(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 January 2025 (30.01.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/024704 A1

(51) International Patent Classification:
A61K 48/00 (2006.01) *C12N 15/85* (2006.01)
C12Q 1/6897 (2018.01) *G01N 33/50* (2006.01)
C12N 15/63 (2006.01)

(21) International Application Number:
PCT/US2024/039618

(22) International Filing Date:
25 July 2024 (25.07.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/515,514 25 July 2023 (25.07.2023) US
63/644,296 08 May 2024 (08.05.2024) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2025/024704 A1

(54) Title: POLYNUCLEOTIDES COMPRISING A MICRO RNA DETARGETING SENSOR AND USES THEREOF

(57) Abstract: The present disclosure relates to polynucleotides comprising (a) a first region encoding a payload and (b) a second region comprising an immune cell detargeting sensor (e.g., a spleen detargeting sensor). Upon recognition of the marker by the sensor, the expression of the encoded payload is reduced or inhibited. Also provided herein are replicons, nanoparticles, synthetic circuits, pharmaceutical compositions comprising such polynucleotides. Methods of using the polynucleotides, replicons, synthetic circuits, nanoparticles, and pharmaceutical compositions are also provided.

POLYNUCLEOTIDES COMPRISING A MICRO RNA DETARGETING SENSOR AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This PCT application claims the priority benefit of U.S. Provisional Application No. 63/515,514, filed July 25, 2023 and U.S. Provisional Application No. 63/644,296, filed May 8, 2024, the entire disclosures of each which are incorporated herein by reference for all purposes.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

[0002] The content of the sequence listing is submitted electronically (Name: 4597_031PC02_Seqlisting_ST26; Size: 38,199 bytes; and Date of Creation: July 25, 2024) and is filed with the application is herein incorporated by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0003] Nucleic acid-based treatment (*e.g.*, mRNA vaccines) is becoming an increasingly important approach for the treatment of a variety of diseases. Upon administration, the encoded protein can be produced to induce an immune response (*e.g.*, tumor antigen to induce anti-tumor immune response) or to provide important function to maintain a healthy state. However, controlling expression of payload proteins from mRNA therapeutics in a tissue-specific and/or cell-specific manner remains a challenge. Additionally, current delivery platforms for delivering the nucleic acid molecules are ineffective or associated with undesirable side effects, limiting the full potential of nucleic acid-based therapy.

[0004] Therefore, there remains a need for improved delivery platforms for nucleic acids (*e.g.*, mRNA) that are more effective and allow for tissue-specific and/or cell-specific delivery.

BRIEF SUMMARY OF THE DISCLOSURE

[0005] Provided herein is an isolated polynucleotide comprising (a) a first region encoding a payload and (b) a second region comprising a sensor that is capable of specifically recognizing a marker expressed by an immune cell (immune cell detargeting sensor), wherein the recognition of

the marker by the immune cell detargeting sensor results in reduced expression of the payload as compared to a reference cell (*e.g.*, non-immune cell).

[0006] In some aspects, the immune cell comprises hematopoietic stem cells and/or derivatives thereof.

[0007] In some aspects, the immune cell comprises T cells, B cells, natural killer (NK) cells, macrophages, monocytes, dendritic cells, neutrophils, basophils, eosinophils, or combinations thereof.

[0008] In some aspects, the immune cell is present within a lymphoid tissue at a greater quantity as compared to a non-lymphoid tissue.

[0009] In some aspects, the number of the immune cell present within the lymphoid tissue is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold or more, as compared to the corresponding value for the non-lymphoid tissue.

[0010] In some aspects, the lymphoid tissue comprises a spleen, lymph node, or both.

[0011] In some aspects, the second region comprises multiple immune cell detargeting sensors.

[0012] In some aspects, each of the multiple immune cell detargeting sensors is the same.

[0013] In some aspects, one or more of the multiple immune cell detargeting sensors are different.

[0014] In some aspects, the marker comprises a microRNA.

[0015] In some aspects, the microRNA comprises a miR-142.

[0016] In some aspects, the payload comprises a cytokine, a ligand-binding protein, or both.

[0017] In some aspects, the cytokine comprises an interleukin (IL)-12 protein.

[0018] In some aspects, the isolated polynucleotide further comprises: (1) an Internal Ribosome Entry Site (IRES), (2) an untranslated region (UTR), (3) a sequence encoding a signal peptide, (4) a translation initiation sequence, (5) a polyA sequence, (6) a sequence encoding a RNA binding protein, (7) a 5'-cap, (8) a sequence encoding a 2A ribosome skip peptide, (9) a translation enhancer element, or (10) any combination of (1) to (9).

[0019] Also provided herein is an isolated polynucleotide comprising (a) a first region encoding a payload and (b) a second region comprising a sensor that is capable of specifically

recognizing a marker expressed within a spleen (spleen detargeting sensor), and wherein the recognition of the marker by the spleen detargeting sensor reduces the expression of the payload.

[0020] In some aspects, the marker comprises a microRNA.

[0021] In some aspects, the microRNA comprises a miR-142.

[0022] In some aspects, the payload comprises a cytokine, a ligand-binding protein, or both.

[0023] In some aspects, the cytokine comprises an interleukin (IL)-12 protein.

[0024] In some aspects, the second region comprises multiple spleen detargeting sensors.

[0025] In some aspects, each of the multiple spleen detargeting sensors are the same.

[0026] In some aspects, one or more of the multiple spleen detargeting sensors are different.

[0027] In some aspects, the isolated polynucleotide further comprises: (1) an Internal Ribosome Entry Site (IRES), (2) an untranslated region (UTR), (3) a sequence encoding a signal peptide, (4) a translation initiation sequence, (5) a polyA sequence, (6) a sequence encoding a RNA binding protein, (7) a 5'-cap, (8) a sequence encoding a 2A ribosome skip peptide, (9) a translation enhancer element, or (10) any combination of (1) to (9).

[0028] Also provided herein is a synthetic circuit comprising any of the isolated polynucleotides described herein.

[0029] Also provided herein is a synthetic circuit consisting essentially of any of the isolated polynucleotides described herein.

[0030] Also provided herein is a synthetic circuit consisting of any of the isolated polynucleotides described herein.

[0031] Also provided herein is a replicon comprising any of the isolated polynucleotides or the synthetic circuits described herein, wherein the replicon is self-replicating.

[0032] In some aspects, the replicon is derived from an alpha virus.

[0033] In some aspects, the alpha virus comprises a Venezuelan equine encephalitis (VEE) virus.

[0034] Also provided herein is a nanoparticle comprising (a) any of the isolated polynucleotides, synthetic circuits, or replicons described herein, and (b) a lipid and/or lipid-like material.

[0035] In some aspects, the lipid comprises an ionizable lipid, cationic lipid, lipidoid, non-cationic helper lipid, phospholipid, sterol or other structural lipids, or combinations thereof.

[0036] In some aspects, the ionizable lipid comprises ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315), heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (SM-102), heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Lipid 5), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), 3-(didodecylamino)-N1,N1,4 tridodecyl-1-piperazineethanamine (KL10), Nl-[2 (didodecylamino)ethyl]-N1,N4,N4-tridodecyl 1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2 dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), or combinations thereof.

[0037] In some aspects, the cationic lipid comprises l,2-dioleoyl-3 -trimethylammonium-propane (DOTAP), lipofectamine, N-[l-(2,3- dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), l-[2- (oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTEVI), 2,3- dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l ,2-dimyristyloxyprop-3 -yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DMRIE), N-(l,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DORIE), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), l,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), l,2-distearoyl-3- trimethylammonium-propane (DSTAP), l,2-dipalmitoyl-3 -trimethylammonium-propane (DPTAP), l,2-dilinoleoyl-3 -trimethylammonium-propane (DLTAP), l,2-dimyristoyl-3- trimethylammonium-propane (DMTAP), 1,2-distearoyl -sn-glycero-3- ethylphosphocholine (DSePC), l,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC), 1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC), 1,2-dioleoyl-sn- glycero-3-ethylphosphocholine (DOePC), l,2-di-(9Z-tetradecenoyl)-sn-glycero-3- ethylphosphocholine (14: 1 EPC), l-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18: 1 EPC), or any combination thereof.

[0038] In some aspects, the lipidoid comprises 1,1′-((2-(4-(2-((2-(bis(2-hydroxydodecyl) amino)ethyl) (2- hydroxydodecyl)amino)ethyl) piperazin-1-yl)ethyl)azanediyl) bis(dodecan-2-ol) (C12-200), 3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine2,5-dione (cKK-E12), tetrakis(8-methylnonyl) 3,3′,3″,3‴- (((methylazanediyl) bis(propane-3,1 diyl))bis (azanetriyl))tetrapropionate (306Oi10), G0-C14, 5A2-SC8, 3,6-bis(4-(bis((9Z,12Z)-2-hydroxyoctadeca9,12-dien-1-yl)amino)butyl)piperazine-2,5-dione (OF-02), (((3,6-dioxopiperazine-2,5-diyl)bis (butane-4,1-diyl))bis(azanetriyl))tetrakis(ethane2,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-Deg-Lin), (((3,6-dioxopiperazine-2,5-diyl)bis(butane-4,1-diyl)) bis(azanetriyl))tetrakis (butane-4,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-C4-Deg-Lin), N1,N3,N5-tris(3-(didodecylamino)propyl)benzene1,3,5-tricarboxamide (TT3), Hexa(octan-3-yl) 9,9′,9″,9‴,9⁗,9⁗′- ((((benzene-1,3,5-tricarbonyl)ris(azanediyl)) tris (propane-3,1-diyl))tris(azanetriyl))hexanonanoate (FTT5), PL-1, 98N12-5, ethyl 5,5-di((Z)-heptadec-8-en-1-yl)-1-(3-(pyrrolidin-1-yl)propyl)-2,5-dihydro-1H-imidazole-2-carboxylate (A2-Iso5-2DC18 (A2)), A12-Iso5−2DC18 (A12), or any combination thereof.

