therapeutics. Also CpG ODNs often exhibit uneven tissue distribution *in vivo*, with primary sites of accumulation being in liver, kidney, and spleen. Such distribution can elicit off-target activity and local toxicity associated with PAMPs. Accordingly, there is a need for an effective method to stabilize and deliver a CpG ODN for therapeutic applications.

SUMMARY

[0011] Provided herein is a conjugate for modulating a natural killer cell or myeloid cell, comprising a targeting moiety and an immunomodulating polynucleotide.

[0011a] In one aspect, the present disclosure refers to a conjugate comprising a targeting moiety, an immunomodulating polynucleotide, and a linker; wherein the targeting moiety binds to an antigen expressed by a NK cell or a myeloid cell; wherein the linker connects the targeting moiety covalently to the immunomodulating polynucleotide; wherein the immunomodulating polynucleotide is a cytosine-guanine dinucleotide (CpG)-containing oligodeoxynucleotide (ODN) that activates Toll-like receptor 9 (TLR9); and wherein the antigen is selected from the group consisting of CD11b, CD11c, CD16/32, CD49b, CD56 (NCAM), CD57, CD69, CD94, CD122, CD158 (Kir), CD161 (NK-1.1), CD244 (2B4), CD314 (NKG2D), CD319 (CRACC), CD328 (Siglec-7), CD335 (NKp46), Ly49, Ly108, Va24-Ja18 TCR (iNKT), granulysin, granzyme, perforin, SIRPα, SIRPβ, SIRPγ, LAIR1, SIGLEC-3 (CD33), SIGLEC-7, SIGLEC-9, LIR1 (ILT2, LILRB1), NKR-P1A (KLRB1), CD94–NKG2A, KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, CD160 (BY55), CD16 (FcgRIIIA), NKp46 (NCR1), NKp30 (NCR3), NKp44 (NCR2), DNAM1(CD226), CRTAM, CD2, CD7, CD11a, CD18, CD25, CD27, CD28, NTB-A (SLAMF6), PSGL1, CD96 (Tactile), CD100 (SEMA4D), NKp80 (KLRF1, CLEC5C), SLAMF7 (CRACC, CS1, CD319), and CD244 (2B4, SLAMF4).

[0012] Also provided herein is a pharmaceutical composition for modulating a natural killer cell or myeloid cell, comprising a conjugate that comprises a targeting moiety and an immunomodulating polynucleotide; and a pharmaceutically acceptable carrier.

[0012a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising the conjugate as disclosed herein, and a pharmaceutically acceptable excipient

[0013] Additionally provided herein is a method of modulating a natural killer cell or myeloid cell, comprising contacting the cell with a conjugate comprising a targeting moiety and an immunomodulating polynucleotide.

[0013a] In one aspect, the present disclosure refers to the conjugate as disclosed herein for use in modulating a natural killer cell in a subject having cancer.

[0013b] In one aspect, the present disclosure refers to the conjugate as disclosed herein for use in modulating a myeloid cell in a subject having cancer.

[0014] Further provided herein is a method of treating a proliferative disease in a subject, comprising administering to the subject a conjugate comprising a targeting moiety and an immunomodulating polynucleotide.

[0014a] In one aspect, the present disclosure refers to the conjugate as disclosed herein for use in treating, preventing, or ameliorating one or more symptoms of a proliferative disease in a subject.

[0014b] In one aspect, the present disclosure refers to use of the conjugate as disclosed herein in the manufacture of a medicament for treating, preventing, or ameliorating one or more symptoms of a proliferative disease in a subject.

[0015] Provided herein is a conjugate of Formula (C):

$$Ab\left[L^N\text{-}(Q)_e\right]_f \qquad (C)$$

or a stereoisomer, a mixture of two or more diastereomers, a tautomer, or a mixture of two or more tautomers thereof; or a pharmaceutically acceptable salt, solvate, or hydrate thereof; wherein:

Ab is an anti-CD56 or anti-SIRP antibody;
each L^N is independently a linker;
each Q is independently an immunomodulating polynucleotide;
each e is independently an integer of about 1, about 2, about 3, or about 4; and
f is an integer of about 1, about 2, about 3, or about 4.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 shows the activation of NK cells as measured by an increase in CD69 expression upon 24-hour treatment of peripheral blood mononuclear cells (PBMC) with an anti-CD56-CpG nucleotide (SEQ. ID NO: 425) conjugate (anti-CD56-CPG) in comparison with controls: the CpG nucleotide (p425) alone, the anti-CD56 antibody (anti-CD56) alone, and media (the horizontal dashed line).

