the LNP.

39. A method of genetically modifying a hematopoietic stem cell (HSC), optionally *ex* vivo in a subject, the method comprising to be administered to the subject, the LNP of any one of claims 1-36, wherein the one or more nucleic acids disposed in the LNP comprise an mRNA encoding a site-directed nuclease, a chemical base editor, a prime editor, or an epigenome editor.

40. The LNP of any one of claims 1-36 for use in treating a disease in a subject, wherein the one or more nucleic acids disposed in the LNP comprise a sequence, optionally an mRNA, encoding a site-directed nuclease, a chemical base editor, a prime editor, or an epigenome editor.

41. The method of claim 39 or the LNP for use of claim 40, wherein the subject is to be further administered with an HSC mobilization agent, and wherein the LNP is to be administered to the subject intravenously; optionally wherein the HSC mobilization agent is to be administered to the subject before, during, or before and during administration of the LNP.

42. The method of claim 39 or 41 or the LNP for use of claim 40 or 41, wherein

(i) the disease is a blood disease,

(ii) the disease is a hemoglobinopathy, a primary immune deficiency (PID), a congenital cytopenia, a hemophilia, a thrombophilia, an inborn error of metabolism, a neuropathy, or a viral disease, or

(iii) the disease is an α-hemoglobinopathy or a β-hemoglobinopathy, optionally wherein the β-hemoglobinopathy is β-thalassemia or sickle cell disease.

43. The method of any one of claims 39, 41-42 or the LNP for use of any one of claims 40-42, wherein administration of the LNPs results in one or more of:

(a) insertion of an *HBB* transgene, or a fragment thereof, into at least one HSC of the subject;

(b) increased expression of β-globin in the subject;

(c) an increased amount of $\alpha_2\beta_2$ adult hemoglobin (HbA) in the subject;

(d) insertion of an *HBG1* transgene, or a fragment thereof, into at least one HSC of the subject;

(e) insertion of an *HBG2* transgene, or a fragment thereof, into at least one HSC of the subject;

(f) increased expression of γ-globin in the subject;

(g) an increased amount of $\alpha_2\gamma_2$ fetal hemoglobin (HbF) in the subject;

(h) disruption of the *HBA1* gene, the *HBA2* gene, or a combination thereof in at least one HSC of the subject;

(i) decreased expression of α-globin in the subject; and

(j) a decreased amount of α_4 α-globin heterotetramers in the subject.

44. The method of any one of claims 37-39 or 41-43, or the LNP for use of any one of claims 40-43, wherein the one or more nucleic acids disposed in the LNP comprise an mRNA encoding a site-directed nuclease, wherein

(i) the site-directed nuclease is a CRISPR-associated (Cas) nuclease, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), or a megaTAL; or

(ii) the site-directed nuclease is a Cas nuclease, ZFN, TALEN, or megaTAL comprising an amino acid sequence that confers binding to a target nucleotide sequence.

45. The method of any one of claims 37-39 or 41-44, or the LNP for use of any one of claims 40-44, wherein the one or more nucleic acids disposed in the LNP comprise:

(a) an mRNA encoding a CRISPR-associated (Cas) nuclease or a chemical base editor; and

(b) a guide RNA (gRNA) comprising a nucleotide sequence that confers binding to a target nucleotide sequence.

46. The method of any one of claims 37-39 or 41-45, or the LNP for use of any one of claims 40-45, wherein the one or more nucleic acids disposed in the LNP comprise:

(a) an mRNA encoding a prime editor; and

(b) a prime editing guide RNA (pegRNA) comprising a nucleotide sequence that confers binding to a target nucleotide sequence.

47. The method or the LNP for use of any one of claims 44-46, wherein the Cas nuclease is a

(i) Type II or a Type V Cas enzyme, or a variant thereof; or

(ii) a Cas9 enzyme, a Cas12 enzyme, a CasX enzyme, or a Cas 14 enzyme, or a variant thereof.

48. The method of any one of claims 37-39 or 41-47, or the LNP for use of any one of claims 40-47, wherein the gRNA or pegRNA comprises a sequence having at least 80% identity to at least 15 consecutive nucleotides of the target nucleotide sequence, optionally wherein the target nucleotide sequence is within the BCL11A erythroid enhancer.

49. The method of any one of claims 37-39 or 41-48, or the LNP for use of any one of claims 40-48, wherein the one or more nucleic acids disposed in the LNP further comprise a donor template nucleic acid comprising a sequence having at least 80% identity to at least 15 consecutive nucleotides of the target nucleotide sequence, optionally wherein the target nucleotide sequence is within the *BCL11A* erythroid enhancer.

50. The method of any one of claims 37-39 or 41-49, or the LNP for use of any one of claims 40-49, wherein the subject is a human.