[0039] In some aspects, the phospholipid is selected from the group consisting of 1,2-dilinoleoyl-sn-glycero-3 phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanola mine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (14:0-16:0 PC, MPPC), 1-myristoyl-2 stearoyl-sn-glycero-3-phosphocholine (14:0-18:0 PC, MSPC), 1-palmitoyl 2-acetyl-sn-glycero-3-phosphocholine (16:0-02:0 PC), 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine (16:0-14:0 PC, PMPC), 1-palmitoyl-2-

stearoyl-sn-glycero-3-phosphocholine (16:0-18:0 PC, PSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18: 1 PC, POPC), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (16:0-18:2 PC, PLPC), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (16:0-20:4 PC), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (14:0-22:6 PC), 1-stearoyl-2-myristoyl-sn-glycero-3-phosphocholine (18:0-14:0 PC, SMPC), 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18:0-16:0 PC, SPPC), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (18:0-18: 1 PC, SOPC), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (18:0-18:2 PC), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (18:0-20:4 PC), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (18:0-22:6 PC), 1-oleoyl-2-myristoyl-sn-glycero-3-phosphocholine (18: 1-14:0 PC, OMPC), 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18: 1-16:0 PC, OPPC), 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (18: 1-18:0 PC, OSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (16:0- 18: 1 PE, POPE), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (16:0-18:2 PE), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (16:0-20:4 PE), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (16:0-22:6 PE), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (18:0-18: 1 PE), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (18:0-18:2 PE), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (18:0-20:4 PE), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (18:0-22:6 PE), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), and any combination thereof.

[0040] In some aspects, the sterol comprises a cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and combinations thereof.

[0041] In some aspects, the lipid and/or lipid-like material is pegylated.

[0042] In some aspects, any of the the nanoparticles described herein further comprises a targeting ligand.

[0043] Also provided herein is a pharmaceutical composition comprising (a) any of the isolated polynucleotides, synthetic circuits, replicons, or nanoparticles described herein, and (b) a pharmaceutically acceptable carrier.

[0044] Some aspects of the present disclosure relate to methods of reducing an expression of a payload (payload expression) within an immune cell of a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits (c) the

replicons, (d) nanoparticles, or (e) the pharmaceutical compositions described herein, or any combination of (a) to (e).

[0045] In some aspects of the methods, reducing the payload expression comprises (a) reducing the amount of payload that is expressed by the immune cell, (b) reducing the duration of payload expression by the immune cell, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell detargeting sensor).

[0046] In some aspects of the methods, after the administration, the amount of payload that is expressed by the immune cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0047] In some aspects of the methods, after the administration, the duration of the payload expression by the immune cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0048] In some aspects of the methods, the immune cell comprises a T cell, macrophage, or both.

[0049] Some aspects of the present disclosure relate to methods of selectively expressing a payload in a non-immune cell of a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) nanoparticles, (e) pharmaceutical compositions described herein, or any combination of (a) to (e).

[0050] In some aspects of the methods, after the administration, (a) an amount of the payload that is expressed in the non-immune cell, (b) a duration of the expression of the payload in the non-immune cell, or (c) both (a) and (b) are increased, as compared to the corresponding amount and/or duration in the immune cell of the subject.

[0051] Some aspects of the present disclosure relate to methods of of reducing an expression of a payload (payload expression) within a lymphoid tissue of a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) nanoparticles, (e) pharmaceutical compositions described herein, or any combination of (a) to (e).

[0052] In some aspects of the methods, the lymphoid tissue comprises a spleen, lymph node, or both.

[0053] In some aspects of the methods, reducing the payload expression comprises (a) reducing the amount of payload that is expressed in the lymphoid tissue, (b) reducing the duration of payload expression in the lymphoid tissue, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell detargeting sensor).

[0054] In some aspects of the methods, after the administration, the amount of payload that is expressed in the lymphoid tissue is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0055] In some aspects of the methods, after the administration, the duration of the payload expression in the lymphoid tissue is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0056] Some aspects of the present disclosure relate to methods of reducing expression of a payload (payload expression) within the spleen of a subject in need thereof, wherein then method comprises administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) nanoparticles, or (e) pharmaceutical compositions described herein, or any combination of (a) to (e).

[0057] In some aspects of the methods, reducing the payload expression comprises (a) reducing the amount of payload that is expressed in the spleen, (b) reducing the duration of payload expression in the spleen, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the spleen detargeting sensor).

[0058] In some aspects of the methods, after the administration, the amount of payload that is expressed in the spleen is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0059] In some aspects of the methods, after the administration, the payload is not expressed in the spleen.

[0060] In some aspects of the methods, after the administration, the duration of the payload expression in the spleen is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0061] Some aspects of the present disclosure relate to methods of selectively expressing a payload in a non-lymphoid tissue of a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) nanoparticles, (e) pharmaceutical compositions described herein, or any combination of (a) to (e).

[0062] In some aspects of the methods, after the administration, (a) an amount of the payload that is expressed in the non-lymphoid tissue, (b) a duration of the expression of the payload in the non-spleen tissue, or (c) both (a) and (b) are increased, as compared to the corresponding amount and/or duration in the spleen of the subject.

[0063] Some aspects of the present disclosure relate to methods of selectively expressing a payload in a non-spleen tissue of a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) nanoparticles, or (e) pharmaceutical compositions described herein, or any combination of (a) to (e).

[0064] In some aspects of the methods, after the administration, (a) an amount of the payload that is expressed in the non-spleen tissue, (b) a duration of the expression of the payload in the non-spleen tissue, or (c) both (a) and (b) are increased, as compared to the corresponding amount and/or duration in the spleen of the subject.

[0065] Some aspects of the present disclosure relate to methods of treating a disease or disorder in a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) nanoparticles, or (e) pharmaceutical compositions described herein, or any combination of (a) to (e).

[0066] In some aspects of the methods, the disease or disorder comprises a cancer, inflammatory disorders, monogenic disorders, neurological disorders, psychiatric disorders, or combinations thereof.

[0067] In some aspects of the methods, the cancer comprises a melanoma, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine cancer, salivary

gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, head and neck cancer, or combinations thereof.

[0068] In some aspects of the methods, any of the isolated polynucleotides, synthetic circuits, replicons, nanoparticles, and/or pharmaceutical compositions described herein is administered to the subject via intravenous, intratumoral, intrathecal, intramuscular, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration.

[0069] In some aspects of the methods, the method comprises administering to the subject an additional therapeutic agent.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0070] **FIGs. 1A** and **1B** provide comparison of IL-12 and IFN-γ levels, respectively, in the spleen of mice after a single intravenous administration of a lipid nanoparticle loaded with either (a) a VEE replicon comprising mRNA encoding IL-12 alone (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)) or (b) a VEE replicon comprising mRNA encoding IL-12 and comprising an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor). The LNP was administered at one of two doses: 0.1 mg/kg or 0.3 mg/kg. Control animals were treated with PBS. IL-12 and IFN-γ levels were measured at 6 hours and 24 hours post-administration.

[0071] **FIG. 2** provides comparison of percentage in body weight in mice after administration of a lipid nanoparticle loaded with either (a) a VEE replicon comprising mRNA encoding IL-12 alone (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)) (square) or (b) a VEE replicon comprising mRNA encoding IL-12 and comprising an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (diamond). Control animals were treated with PBS (circle). Body weight was measured over a course of 10 days post-administration.

[0072] **FIGs. 3A** and **3B** provide comparison of IL-12 and IFN-γ levels, respectively, in the plasma of non-human primates after a single intravenous administration of a lipid nanoparticle loaded with one of the following: (a) modified mRNA encoding IL-12 alone (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)) ("mod-IL-12"; circle); (b) modified mRNA encoding IL-12 and comprising an immune cell detargeting sensor

(*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) and a liver detargeting sensor ("mod-IL-12-circuit"; square); (c) VEE replicon comprising mRNA encoding IL-12 alone (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)) ("rep-IL-12"; triangle); and (d) VEE replicon comprising mRNA encoding IL-12 and comprising an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) ("rep-IL-12-circuit"; inverted triangle).

[0073] **FIG. 4** provides a schematic of the RNA circuit described in Example 1.

[0074] **FIG. 5** provides a comparison of miRNA expression in different cell types (two different tumors, splenocytes, and healthy non-splenocytes) as well as comparison of predicted payload expression in the different cell types after treating with a RNA circuit encoding the payload and a sensor specific for the miRNA.

[0075] **FIGs. 6A** and **6B** provide comparison of payload expression in the spleen and a non-spleen tissue, respectively, from mice after systemic treatment with one of the following: (1) PBS alone, (2) mRNA encoding the payload without an immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor) (*i.e.*, uncircuited mRNA), and (3) mRNA encoding the payload with an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA).

[0076] **FIGs. 7A-7F** provide comparison of body weight, serum and spleen IL-12 levels, and serum and spleen IFN-γ levels in mice treated with one of the following: (1) PBS alone, (2) mRNA encoding IL-12 without an immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor) (*i.e.*, uncircuited mRNA), and (3) mRNA encoding IL-12 with an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA). mRNA was administered to the animals at two different doses: 0.1 mg/kg (**FIGs. 7A, 7B,** and **7C**) and 0.3 mg/kg (**FIGs. 7D, 7E,** and **7F**).