[0017] FIG. 2 shows the activation of NK cells as measured by an increase in CD69 expression upon 48-hour treatment of PBMC with an anti-CD56-CpG nucleotide (SEQ. ID NO: 425) conjugate (anti-CD56-CPG) in comparison with controls: the CpG nucleotide (p425) alone, the anti-CD56 antibody (anti-CD56) alone, and media (the horizontal dashed line).

[0018] FIG. 3 shows an increase in $CD14^+$ cells upon treatment of PBMC with anti-SIRPα-CpG nucleotide (SEQ. ID NO: 425) conjugates (anti-Sirpα 1-CpG and anti-Sirpα 2-CpG) with a blocking anti-SIRPα antibody (anti-Sirpα 1) or a non-blocking anti-SIRPα antibody (anti-Sirpα 2) in comparison with controls: the CpG nucleotide (p425) alone, the anti-SIRPα antibodies (anti-Sirpα 1

Claims

What is claimed is:

1. A conjugate comprising a targeting moiety, an immunomodulating polynucleotide, and a linker; wherein the targeting moiety binds to an antigen expressed by a NK cell or a myeloid cell; wherein the linker connects the targeting moiety covalently to the immunomodulating polynucleotide; wherein the immunomodulating polynucleotide is a cytosine-guanine dinucleotide (CpG)-containing oligodeoxynucleotide (ODN) that activates Toll-like receptor 9 (TLR9); and wherein the antigen is selected from the group consisting of CD11b, CD11c, CD16/32, CD49b, CD56 (NCAM), CD57, CD69, CD94, CD122, CD158 (Kir), CD161 (NK-1.1), CD244 (2B4), CD314 (NKG2D), CD319 (CRACC), CD328 (Siglec-7), CD335 (NKp46), Ly49, Ly108, Va24-Ja18 TCR (iNKT), granulysin, granzyme, perforin, SIRPα, SIRPβ, SIRPγ, LAIR1, SIGLEC-3 (CD33), SIGLEC-7, SIGLEC-9, LIR1 (ILT2, LILRB1), NKR-P1A (KLRB1), CD94–NKG2A, KLRG1, KIR2DL5A, KIR2DL5B, KIR2DL1, KIR2DL2, CD160 (BY55), CD16 (FcgRIIIA), NKp46 (NCR1), NKp30 (NCR3), NKp44 (NCR2), DNAM1(CD226), CRTAM, CD2, CD7, CD11a, CD18, CD25, CD27, CD28, NTB-A (SLAMF6), PSGL1, CD96 (Tactile), CD100 (SEMA4D), NKp80 (KLRF1, CLEC5C), SLAMF7 (CRACC, CS1, CD319), and CD244 (2B4, SLAMF4).

2. The conjugate of claim 1, wherein the immunomodulating polynucleotide comprises an internucleoside phosphotriester.

3. The conjugate of claim 1 or 2, wherein the immunomodulating polynucleotide comprises a nucleotide with a modified nucleobase.

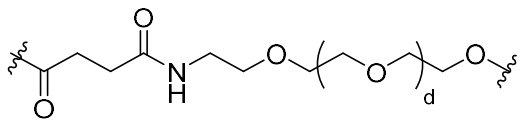
4. The conjugate of any one of claims 1 to 3, wherein the conjugate has the structure of Formula (C):

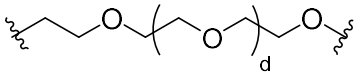
$$Ab\left[L^N\text{-}(Q)_e\right]_f \qquad (C)$$

or a stereoisomer, a mixture of two or more diastereomers, a tautomer, or a mixture of two or more tautomers thereof; or a pharmaceutically acceptable salt, solvate, or hydrate thereof;
wherein:

Ab is a targeting moiety;
each L^N is independently a linker;
each Q is independently an immunomodulating polynucleotide;
each e is independently an integer of 1, 2, 3, or 4; and
f is an integer of 1, 2, 3, or 4.

5. The conjugate of claim 4, wherein L^N is a linker comprising a polyethylene glycol.

6. The conjugate of claim 5, wherein L^N is , wherein d is an integer ranging from 0 to 50, an integer ranging from 0 to 10, an integer ranging from 0 to 5, or d is an integer of 0, 1, or 3.

7. The conjugate of claim 5, wherein L^N is , wherein d is an integer ranging from 0 to 50, an integer ranging from 0 to 10, an integer ranging from 0 to 5, or d is an integer of 0, 1, or 3.