[0077] **FIGs. 8A-8C** provide comparison of tumor volume, IL-12 level in the tumor, and IL-12 level in the spleen, respectively, in mice treated with one of the following: (1) PBS alone, (2) mRNA encoding the payload without an immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor) (*i.e.*, uncircuited mRNA), and (3) mRNA encoding the payload with an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA).

[0078] **FIGs. 9A** and **9B** provide comparison of IL-12 protein level in the supernatant after transfection of patient-derived breast cancer organoids (**FIG. 9A**) and A549 cells (**FIG. 9B**) with one of the following: (1) mRNA encoding the payload without an immune cell detargeting sensor

(*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor) (*i.e.*, uncircuited mRNA) (left bar) and (2) mRNA encoding the payload with an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA) (right bar).

[0079] **FIG. 10** provides a comparison of miR-142 expression profiles (counts per million) across different cell types (*i.e.*, A549, BT-20, HeLa, HEK293T, HEK293, CD3+ T, CD4+ T, CD8+ T, CD14+ monocyte, CD19+ B, CD56+ NK, and B16F10).

[0080] **FIG. 11** provides an *in vitro* comparison of luciferase expression in human Kupffer cells (liver macrophages) transfected with either a VEE replicon comprising mRNA encoding firefly luciferase ("Fluc") without an immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor) (*i.e.*, uncircuited mRNA) ("Rep-Fluc") or a VEE replicon comprising mRNA encoding Fluc with an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA) ("Rep-Fluc-miRNA sensor").

[0081] **FIG. 12** provides a comparison of IL-12 expression in the spleen, mesenteric lymph nodes (LN), tracheobronchial LN, mandibular LN, axillary LN, and bone marrow of non-human primates following intravenous delivery of a lipid nanoparticle loaded with one of the following: (a) modified mRNA encoding IL-12 alone (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)) ("mod-IL-12"; circle); (b) modified mRNA encoding IL-12 and comprising an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) and a liver detargeting sensor ("mod-IL-12-circuit"; square); (c) VEE replicon comprising mRNA encoding IL-12 alone (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)) ("rep-IL-12"; triangle); and (d) VEE replicon comprising mRNA encoding IL-12 and comprising an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) ("rep-IL-12-circuit"; inverted triangle). The dotted line denotes the lower limit of quantification (LLOQ).

[0082] **FIG. 13** shows staining of miR-142-3p expression within the white pulp (containing lymphocytes and macrophages) of non-human primate spleen.

[0083] **FIG. 14** provides a comparison of payload expression (*i.e.*, reporter) for the following groups: control and miR-142-3p Sensor in T cells.

[0084] **FIGs. 15A** and **15B** provide miR-142 sensor activity in A549 (**FIG. 15A**) and B16.F10 cells (**FIG. 15B**). **FIG. 15A** provides a comparison of payload expression (*i.e.*, luminescence) for the following groups: (1) hsa-miR-142-3p (circuited; left bar) (*i.e.*, A549 cells treated with a construct comprising a payload-encoding mRNA and a miR-142 sensor), (2) no miR (uncircuited; middle bar) (*i.e.*, A549 cells treated with a construct comprising a payload-encoding

mRNA but no miR-142 sensor), and (3) no reporter (control; right bar) (*i.e.*, non-treated A549 cells). **FIG. 15B** provides a comparison of payload expression (*i.e.*, mIL-12) for the following groups: (1) no sensor (uncircuited; left bar) (*i.e.*, B16.F10 cells treated with a construct comprising an IL-12-encoding mRNA but no miR-142 sensor) and (2) hsa-miR-142-3p-Sensor (circuited; right bar) (*i.e.*, B16.F10 cells treated with a construct comprising an IL-12-encoding mRNA and a miR-142 sensor).

[0085] **FIG. 16** is a schematic of a plasmid encoding a VEE-derived replicon described throughout the Examples.

[0086] **FIG. 17** provides a comparison of miR-142 sensor activity in RAW264.7 macrophage cells. A VEE replicon (Rep) expressing a firefly luciferase (Fluc) payload protein and bearing a sensor responsive to miR-142-3p (Rep with Sensor) or without such a sensor (Rep without Sensor) were transfected into RAW264.7 macrophages. Some samples were co-transfected with a miRNA inhibitor targeting miR-142-3p (anti-miR-142-3p) or a negative control miRNA inhibitor (anti-cel-miR-67).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0087] The present disclosure is generally directed to isolated polynucleotides that allow for selective regulation of the expression of a gene (*e.g.*, encoding a payload) in a tissue. More specifically, the polynucleotides described herein comprise (a) a first region encoding a payload and (b) a second region comprising a sensor that has been programmed to recognize a specific marker, such as that expressed within the spleen. As further described herein, depending on the presence or absence of the specific marker, the sensor can regulate the expression of the encoded payload. Such polynucleotides can be particularly useful in reducing and/or preventing the expression of a payload within a specific tissue, *e.g.*, the spleen. As will be apparent to those skilled in the arts, tissues such as the spleen comprise large population of immune cells (*e.g.*, T cells). In some aspects, the specific marker recognized by the sensor is expressed by an immune cell present within such tissues. Not to be bound by any one theory, in some aspects, the expression of a gene (*e.g.*, encoding a payload) within a specific tissue (*e.g.*, spleen) is directly correlated with the expression of the gene by the immune cells present within the specific tissue. By regulating the expression of a gene (*e.g.*, encoding a payload) by an immune cell, in some aspects, the expression of the gene within a specific tissue can also be regulated. Additional aspects of the present disclosure are provided throughout the present application.

[0088] Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to the particular compositions or process steps described, as such can, of course, vary. As will be apparent to those of skill in the art upon reading this disclosure, each of the individual aspects described and illustrated herein has discrete components and features which can be readily separated from or combined with the features of any of the other several aspects without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[0089] The headings provided herein are not limitations of the various aspects of the disclosure, which can be defined by reference to the specification as a whole. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

I. Definitions

[0090] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In case of conflict, the present application, including the definitions, will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0091] Throughout this disclosure, the term "**a**" or "**an**" entity refers to one or more of that entity; for example, "a polynucleotide," is understood to represent one or more polynucleotides. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0092] Furthermore, "**and/or**" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0093] It is understood that wherever aspects are described herein with the language "**comprising**," otherwise analogous aspects described in terms of "**consisting of**" and/or "**consisting essentially of**" are also provided.

[0094] The term "**about**" is used herein to mean approximately, roughly, around, or in the regions of. When the term is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower), unless indicated otherwise.

[0095] The term "**at least**" prior to a number or series of numbers is understood to include the number adjacent to the term "at least," and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21-nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range. "At least" is also not limited to integers (*e.g.*, "at least 5%" includes 5.0%, 5.1%, 5.18% without consideration of the number of significant figures.

[0096] As used herein, the term "**sensor**" refers to any moiety that is capable of recognizing a marker described herein. As used herein, "**recognizing**" a marker can comprise the physical interaction between the marker and the sensor (*e.g.*, the marker binds to a specific marker recognition site within the sensor). As described herein, in some aspects, sensors useful for the present disclosure are capable of specifically recognizing a marker expressed by an immune cell (*i.e.*, immune cell marker). Upon recognition of the marker, the sensor is "**turned on**" and thereby, reduce or inhibit the expression of the encoded payload by the immune cell, *e.g.*, when administered to a subject. Such sensors are also referred to herein as "**immune cell detargeting sensor**." As further described elsewhere in the present disclosure, such immune cells are present in many tissues, *e.g.*, lymphoid tissues, such as the spleen. In some aspects, by reducing or inhibiting the expression of the encoded payload by such immune cells, the sensors described herein can also be useful in reducing or inhibiting the expression of the encoded polypeptide within certain tissues, *e.g.*, lymphoid tissues. Where the sensors result in the reduction or inhibition of the expression of the encoded payload within the spleen (*e.g.*, by recognizing markers expressed by immune cells present within the spleen), such sensors are also referred to herein as "**spleen detargeting sensor**." Where the sensors specifically recognize miR-142-3p, such sensors are also referred to herein as "**miR-142 sensor**."

[0097] As used herein, the term "**marker**" refers to any molecule that is expressed by an immune cell (*e.g.*, present within a tissue (*e.g.*, spleen)) and can be recognized by a sensor

described herein. Non-limiting examples of such markers are provided elsewhere in the present disclosure.

[0098] As used herein, the term "**immune cell**" refers to those cells that are part of the immune system and helps the body fight infections and other diseases. Non-limiting examples of immune cells useful for the present disclosure include cells that are derivatives of hematopoietic stem cells. In some aspects, cells that are derivatives of hematopoietic stem cells comprise lymphoid lineage cells, myeloid lineage cells, or both. In some aspects, lymphoid lineage cells comprise lymphocytes, plasma cells, or both. In some aspects, lymphocytes comprise T cells, B cells, natural killer (NK) cells, or combinations thereof. In some aspects, myeloid lineage cells comprise macrophages, monocytes, dendritic cells, neutrophils, basophils, eosinophils, or combinations thereof. As will be apparent to those skilled in the art, immune cells are found predominantly within a lymphoid tissue or in circulation (*e.g.*, within the blood). As used herein, "**lymphoid tissue**" refers to any tissue within a subject that is rich in immune cells. Non-limiting examples of lymphoid tissues include the bone marrow, thymus, lymph nodes, spleen, tonsils, appendix, Peyer's patches, and combinations thereof.

[0099] As used herein, the term "**non-immune cell**" refers to any cells that are not derived from hematopoietic stem cells.

[0100] As used herein, the term "**payload**" refers to any agent that can be encoded by a polynucleotide described. Non-limiting examples of useful payloads are provided elsewhere in the present disclosure.

[0101] The term "**derived from**," as used herein, refers to a component that is isolated from or made using a specified molecule or organism, or information (*e.g.*, amino acid or nucleic acid sequence) from the specified molecule or organism. For example, a nucleic acid sequence that is derived from a second nucleic acid sequence can include a nucleotide sequence that is identical or substantially similar to the nucleotide sequence of the second nucleic acid sequence. In the case of nucleotides or polypeptides, the derived species can be obtained by, for example, naturally occurring mutagenesis, artificial directed mutagenesis or artificial random mutagenesis. The mutagenesis used to derive nucleotides or polypeptides can be intentionally directed or intentionally random, or a mixture of each. The mutagenesis of a nucleotide or polypeptide to create a different nucleotide or polypeptide derived from the first can be a random event (*e.g.*, caused by polymerase infidelity) and the identification of the derived nucleotide or polypeptide can be made by appropriate screening methods, *e.g.*, as discussed herein. In some aspects, a nucleotide or amino acid sequence that is derived from a second nucleotide or amino acid sequence

has a sequence identity of at least about 50%, at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% to the second nucleotide or amino acid sequence, respectively, wherein the first nucleotide or amino acid sequence retains the biological activity of the second nucleotide or amino acid sequence.

[0102] "**Nucleic acid**," "**nucleic acid molecule**," "**nucleotide sequence**," "**nucleic acid sequence**," "**polynucleotide**," and grammatical variants thereof are used interchangeably and refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Single stranded nucleic acid sequences refer to single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA). Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, supercoiled DNA and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation. DNA includes, but is not limited to, cDNA, genomic DNA, plasmid DNA, synthetic DNA, and semi-synthetic DNA. A "nucleic acid composition" of the disclosure comprises one or more nucleic acids as described herein. As described herein, a polynucleotide of the present disclosure comprises DNA, RNA, or both. In some aspects, the term "polynucleotide" includes

polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, shRNA, siRNA, miRNA and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (*e.g.*, peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

[0103] The term "**replicon**," as used herein, refers to a polynucleotide comprising an origin of replication which allows for replication of the polynucleotide in a cell of interest. As further described herein, in some aspects, the replicon is a self-replicating mRNA (*i.e.*, capable of directing its own amplification or replication within a target cell) (also referred to herein as "**repRNA**" or "**RNA-VEE**"). To direct its own amplification, the RNA molecule should encode the enzyme(s) necessary to catalyze RNA amplification (*e.g.*, alphavirus nonstructural proteins nsP1, nsP2, nsP3, nsP4) and also contain cis RNA sequences required for replication which are recognized and utilized by the encoded enzymes(s). An alphavirus RNA vector replicon should contain the following ordered elements: 5′ viral or cellular sequences required for nonstructural protein-mediated amplification (may also be referred to as 5′CSE, or 5′ cis replication sequence, or 5′ viral sequences required in cis for replication, or 5′ sequence which is capable of initiating transcription of an alphavirus), sequences which, when expressed, code for biologically active alphavirus nonstructural proteins (*e.g.*, nsP1, nsP2, nsP3, nsP4), and 3′ viral or cellular sequences required for nonstructural protein-mediated amplification (may also be referred as 3′CSE, or 3′ viral sequences required in cis for replication, or an alphavirus RNA polymerase recognition sequence). The alphavirus RNA vector replicon may contain a means to express one or more heterologous sequence(s), such as for example, an IRES or a viral (e.g., alphaviral) subgenomic promoter (*e.g.*, junction region promoter) which may, in certain aspects, be modified in order to increase or reduce viral transcription of the subgenomic fragment, or to decrease homology with defective helper or structural protein expression cassettes, and one or more heterologous sequence(s) to be expressed. A replicon can also contain additional sequences, for example, one or more heterologous sequence(s) encoding one or more polypeptides (*e.g.*, a protein-encoding gene or a 3′ proximal gene) and/or a polyadenylate tract. The replicon should not contain sequences encoding all of the alphavirus structural proteins (capsid, E1, E2). Non-limiting examples of heterologous sequences that can be expressed by replicon vectors are described, for example in U.S. Pat. No. 6,015,686, incorporated by reference in its entirety herein, and include, for example, antigens, lymphokines,

cytokines, etc. As is apparent from the present disclosure, VEE replicons provided herein are repRNA. Accordingly, "**VEE replicon**," "**RNA-VEE**," and "**repRNA**" are used interchangeably in the present disclosure.

[0104] As used herein, the term "**modRNA**" refers to a synthetic modified RNA which can encode a payload (*e.g.*, described herein) and when delivered to a cell, can induce the expression of the encoded payload. Unless indicated otherwise, modRNAs described herein are not self-replicating. In some aspects, a modRNA described herein comprises a modification which can help improve one or more properties of the RNA (*e.g.*, increased stability and/or transcription). Non-limiting examples of such modifications are known in the art (*e.g.*, chemical modification of a nucleobase). *See, e.g.*, US 2020/0000881, which is incorporated herein by reference in its entirety. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising sensor, *e.g.*, an immune cell detargeting sensor) is not a modRNA.

[0105] The terms "**pharmaceutically acceptable carrier**," "**pharmaceutically acceptable excipient**," and grammatical variations thereof, encompass any of the agents approved by a regulatory agency of the U.S. Federal government or listed in the U.S. Pharmacopeia for use in animals, including humans, as well as any carrier or diluent that does not cause the production of undesirable physiological effects to a degree that prohibits administration of the composition to a subject and does not abrogate the biological activity and properties of the administered compound. Included are excipients and carriers that are useful in preparing a pharmaceutical composition and are generally safe, non-toxic, and desirable.

[0106] As used herein, the term "**pharmaceutical composition**" refers to one or more of the compounds described herein, such as, *e.g.*, a polynucleotide of the present disclosure, mixed or intermingled with, or suspended in one or more other chemical components, such as pharmaceutically acceptable carriers and excipients.

[0107] The terms "**polypeptide**," "**peptide**," and "**protein**" are used interchangeably herein to refer to polymers of amino acids of any length, *e.g.*, that are encoded by a polynucleotide described herein. The polymer can comprise modified amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids such as homocysteine, ornithine, p-

acetylphenylalanine, D-amino acids, and creatine), as well as other modifications known in the art. The term "polypeptide," as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function.

[0108] Polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing.

[0109] A polypeptide can be a single polypeptide or can be a multi-molecular complex such as a dimer, trimer or tetramer. They can also comprise single chain or multi-chain polypeptides. Most commonly disulfide linkages are found in multi-chain polypeptides. The term polypeptide can also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid. In some aspects, a "peptide" can be less than or equal to about 50 amino acids long, *e.g.,* about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 amino acids long.

[0110] The term "**RNA**" is used herein to mean a molecule which comprises at least one ribonucleotide residue. "**Ribonucleotide**" relates to a nucleotide with a hydroxyl group at the 2′-position of a β-D-ribofuranosyl group. The term comprises double-stranded RNA, single-stranded RNA, isolated RNA such as partially or completely purified RNA, essentially pure RNA, synthetic RNA, recombinantly generated RNA differs from naturally occurring RNA by addition, deletion, substitution and/or alteration of one or more nucleotides. In some aspects, the RNA comprises a linear RNA, circular RNA, self-replicating RNA, non-replicating RNA, or combinations thereof. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a sensor, *e.g.*, an immune cell detargeting sensor, *e.g.*, a spleen detargeting sensor) is a linear RNA. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a sensor, *e.g.*, an immune cell detargeting sensor, *e.g.*, a spleen detargeting sensor) is a circular RNA. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a sensor, *e.g.*, an immune cell detargeting sensor, *e.g.*, a spleen detargeting sensor) is a self-replicating RNA. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a sensor, *e.g.*, an immune cell detargeting sensor, *e.g.*, a spleen detargeting sensor) is a non-self-replicating RNA.

[0111] The term "**mRNA**" means "**messenger-RNA**" and relates to a "**transcript**" which is generated by using a DNA template and encodes a peptide or protein (*e.g.*, payload described herein). Typically, an mRNA comprises a 5′-UTR, a protein coding region and a 3′-UTR. mRNA

only possesses limited half-life in cells and in vitro. In the context of the present disclosure, mRNA can be generated by in vitro transcription from a DNA template. The in vitro transcription methodology is known to the skilled person. For example, there is a variety of in vitro transcription kits commercially available. In some aspects of the disclosure, the RNA, preferably the mRNA, is modified with a 5′-cap structure.

[0112] As used herein, the term "**identity**" (*e.g.*, sequence identity) refers to the overall monomer conservation between polymeric molecules, *e.g.*, between polynucleotide molecules. The term "identical" without any additional qualifiers, *e.g.*, polynucleotide A is identical to polynucleotide B, implies the polynucleotide sequences are 100% identical (100% sequence identity). Describing two sequences as, *e.g.*, "70% identical," is equivalent to describing them as having, *e.g.*, "70% sequence identity."

[0113] Calculation of the percent identity of two polypeptide or polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second polypeptide or polynucleotide sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In some aspects, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The amino acids at corresponding amino acid positions, or bases in the case of polynucleotides, are then compared.