8. The conjugate of any one of claims 4 to 7, wherein e is an integer of 1.

9. The conjugate of any one of claims 4 to 8, wherein each Q independently has the structure of Formula (D):

$$X^{5'}-(X^N)_b-Y^P-(X^N)_c-X^{3'} \qquad (D)$$

wherein:

each X^N is independently a nucleotide;

$X^{3'}$ is a 3' terminal nucleotide;

$X^{5'}$ is a 5' terminal nucleotide;

Y^P is an internucleoside phosphotriester; and

b and c are each an integer ranging from about 0 to 25; with the proviso that their sum is no less than 5;

wherein optionally b is an integer ranging from 1 to 15 or an integer of 3, 4, 11, or 14; and/or c is an integer ranging from 0 to 10 or an integer of 0 or 8.

10. The conjugate of claim 9, wherein the sum of b and c is ranging from 5 to 20, from 5 to 15, or is 8, 9, 10, 11, 12, 13, or 14.

11. The conjugate of any one of claims 9 to 10, wherein each X^N is independently a 2'-deoxyribonucleotide; wherein optionally:

(a) each X^N is independently 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, a 5-halo-2'-deoxycytidine, 2'-deoxythymidine, 2'-deoxyuridine, or a 5-halo-2'-deoxyuridine; or

(b) each X^N is independently 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, 2'-deoxythymidine, 5-bromo-2'-deoxyuridine, or 5-iodo-2'-deoxyuridine.

12. The conjugate of any one of claims 9 to 11, wherein $X^{3'}$ is a 2'-deoxyribonucleotide; wherein optionally:

(a) $X^{3'}$ is 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, a 5-halo-2'-deoxycytidine, 2'-deoxythymidine, 2'-deoxyuridine, or a 5-halo-2'-deoxyuridine;

(b) $X^{3'}$ is 2'-deoxythymidine;

(c) $X^{3'}$ is a 2'-modified ribonucleotide; or

(d) $X^{3'}$ is a 2'-methoxy ribonucleotide or 2'-ethoxymethoxy ribonucleotide.

13. The conjugate of any one of claims 9 to 12, wherein $X^{5'}$ is a 2'-deoxyribonucleotide; wherein optionally:

(a) $X^{5'}$ is 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, 5-halo-2'-deoxycytidine, 2'-deoxythymidine, 2'-deoxyuridine, or 5-halo-2'-deoxyuridine;

(b) $X^{5'}$ is a 2'-deoxyribonucleotide with a substituted pyrimidine base;

(c) $X^{5'}$ is a 2'-deoxyribonucleotide with a 5-substituted pyrimidine base;

(d) $X^{5'}$ is 2'-deoxythymidine, a 5-halo-2'-deoxycytidine, or a 5-halo-2'-deoxyuridine;

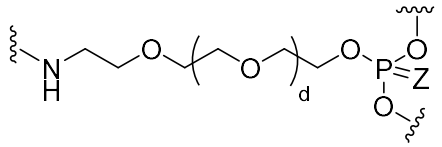
(e) $X^{5'}$ is 2'-deoxythymidine, 5-bromo-2'-deoxycytidine, 5-iodo-2'-deoxycytidine, 5-bromo-2'-deoxyuridine, or 5-iodo-2'-deoxyuridine; or

(f) $X^{5'}$ is 2'-deoxythymidine, 5-bromo-2'-deoxyuridine, or 5-iodo-2'-deoxyuridine.

14. The conjugate of any one of claims 9 to 13, wherein $X^{5'}$ has a 3'-phosphorothioate group; wherein optionally the 3'-phosphorothioate is chiral; and wherein optionally the 3'-phosphorothioate has a chirality of Rp or Sp.

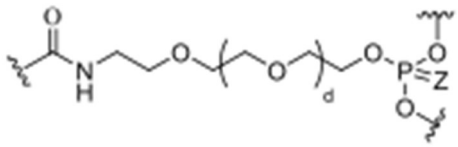
15. The conjugate of any one of claims 9 to 14, wherein $X^{5'}$ has a 3'-phosphorothioate group having a chirality of Rp and $X^{3'}$ is a 2'-methoxy ribonucleotide or 2'-ethoxymethoxy ribonucleotide; or wherein $X^{5'}$ has a 3'-phosphorothioate group having a chirality of Sp and $X^{3'}$ is a 2'-methoxy ribonucleotide or 2'-ethoxymethoxy ribonucleotide.

16. The conjugate of any one of claims 9 to 15, wherein Y^P is:



wherein Z is O or S; and d is an integer ranging from 0 to 50, ranging from 0 to 10, ranging from 0 to 5, 0, 1, or 3.

17. The conjugate of any one of claims 9 to 15, wherein Y^P is:



wherein Z is O or S; and d is an integer ranging from 0 to 50, ranging from 0 to 10, ranging from 0 to 5, 0, 1, or 3.