[0114] When a position in the first sequence is occupied by the same amino acid or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm.

[0115] Suitable software programs that can be used to align different sequences (*e.g.*, polynucleotide sequences) are available from various sources. One suitable program to determine percent sequence identity is bl2seq, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, *e.g.*, Needle,

Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at worldwideweb.ebi.ac.uk/Tools/psa.

[0116] Sequence alignments can be conducted using methods known in the art such as MAFFT, Clustal (ClustalW, Clustal X or Clustal Omega), MUSCLE, etc.

[0117] Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[0118] In some aspects, the percentage identity (%ID) or of a first amino acid sequence (or nucleic acid sequence) to a second amino acid sequence (or nucleic acid sequence) is calculated as %ID = 100 x (Y/Z), where Y is the number of amino acid residues (or nucleobases) scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence.

[0119] One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data *(e.g.,* crystallographic protein structures), functional data *(e.g.,* location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, *e.g.,* from the EBI. It will also be appreciated that the final alignment used to calculate percent sequence identity can be curated either automatically or manually.

[0120] As used herein, the terms "**isolated**," "**purified**," "**extracted**," and grammatical variants thereof are used interchangeably and refer to the state of a preparation of desired composition of the present disclosure, *e.g.*, a polynucleotide of the present disclosure, that has undergone one or more processes of purification. In some aspects, isolating or purifying as used herein is the process of removing, partially removing (*e.g.*, a fraction) of a composition of the present disclosure from a sample containing contaminants.

[0121] The term "**expression**," as used herein, refers to a process by which a polynucleotide produces a gene product, *e.g.*, RNA or a polypeptide (*e.g.*, therapeutic protein, *e.g.*, coronavirus protein). It includes without limitation transcription of the polynucleotide into micro RNA binding site, small hairpin RNA (shRNA), small interfering RNA (siRNA), or any other RNA product. It includes, without limitation, transcription of the polynucleotide into messenger RNA (mRNA), and the translation of mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be, *e.g.*, a nucleic acid, such as an RNA produced by transcription of a gene. As used herein, a gene product can be either a nucleic acid, RNA or miRNA produced by the transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.*, phosphorylation, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

II. Polynucleotides

[0122] Provided herein is a polynucleotide (*e.g.*, isolated polynucleotide) comprising (a) a first region encoding a payload and (b) a second region comprising a sensor, wherein the sensor is capable of specifically recognizing a marker expressed by an immune cell (*i.e.*, immune cell detargeting sensor). As is apparent from the present disclosure, in some aspects, a tissue (*e.g.*, lymphoid tissue) comprises a population of such immune cells, such that the expression of the encoded payload within the tissue is directly correlated with the expression of the encoded payload by the immune cells. As described herein, when the sensor recognizes (*e.g.*, binds to) the marker, the sensor is "**turned on**" (*i.e.*, in an active form) and thereby, inhibits or reduces the expression of the encoded payload. In the absence of the specific marker, the sensor is "**turned off**" (*i.e.*, in an inactive form) and there is no inhibition or reduction in the expression of the encoded payload.

II.A. Sensors and Markers

[0123] A sensor useful for the present disclosure can be programmed to recognize a wide range of markers expressed by an immune cell (*e.g.*, present within a lymphoid tissue, such as the spleen). In some aspects, the marker is expressed only by an immune cell (*e.g.*, present within a lymphoid tissue, such as the spleen). In some aspects, the marker is expressed by an immune cell (*e.g.*, present within a lymphoid tissue, such as the spleen) and one or more non-immune cells. Where the marker is also expressed by a non-immune cell, in some aspects, the expression of the marker is increased in the immune cell as compared to the non-immune cell. Unless indicated

otherwise, increased expression of the marker can comprise (a) an increase in the total amount of the marker expressed, (b) an increase in how long the marker is expressed, or (c) both (a) and (b).

[0124] In some aspects, the expression of the marker by an immune cell (*e.g.*, present in a lymphoid tissue, such as the spleen) is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or at least about 100% or more, as compared to the corresponding expression by the non-immune cell. In some aspects, the expression of the marker by an immune cell (*e.g.*, present in a lymphoid tissue, such as the spleen) is increased by at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10 fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, or at least about 50-fold or more, as compared to the corresponding expression by the non-immune cell.

[0125] In some aspects, the marker comprises a microRNA. In some aspects, a polynucleotide described herein comprises (a) a first region encoding a payload and (b) a second region comprising an immune cell detargeting sensor, wherein the immune cell detargeting sensor is capable of recognizing a microRNA expressed within an immune cell. In some aspects, the immune cell is present within a lymphoid tissue, such as the spleen. Accordingly, in some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising a spleen detargeting sensor, wherein the spleen detargeting sensor is capable of recognizing a microRNA expressed in the spleen (*e.g.*, a microRNA expressed within an immune cell within the spleen). In some aspects, the microRNA comprises a microRNA-142 (miR-142). Accordingly, in some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising an immune cell detargeting sensor, wherein the immune cell detargeting sensor is capable of recognizing a miR-142. In some aspects, an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) comprises the nucleotide sequence TCCATAAAGTAGGAAACACTACA (SEQ ID NO: 6). In some aspects, an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) consists essentially of the nucleotide sequence TCCATAAAGTAGGAAACACTACA (SEQ ID NO: 6). In some aspects, an immune cell detargeting sensor (*e.g.*, spelen detargeting sensor, *e.g.*, miR-142 sensor) consists of the nucleotide sequence TCCATAAAGTAGGAAACACTACA (SEQ ID NO: 6).

[0126] Unless indicated otherwise, miR-142 useful for the present disclosure comprises any miR-142 from any vertebrate source (*e.g.*, humans). In some aspects, the miR-142 comprises miR-142-3p, miR-142-5p, or both. An exemplary sequence for miR-142-3p is set forth in SEQ ID NO: 1 (UGUAGUGUUUCCUACUUUAUGGA). An exemplary sequence for miR-142-5p is set forth in SEQ ID NO: 2 (CAUAAAGUAGAAAGCACUACU).

[0127] In some aspects, a polynucleotide provided herein comprises multiple immune cell detargeting sensors (*e.g.*, spleen detargeting sensors). For instance, in some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising multiple immune cell detargeting sensors (*e.g.*, spleen detargeting sensors), wherein the recognition of the marker by the multiple immune cell detargeting sensors reduces the expression of the payload. In some aspects, the multiple immune cell detargeting sensors comprise about two immune cell detargeting sensors, about three immune cell detargeting sensors, about four immune cell detargeting sensors, about five immune cell detargeting sensors, about six immune cell detargeting sensors, about seven immune cell detargeting sensors, about eight immune cell detargeting sensors, about nine immune cell detargeting sensors, about 10 immune cell detargeting sensors, about 11 immune cell detargeting sensors, about 12 immune cell detargeting sensors, about 13 immune cell detargeting sensors, about 14 immune cell detargeting sensors, about 15 immune cell detargeting sensors, about 16 immune cell detargeting sensors, about 17 immune cell detargeting sensors, about 18 immune cell detargeting sensors, about 19 immune cell detargeting sensors, or about 20 or more immune cell detargeting sensors.

[0128] In some aspects, each of the multiple immune cell detargeting sensors (*e.g.*, spleen detargeting sensors) is the same (*e.g.*, all have the same sequence and recognize the same marker expressed by an immune cell (*e.g.*, present within the spleen)). In some aspects, one or more of the immune cell detargeting sensors are different. For example, in some aspects, one or more of the multiple immune cell detargeting sensors (*e.g.*, spleen detargeting sensors) can recognize the same marker expressed by an immune cell (*e.g.*, present within the spleen) but differ in their sequence. In some aspects, one or more of the multiple immune cell detargeting sensors (*e.g.*, spleen detargeting sensors) differ in their sequence and recognize a different marker expressed by an immune cell (*e.g.*, present within the spleen).

[0129] In some aspects, a polynucleotide described herein (*e.g.*, comprising an immune cell detargeting sensor) can comprise one or more additional sensors that recognize a marker expressed by a non-immune cell. Such additional sensors are also referred to herein as "**non-immune cell detargeting sensor**." For instance, in some aspects, a polynucleotide useful for the

present disclosure comprises (a) a first region encoding a payload and (b) a second region comprising (i) an immune cell detargeting sensor (*e.g.*, a spleen detargeting sensor) and (ii) an additional sensor that recognize a marker expressed by a non-immune cell.. As it is apparent from the present disclosure, in some aspects, such a polynucleotide can be useful in regulating (*e.g.*, reducing or inhibiting) the expression of the payload within multiple cell types.

[0130] Where a polynucleotide comprises both an immune cell detargeting sensor and a non-immune cell detargeting sensor, in some aspects, (a) the immune cell detargeting sensor recognizes a microRNA expressed by an immune cell (*e.g.*, present within the spleen), (b) the non-immune cell detargeting sensor recognizes a microRNA expressed by a non-immune cell, or (c) both (a) and (b). In some aspects, the microRNA expressed by an immune cell and the microRNA expressed by a non-immune cell are not the same. In some examples, the non-immune cell is present within a non-lymphoid tissue. Examples of non-lymphoid tissues where microRNA are known to regulate mRNA, and thereby protein expression, include, but are not limited to, liver (miR-122), muscle (miR-133, miR-206, miR-208), endothelial cells (miR-17-92, miR-126), myeloid cells (miR-16, miR-21, miR-223, miR-24, miR-27), adipose tissue (let-7, miR-30c), heart (miR-1d, miR-149), kidney (miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126).

II.B. Payloads

[0131] As described herein, polynucleotides provided herein (*e.g.*, comprising an immune cell detargeting sensor, *e.g.*, a spleen detargeting sensor) comprises a first region that encodes a payload. As will be apparent to those skilled in the arts, any suitable payloads known in the art can be used with the present disclosure. Non-limiting examples of such payloads include a therapeutic protein, a detectable protein (*e.g.*, reporter or fluorescent protein), immunomodulatory protein, a ligand-binding protein, or combinations thereof.

[0132] In some aspects, the payload comprises a therapeutic protein. A non-limiting example of such a therapeutic protein comprises a cytokine. In some aspects, a polynucleotide described herein comprises (a) a first region encoding a cytokine and (b) a second region comprising an immune cell detargeting sensor, wherein recognition of the marker by the immune cell detargeting sensor results in reduced expression of the cytokine in the immune cell. In some aspects, the immune cell is present within a lymphoid tissue, such as the spleen. Accordingly, some aspects of the present disclosure is directed to an isolated polynucleotide comprising (a) a first region encoding a cytokine and (b) a second region comprising a spleen detargeting sensor,

wherein the recognition of the marker by the spleen detargeting sensor reduces the expression of the cytokine within the spleen.

[0133] In some aspects, the therapeutic protein is a cytokine and the marker comprises a microRNA. In some aspects, a polynucleotide described herein comprises (a) a first region encoding a cytokine and (b) a second region comprising an immune cell detargeting sensor that is capable of recognizing a microRNA expressed by an immune cell, wherein the recognition of the microRNA by the sensor results in reduced expression of the cytokine in the immune cell. In some aspects, the immune cell is present within a lymphoid tissue, such as the spleen. Accordingly, in some aspects, an isolated polynucleotide provided herein comprises (a) a first region encoding a cytokine and (b) a second region comprising a spleen detargeting sensor that is capable of recognizing a microRNA expressed in the spleen, wherein the recognition of the microRNA by the sensor reduces the expression of the cytokine within the spleen.

[0134] In some aspects, the cytokine comprises an interleukin (IL)-12. In some aspects, a polynucleotide described herein comprises (a) a first region encoding an IL-12 protein and (b) a second region comprising an immune cell detargeting sensor, wherein the recognition of the marker by the sensor results in reduced expression of the IL-12 protein by the immune cell. In some aspects, the immune cell is present within a lymphoid tissue, such as the spleen. In some aspects, provided herein is an isolated polynucleotide comprising (a) a first region encoding an IL-12 protein and (b) a second region comprising a spleen detargeting sensor (*e.g.*, which is capable of recognizing a microRNA expressed in the spleen), wherein the recognition of the marker by the sensor reduces the expression of the IL-12. Exemplary amino acid and/or nucleic acid sequences of IL-12 are provided in WO2022150712A1, which is incorporated herein by reference in its entirety.

[0135] In some aspects, the cytokine is not IL-12. In some aspects, the cytokine is selected from (i) common gamma chain family of cytokines; (ii) IL-1 family of cytokines; (iii) hematopoietic cytokines; (iv) interferons (*e.g.*, type I, type II, or type III); (v) TNF family of cytokines; (vi) IL-17 family of cytokines; (vii) damage-associated molecular patterns (DAMPs); (viii) tolerogenic cytokines; or (ix) combinations thereof. In some aspects, the cytokine comprises IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36ra, IL-37, IL-38, IL-3, IL-5, IL-6, IL-11, IL-13, IL-23, granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), leukemia inhibitory factor (LIF), stem cell factor (SCF), thrombopoietin (TPO), macrophage-colony stimulating factor (M-CSF), erythropoieticn (EPO), Flt-3, IFN-α, IFN-β, IFN-γ, IL-19, IL-20, IL-

22, IL-24, TNF-α, TNF-β, BAFF, APRIL, lymphotoxin beta (TNF-γ), IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F, IL-25, TSLP, IL-35, IL-27, TGF-β, or combinations thereof.

[0136] In some aspects, the payload comprises a ligand-binding protein. Non-limiting examples of ligand binding proteins include a chimeric antigen receptor (CAR), T cell receptor (TCR), chimeric antibody-T cell receptor (caTCR), chimeric signaling receptor (CSR), T cell receptor mimic (TCR mimic), or combinations thereof.

[0137] In some aspects, the payload comprises an antibody or an antigen-binding fragment thereof (collectively referred to herein as "**antibody**"). The antibody can be derived from natural sources, or partly or wholly synthetically produced. In some aspects, the antibody is a monoclonal antibody. In some aspects, the monoclonal antibody is an IgG antibody. In some aspects, the monoclonal antibody is an IgG1, IgG2, IgG3, or IgG4. In some aspects, the antibody is a polyclonal antibody. In some aspects, the antibody is selected from Fab, Fab', and $F(ab')_2$, $F(ab1)_2$, Fv, dAb, and Fd fragments. In some aspects, the antibody is an scFv or $(scFv)_2$ fragment. In some aspects, the antibody is a NANOBODY® (single-domain antibody). In some aspects, the antibody is a bispecific or multi-specific antibody.

[0138] In some aspects, the payload comprises a detectable protein (*e.g.*, reporter or fluorescent protein). Non-limiting examples of such detectable proteins include: luciferase, wt-GFP, green fluorescent protein (*e.g.*, EGFP, Emerald, Superfolder GFP, Azami Green, mWasabi, TagGFP, TurboGFP, AcGFP, ZsGreen, T-Sapphire, etc.), blue fluorescent protein, (*e.g.*, EBFP, EBFP2, Azurite, mTagBFP, etc.), cyan fluorescent protein (*e.g.*, ECFP, mECFP, Cerulean, mTurquoise, CyPet, AmCyan1, Midori-Ishi Cyan, TagCFP, mTFP1 (Teal), etc.), yellow fluorescent protein (*e.g.*, EYFP, Topaz, Venus, mCitrine, YPet, TagYFP, PhiYFP, ZsYellow1, mBanana, etc.), orange fluorescent protein (*e.g.*, Kusabira Orange, Kusabira Orange2, mOrange, mOrange2, dTomato, dTomato-Tandem, TagRFP, TagRFP-T, DsRed, DsRed2, DsRed-Express (T1), DsRed-Monomer, mTangerine, etc.), or red fluorescent protein (*e.g.*, mRuby, mApple, mStrawberry, AsRed2, mRFP1, JRed, mCherry, HcRedl, mRaspberry, dKeima-Tandem, HcRed-Tandem, mPlum, AQ143, etc.).

II.C. Additional Components

[0139] In some aspects, a polynucleotide provided herein (*e.g.*, encoding a payload and comprising an immune cell detargeting sensor) further comprises one or more additional components. In some aspects, such additional components can help in the function and/or expression of the polynucleotide. Accordingly, in some aspects, a polynucleotide of the present disclosure comprises (a) a first region encoding a payload, (b) a second region comprising an

immune cell detargeting sensor (*e.g.*, a spleen detargeting sensor), and (c) one or more additional component described herein, wherein the recognition of the marker by the sensor reduces the expression of the payload. Non-limiting examples of such additional components include: (1) an Internal Ribosome Entry Site (IRES), (2) an untranslated region (UTR), (3) a sequence encoding a signal peptide, (4) a translation initiation sequence, (5) a polyA sequence, (6) a sequence encoding a RNA binding protein, (7) a 5'-cap, (8) a sequence encoding a 2A ribosome skip peptide, (9) a translation enhancer element, (10) a spacer sequence, or (11) any combination of (1) to (10). Additional disclosure related to such additional components are provided below.

Untranslated Regions (UTRs)

[0140] In some aspects, a polynucleotide described herein further comprises a UTR. In some aspects, the UTR is a 5'-UTR. In some aspects, the UTR is a 3'-UTR. In some aspects, the UTR comprises both a 5'-UTR and a 3'-UTR.

[0141] Natural 5′-UTRs bear features which play roles in translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5′-UTR also have been known to form secondary structures which are involved in elongation factor binding.

[0142] 5′-UTR secondary structures involved in elongation factor binding can interact with other RNA binding molecules in the 5′-UTR or 3′-UTR to regulate gene expression. For example, the elongation factor EIF4A2 binding to a secondarily structured element in the 5′-UTR is necessary for microRNA mediated repression (Meijer H A et al., Science, 2013, 340, 82-85, herein incorporated by reference in its entirety). The different secondary structures in the 5′-UTR can be incorporated into the flanking region to either stabilize or selectively destalized mRNAs in specific tissues or cells.

[0143] By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and protein production. For example, introduction of 5′-UTR of liver-expressed mRNA, such as albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII, could be used to enhance expression of a nucleic acid molecule, such as a mRNA, in hepatic cell lines or liver. Likewise, use of 5′-UTR from other tissue-specific mRNA to improve expression in that tissue is possible—for muscle (MyoD, Myosin, Myoglobin, Myogenin, Herculin), for endothelial cells (Tie-1, CD36), for myeloid cells (C/EBP, AML1, G-CSF, GM-CSF, CD11b, MSR, Fr-1, i-NOS),

for leukocytes (CD45, CD18), for adipose tissue (CD36, GLUT4, ACRP30, adiponectin) and for lung epithelial cells (SP-A/B/C/D).

[0144] Other non-UTR sequences can also be incorporated into the UTRs (*e.g.*, 5'-UTR and/or 3'-UTR). For example, introns or portions of introns sequences can be incorporated into the flanking regions of a polynucleotide described herein.