18. The conjugate of any one of claims 1 to 17, wherein the immunomodulating polynucleotide comprises an additional internucleoside phosphotriester; wherein optionally the additional internucleoside phosphotriester is an alkylphosphotriester or ethylphosphotriester.

19. The conjugate of any one of claims 1 to 18, wherein the immunomodulating polynucleotide comprises one 5-halo-2'-deoxyuridine; wherein optionally the 5-halo-2'-deoxyuridine is 5-bromo-2'-deoxyuridine or 5-iodo-2'-deoxyuridine.

20. The conjugate of any one of claims 1 to 19, wherein the immunomodulating polynucleotide comprises three or more 2'-deoxycytidines; wherein optionally the immunomodulating polynucleotide comprises three 2'-deoxycytidines.

21. The conjugate of any one of claims 1 to 20, wherein the immunomodulating polynucleotide comprises four or more 2'-deoxyguanosines; wherein optionally the immunomodulating polynucleotide comprises four 2'-deoxyguanosines.

22. The conjugate of any one of claims 1 to 21, wherein the immunomodulating polynucleotide comprises:

(a) three 2'-deoxycytidines and four 2'-deoxyguanosines, or

(b) three or more 2'-deoxythymidines; wherein optionally the immunomodulating polynucleotide comprises three, four, five, six, seven, or eight 2'-deoxythymidines.

23. The conjugate of any one of claims 1 to 22, wherein the immunomodulating polynucleotide comprises zero, one, or two 2'-deoxyadenosines.

24. The conjugate of any one of claims 1 to 23, wherein the immunomodulating polynucleotide comprises one or more internucleoside phosphorothioates; wherein optionally the immunomodulating polynucleotide comprises about 12 internucleoside phosphorothioates.

25. The conjugate of any one of claims 1 to 24, wherein the targeting moiety is an antibody to an antigen expressed on the NK cell or the myeloid cell.

26. The conjugate of any one of claims 1 to 25, wherein the targeting moiety is a human antibody.

27. The conjugate of any one of claims 1 to 26, wherein the targeting moiety is a human anti-CD56 antibody; wherein optionally the antibody is a human anti-CD56 antibody derived from clone 5.1H11.

28. The conjugate of any one of claims 1 to 26, wherein the antibody is an anti-SIRPα antibody.

29. The conjugate of claim 28, wherein the targeting moiety is a blocking anti-SIRPα antibody; wherein optionally the blocking anti-SIRPα antibody comprises:

(a) a heavy chain variable (VH) domain comprising an HVR-H1 comprising a sequence selected from the group consisting of SEQ ID NOs:498-500, an HVR-H2 comprising the sequence of SEQ ID NO:501, and an HVR-H3 comprising the sequence of SEQ ID NO:502; and a light chain variable (VL) domain comprising an HVR-L1 comprising the sequence of SEQ ID NO:503, an HVR-L2 comprising the sequence of SEQ ID NO:504, and an HVR-L3 comprising the sequence of SEQ ID NO:505;

(b) a VH domain comprising a sequence selected from the group consisting of SEQ ID NOs:490-495 and a VL domain comprising the sequence of SEQ ID NO:496 or 497;

(c) a VH domain comprising an HVR-H1 comprising a sequence selected from the group consisting of SEQ ID NOs:512-514, an HVR-H2 comprising the sequence of SEQ ID NO:515, and an HVR-H3 comprising the sequence of SEQ ID NO:516; and a VL domain comprising an HVR-L1 comprising the sequence of SEQ ID NO:517, an HVR-L2 comprising the sequence of SEQ ID NO:518, and an HVR-L3 comprising the sequence of SEQ ID NO:519;

(d) a VH domain comprising a sequence selected from the group consisting of SEQ ID NOs:506-509 and a VL domain comprising the sequence of SEQ ID NO:510 or 511;

(e) a VH domain comprising an HVR-H1 comprising a sequence selected from the group consisting of SEQ ID NOs:533-535, an HVR-H2 comprising the sequence of SEQ ID NO:536, and an HVR-H3 comprising the sequence of SEQ ID NO:537; and a VL domain comprising an HVR-L1 comprising a sequence selected from the group consisting of SEQ ID NOs:538-542, an HVR-L2 comprising the sequence of SEQ ID NO:543, and an HVR-L3 comprising a sequence selected from the group consisting of SEQ ID NOs:544-546; or

(f) a VH domain comprising a sequence selected from the group consisting of SEQ ID NOs:520-523 and a VL domain comprising a sequence selected from the group consisting of SEQ ID NOs:525-532.