[0145] In some aspects, one or more nucleotides within a UTR (*e.g.*, 5'-UTR and/or 3'-UTR) can be mutated, replaced and/or removed. For example, one or more nucleotides upstream of the start codon can be replaced with another nucleotide. The nucleotide or nucleotides to be replaced can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 nucleotides upstream of the start codon. As another example, one or more nucleotides upstream of the start codon can be removed from the UTR.

[0146] 3′-UTRs are known to have stretches of adenosines and uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF-a. Class III ARES are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3′-UTR of nucleic acid molecules can lead to HuR binding and thus, stabilization of the message *in vivo*.

[0147] In some aspects, introduction, removal, or modification of 3′-UTR AU rich elements (AREs) can be used to modulate the stability of a nucleic acid sequence. When engineering specific polynucleotides, one or more copies of an ARE can be introduced to make the nucleic acid sequence less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein.

Translation Enhancer Elements (TEEs)

[0148] In some aspects, a polynucleotide provided herein further comprises a translational enhancer element (TEE). As used herein, the term "**translational enhancer element**" refers to cis-acting sequences that increase the expression of a protein encoded by a nucleotide sequence. Non-

limiting examples of TEEs that can be used with the present disclosure are known in the art, *see, e.g.*, US20130177581A, which is incorporated herein by reference in its entirety.

[0149] In some aspects, the TEE is positioned between the transcription promoter and the start codon of a sequence. In some aspects, a TEE useful for the present disclosure has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% sequence identity with any of the TEEs provided in U.S. Publication Number US 20140147454, US20090226470, US20070048776, US20130177581, US20110124100, WO1999024595, WO2012009644, WO2009075886, WO2007025008, U.S. Pat. No. 6,310,197, U.S. Pat. No. 6,849,405, U.S. Pat. No. 7,456,273, U.S. Pat. No. 7,183,395, each of which is herein incorporated by reference in its entirety.

[0150] In some aspects, a polynucleotide provided herein comprises multiple TEEs. For example, in some aspects, a polynucleotide of the present disclosure (*e.g.*, encoding a payload and comprising a spleen detargeting sensor) comprises at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18 at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, or more than about 60 TEE sequences. In some aspects, the TEE sequences in the 5′UTR of the RNA (*e.g.*, modified RNA) are the same or different TEE sequences. In some aspects, the TEE sequences are in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

RNA Binding Proteins (RBPs)

[0151] In some aspects, a polynucleotide provided herein further comprises a sequence encoding a RNA binding protein. RNA binding proteins (RBPs) can regulate numerous aspects of co- and post-transcription gene expression such as, but not limited to, RNA splicing, localization, translation, turnover, polyadenylation, capping, modification, export and localization. RNA-binding domains (RBDs), such as, but not limited to, RNA recognition motif (RR) and hnRNP K-homology (KH) domains, typically regulate the sequence association between RBPs and their RNA targets (Ray *et al., Nature* 2013. 499:172-177; herein incorporated by reference in its entirety). In

some aspects, the canonical RBDs bind short RNA sequences. In some aspects, the canonical RBDs recognize RNA structure.

[0152] Non limiting examples of RNA binding proteins and related nucleic acid and protein sequences are described in US 2014/0147454, which is herein incorporated by reference in its entirety.

5′-Capping

[0153] In some aspects, a polynucleotide described herein further comprises a 5'-cap structure. The 5′ cap structure of a mRNA is involved in nuclear export, increasing mRNA stability and binds the mRNA Cap Binding Protein (CBP), which is responsible for mRNA stability in the cell and translation competency through the association of CBP with poly(A) binding protein to form the mature cyclic mRNA species. The cap further assists the removal of 5′ proximal introns removal during mRNA splicing.

[0154] Modifications to the RNA of the present disclosure can generate a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5′-ppp-5′ phosphorodiester linkages, modified nucleotides can be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, Mass.) can be used with α-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5′-ppp-5′ cap. Additional modified guanosine nucleotides can be used such as α-methyl-phosphonate and seleno-phosphate nucleotides.

[0155] Additional modifications include, but are not limited to, 2′-O-methylation of the ribose sugars of 5′-terminal and/or 5′-anteterminal nucleotides of the mRNA (as mentioned above) on the 2′-hydroxyl group of the sugar ring. Multiple distinct 5′-cap structures can be used to generate the 5′-cap of a nucleic acid molecule, such as an mRNA molecule.

[0156] Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (*i.e.*, endogenous, wild-type or physiological) 5′-caps in their chemical structure, while retaining cap function. Cap analogs can be chemically (*i.e.*, non-enzymatically) or enzymatically synthesized and/linked to a nucleic acid molecule.

[0157] For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5′-5′-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3′-O-methyl group (*i.e.*, N7,3′-O-dimethyl-guanosine-5′-triphosphate-5′-guanosine (m7G-3′ mppp-G; which can equivalently be designated 3′ O-Me-m7G(5′)ppp(5′)G). The 3′-O atom of the

other, unmodified, guanine becomes linked to the 5′-terminal nucleotide of the capped nucleic acid molecule (*e.g.,* an mRNA or mmRNA). The N7- and 3′-O-methylated guanine provides the terminal moiety of the capped nucleic acid molecule (*e.g.,* mRNA or mmRNA).

[0158] Another exemplary cap is mCAP, which is similar to ARCA but has a 2′-β-methyl group on guanosine (*i.e.*, N7,2′-O-dimethyl-guanosine-5′-triphosphate-5′-guanosine, m7Gm-ppp-G).

[0159] In some aspects, the cap is a dinucleotide cap analog. In some aspects, the dinucleotide cap analog is modified at different phosphate positions with a boranophosphate group or a phosphoroselenoate group such as the dinucleotide cap analogs described in U.S. Pat. No. 8,519,110, the contents of which are herein incorporated by reference in its entirety.

[0160] In some aspects, the cap is a cap analog is a N7-(4-chlorophenoxyethyl) substituted dicucleotide form of a cap analog known in the art and/or described herein. Non-limiting examples of a N7-(4-chlorophenoxyethyl) substituted dinucleotide form of a cap analog include a N7-(4-chlorophenoxyethyl)-G(5′)ppp(5′)G and a N7-(4-chlorophenoxyethyl)-m3′-OG(5′)ppp(5′)G cap analog (See *e.g.,* the various cap analogs and the methods of synthesizing cap analogs described in Kore et al. Bioorganic & Medicinal Chemistry 2013 21:4570-4574; the contents of which are herein incorporated by reference in its entirety). In some aspects, a cap analog of the present disclosure is a 4-chloro/bromophenoxyethyl analog.

[0161] While cap analogs allow for the concomitant capping of a nucleic acid molecule in an *in vitro* transcription reaction, up to about 20% of transcripts remain uncapped. This, as well as the structural differences of a cap analog from an endogenous 5′-cap structures of nucleic acids produced by the endogenous, cellular transcription machinery, can lead to reduced translational competency and reduced cellular stability.

[0162] In some aspects, providing an RNA with a 5′-cap or 5′-cap analog is achieved by in vitro transcription of a DNA template in the presence of said 5′-cap or 5′-cap analog, wherein said 5′-cap is co-transcriptionally incorporated into the generated RNA strand,

[0163] In some aspects, RNA can be generated, for example, by in vitro transcription, and the 5′-cap can be attached to the RNA post-transcriptionally using capping enzymes, for example, capping enzymes of vaccinia virus. In some aspects, the nucleotide sequence encoding IL-12 is capped post-transcriptionally, using enzymes, in order to generate more authentic 5′-cap structures. As used herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an endogenous, wild-type, natural or physiological cellular function

and/or structure as compared to synthetic features or analogs, etc., of the prior art, or which outperforms the corresponding endogenous, wild-type, natural or physiological feature in one or more respects. Non-limiting examples of more authentic 5′ cap structures of the present disclosure are those which, among other things, have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5′ endonucleases and/or reduced 5′ decapping, as compared to synthetic 5′ cap structures known in the art (or to a wild-type, natural or physiological 5′ cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2′-O-methyltransferase enzyme can create a canonical 5′-5′-triphosphate linkage between the 5′-terminal nucleotide of an mRNA and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5′-terminal nucleotide of the mRNA contains a 2′-O-methyl. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, *e.g.,* to other 5′ cap analog structures known in the art. Cap structures include 7mG(5′)ppp(5′)N,pN2p, 7mG(5′)ppp(5′)NlmpNp, 7mG(5′)-ppp(5′)NlmpN2 mp and m(7)Gpppm(3)(6,6,2′)Apm(2′)Apm(2′)Cpm(2)(3,2′)Up.

[0164] In some aspects, 5′ terminal caps include endogenous caps or cap analogs. In some aspects, a 5′ terminal cap comprises a guanine analog. Useful guanine analogs include inosine, N1-methyl-guanosine, 2′ fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[0165] In some aspects, the 5' cap comprises a 5' to 5' triphosphate linkage. In some aspects, the 5' cap comprises a 5' to 5' triphosphate linkage including thiophosphate modification. In some aspects, the 5' cap comprises a 2´-O or 3´-O-ribose-methylated nucleotide. In some aspects, the 5' cap comprises a modified guanosine nucleotide or modified adenosine nucleotide. In some aspects, the 5' cap comprises 7- methylguanylate. Exemplary cap structures include m7G(5')ppp(5')G, m7,2`O-mG(5')ppSp(5')G, m7G(5')ppp(5')2`O-mG, and m7,3`O-mG(5')ppp(5')2`O-mA.