30. The conjugate of claim 28, wherein the targeting moiety is a non-blocking anti-SIRPα antibody; wherein optionally the non-blocking anti-SIRPα antibody comprises:

(a) a heavy chain variable (VH) domain comprising an HVR-H1 comprising a sequence selected from the group consisting of SEQ ID NOs:554-556, an HVR-H2 comprising the sequence of SEQ ID NO:557, and an HVR-H3 comprising the sequence of SEQ ID NO:558; and a light chain variable (VL) domain comprising an HVR-L1 comprising the sequence of SEQ ID NO:559, an HVR-L2 comprising the sequence of SEQ ID NO:560, and an HVR-L3 comprising the sequence of SEQ ID NO:561;

(b) a VH domain comprising a sequence selected from the group consisting of SEQ ID NOs:547-550 and a VL domain comprising a sequence selected from the group consisting of SEQ ID NOs:551-553; or

(c) a VH domain comprising the sequence of SEQ ID NO:584 and a VL domain comprising a sequence selected from the group consisting of SEQ ID NOs:585, 562, and 563.

31. The conjugate of any one of claims 1 to 26, wherein the antibody is an anti-SIRPβ antibody; wherein optionally the targeting moiety is a blocking or non-blocking anti-SIRPβ antibody.

32. The conjugate of any one of claims 1 to 26, wherein the antibody is an anti-SIRPγ antibody; wherein optionally the targeting moiety is a blocking or non-blocking anti-SIRPγ antibody.

33. The conjugate of any one of claims 25-32, wherein the antibody comprises a human Fc region; wherein optionally the Fc region is:

(a) a human IgG1, IgG2, or IgG4 Fc region;

(b) a human IgG1 Fc region comprising L234A, L235A, and G237A mutations, according to EU numbering;

(c) a human IgG1 Fc region comprising L234A, L235A, G237A, and N297A mutations, according to EU numbering;

(d) a human IgG1 Fc region comprising an N297A mutation, according to EU numbering;

(e) a human IgG1 Fc region comprising a D265A mutation, according to EU numbering;

(f) a human IgG1 Fc region comprising D265A and N297A mutations, according to EU numbering;

(g) a human IgG2 Fc region comprising A330S and P331S mutations, according to EU numbering;

(h) a human IgG2 Fc region comprising A330S, P331S, and N297A mutations, according to EU numbering;

(i) a human IgG2 Fc region comprising an N297A mutation, according to EU numbering;

(j) a human IgG4 Fc region comprising an S228P mutation, according to EU numbering;

(k) a human IgG4 Fc region comprising S228P and D265A mutations, according to EU numbering;

(l) a human IgG4 Fc region comprising S228P and L235E mutations, according to EU numbering;

(m) a human IgG4 Fc region comprising S228P and N297A mutations, according to EU numbering;

(n) a human IgG4 Fc region comprising S228P, E233P, F234V, L235A, delG236, and N297A mutations, according to EU numbering;

(o) an Fc region that comprises a sequence selected from the group consisting of SEQ ID NOs:564-578.

34. The conjugate of any one of claims 25-33, wherein the antibody comprises:

(a) a human kappa light chain constant domain, wherein optionally the light chain constant domain comprises the sequence of SEQ ID NO:579; or

(b) a human lambda light chain constant domain, wherein optionally the light chain constant domain comprises the sequence of SEQ ID NO:580 or 581.

35. A pharmaceutical composition comprising the conjugate of any one of claims 1 to 34, and a pharmaceutically acceptable excipient.

36. The pharmaceutical composition of claim 35, wherein the composition is formulated for parenteral administration.

37. The pharmaceutical composition of claim 35 or 36, wherein the composition is formulated as a single dosage form.

38. The pharmaceutical composition of any one of claims 35 to 37, wherein the composition is formulated as an intravenous dosage form.

39. The pharmaceutical composition of any one of claims 35 to 38, further comprising a second therapeutic agent.

40. The conjugate of any one of claims 1 to 34 for use in treating, preventing, or ameliorating one or more symptoms of a proliferative disease in a subject.

41. Use of the conjugate of any one of claims 1 to 34 in the manufacture of a medicament for treating, preventing, or ameliorating one or more symptoms of a proliferative disease in a subject.

42. The conjugate for use of claim 40 or the use of claim 41, wherein the proliferative disease is cancer.

43. The conjugate of any one of claims 1 to 27, 33, and 34 for use in modulating a natural killer cell in a subject having cancer.

44. The conjugate of any one of claims 1 to 26 and 28 to 34 for use in modulating a myeloid cell in a subject having cancer.

45. The conjugate for use of claim 44, wherein the myeloid cell is a monocyte.