[0166] In some aspects, a polynucleotide described herein comprises a modified 5′ cap. A modification on the 5′ cap can increase the stability of mRNA, increase the half-life of the mRNA, and could increase the mRNA translational efficiency. In some aspects, the modified 5′ cap comprises one or more of the following modifications: modification at the 2′ and/or 3′ position of a capped guanosine triphosphate (GTP), a replacement of the sugar ring oxygen (that produced the carbocyclic ring) with a methylene moiety (CH2), a modification at the triphosphate bridge moiety of the cap structure, or a modification at the nucleobase (G) moiety.

[0167] The 5′ cap structure that can be modified includes, but is not limited to, the caps described in U.S. Application No. 2014/0147454 and WO2018/160540 which is incorporated herein by reference in its entirety.

IRES Sequence

[0168] In some aspects, a polynucleotide provided herein comprises an internal ribosome entry site (IRES). First identified as a feature Picorna virus RNA, IRES plays an important role in initiating protein synthesis in absence of the 5′ cap structure. An IRES can act as the sole ribosome binding site, or can serve as one of multiple ribosome binding sites of an mRNA. Nucleic acids or mRNA containing more than one functional ribosome binding site can encode several peptides or polypeptides that are translated independently by the ribosomes ("multicistronic nucleic acid molecules"). When nucleic acids or mRNA are provided with an IRES, further optionally provided is a second translatable region. Examples of IRES sequences that can be used according to the disclosure include without limitation, those from picornaviruses (*e.g.,* FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV).

Poly-A Tails

[0169] In some aspects, a polynucleotide provided herein comprises a poly-A tail.

[0170] In some aspects, the length of the poly-A tail is greater than about 30 nucleotides in length. In some aspects, the poly-A tail is greater than about 35 nucleotides in length. In some aspects, the length is at least about 40 nucleotides. In some aspects, the length is at least about 45 nucleotides. In some aspects, the length is at least about 55 nucleotides. In some aspects, the length is at least about 60 nucleotides. In some aspects, the length is at least 70 nucleotides. In some aspects, the length is at least about 80 nucleotides. In some aspects, the length is at least about 90 nucleotides. In some aspects, the length is at least about 100 nucleotides. In some aspects, the length is at least about 120 nucleotides. In some aspects, the length is at least about 140 nucleotides. In some aspects, the length is at least about 160 nucleotides. In some aspects, the length is at least about 180 nucleotides. In some aspects, the length is at least about 200 nucleotides. In some aspects, the length is at least about 250 nucleotides. In some aspects, the length is at least about 300 nucleotides. In some aspects, the length is at least about 350 nucleotides. In some aspects, the length is at least about 400 nucleotides. In some aspects, the length is at least about 450 nucleotides. In some aspects, the length is at least about 500 nucleotides. In some aspects, the length is at least

about 600 nucleotides. In some aspects, the length is at least about 700 nucleotides. In some aspects, the length is at least about 800 nucleotides. In some aspects, the length is at least about 900 nucleotides. In some aspects, the length is at least about 1000 nucleotides. In some aspects, the length is at least about 1100 nucleotides. In some aspects, the length is at least about 1200 nucleotides. In some aspects, the length is at least about 1300 nucleotides. In some aspects, the length is at least about 1400 nucleotides. In some aspects, the length is at least about 1500 nucleotides. In some aspects, the length is at least about 1600 nucleotides. In some aspects, the length is at least about 1700 nucleotides. In some aspects, the length is at least about 1800 nucleotides. In some aspects, the length is at least about 1900 nucleotides. In some aspects, the length is at least about 2000 nucleotides. In some aspects, the length is at least about 2500 nucleotides. In some aspects, the length is at least about 3000 nucleotides.

[0171] In some aspects, the poly-A tail comprises a polyA-G quartet. The G-quartet is a cyclic hydrogen bonded array of four guanine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In some aspects, the G-quartet is incorporated at the end of the poly-A tail. The resultant nucleic acid or mRNA can be assayed for stability, protein production and other parameters including half-life at various time points. It has been discovered that the polyA-G quartet results in protein production equivalent to at least 75% of that seen using a poly-A tail of 120 nucleotides alone.

Modified Nucleosides

[0172] In some aspects, a polynucleotide provided herein comprises one or more modified nucleosides. In some aspects, the one or more modified nucleosides comprises 6-aza-cytidine, 2-thio-cytidine, α-thio-cytidine, pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodo-uridine, N1-methyl-pseudouridine, 5,6-dihydrouridine, α-thio-uridine, 4-thio-uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, pseudo-uridine, inosine, α-thio-guanosine, 8-oxo-guanosine, O6-methyl-guanosine, 7-deaza-guanosine, N1-methyl adenosine, 2-amino-6-chloro-purine, N6-methyl-2-amino-purine, 6-chloro-purine, N6-methyl-adenosine, α-thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine, pyrrolo-cytidine, 5-methyl-cytidine, N4-acetyl-cytidine, 5-methyl-uridine, 5-iodo-cytidine, and combinations thereof.

[0173] In some aspects, a polynucleotide provided herein comprises one or more uridines which have been replaced by a modified nucleoside. In some aspects, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ) or 5-methyl-uridine (m5U).

[0174] In some aspects, a polynucleotide provided herein further comprises a spacer sequence. Accordingly, in some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload, (b) a second region comprising an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor), and (c) a spacer sequence. In some aspects, the spacer sequence comprises the nucleotide sequence GCGGCCGCTAAA (SEQ ID NO: 7). In some aspects, the spacer sequence consists essentially of the nucleotide sequence GCGGCCGCTAAA (SEQ ID NO: 7). In some aspects, the spacer sequence consists of the nucleotide sequence GCGGCCGCTAAA (SEQ ID NO: 7). In some aspects, the spacer sequence is positioned upstream of the second region comprising an immune cell detargeting sensor. In some aspects, the spacer sequence is positioned in between the first region encoding a payload and the second region comprising an immune cell detargeting sensor.

III. Synthetic Circuits

[0175] Some aspects of the present disclosure relates to genetic circuits that comprise any of the polynucleotides described herein. As used herein, the term "**genetic circuit**" refers to a controllable gene expression system. As described herein, a genetic circuit useful for the present disclosure comprises a synthetic genetic circuit ("**synthetic circuit**"). As used herein, the term "**synthetic circuit**" refers to an engineered, non-natural genetic circuit. As is apparent from the present disclosure, synthetic circuits described herein have been specifically programmed to reduce or inhibit the expression of a payload within the spleen of a subject.

[0176] In some aspects, provided herein is a synthetic circuit comprising an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising an immune cell detargeting sensor (*e.g.*, a spleen detargeting sensor), wherein the recognition of the marker by the sensor reduces or inhibits the expression of the encoded payload. In some aspects, the synthetic circuit consists essentially of an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising an immune cell detargeting sensor (*e.g.*, a spleen detargeting sensor), wherein the recognition of the marker by the sensor reduces or inhibits the expression of the encoded payload. In some aspects, the synthetic circuit consists of an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising an immune cell detargeting sensor (*e.g.*, a spleen detargeting sensor), wherein the recognition of the marker by the sensor reduces or inhibits the expression of the encoded payload. Accordingly, in some aspects, a synthetic circuit useful for the present disclosure is single stranded.

[0177] As provided elsewhere in the present disclosure, in some aspects, a marker that is expressed by an immune cell and thereby, specifically recognized by an immune cell detargeting sensor comprises a microRNA. In some aspects, the immune cell is present within a lymphoid tissue, such as the spleen. In some aspects, a marker that is expressed in the spleen and thereby, specifically recognized by a spleen detargeting sensor described herein comprises a microRNA. Accordingly, in some aspects, a synthetic circuit described herein comprises an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising an immune cell detargeting sensor, which is capable of specifically recognizing a microRNA expressed in an immune cell. In some aspects, the payload is IL-12. In some aspects, provided herein is a synthetic circuit comprising an isolated polynucleotide, which comprises (a) a first region encoding an IL-12 protein and (b) a second region comprising an immune cell detargeting sensor, which is capable of specifically recognizing a microRNA expressed in an immune cell. In some aspects, the microRNA comprises miR-142. Accordingly, in some aspects, a synthetic circuit described herein comprises an isolated polynucleotide, which comprises (a) a first region encoding an IL-12 protein and (b) a second region comprising an immune cell detargeting sensor, which is capable of specifically recognizing a miR-142. As is apparent from the present disclosure, the recognition of the miR-142 by the immune cell detargeting sensor can result in reduced expression of the IL-12 protein by the immune cell.

[0178] As is apparent from the present disclosure, in some aspects, the immune cell is present within a lymphoid tissue. In some aspects, the lymphoid tissue comprises the spleen. Accordingly, in some aspects, a synthetic circuit comprises an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising a spleen detargeting sensor, which is capable of specifically recognizing a microRNA expressed in the spleen. In some aspects, the payload is IL-12. For such aspects, a synthetic circuit described herein can comprise an isolated polynucleotide, which comprises (a) a first region encoding IL-12 and (b) a second region comprising a spleen detargeting sensor, which is capable of specifically recognizing a microRNA expressed in the spleen. In some aspects, the microRNA comprises miR-142. Accordingly, in some aspects, a synthetic circuit comprises an isolated polynucleotide, which comprises (a) a first region encoding an IL-12 and (b) a second region comprising a spleen detargeting sensor, which is capable of specifically recognizing a miR-142. In some aspects, a synthetic circuit comprises an isolated polynucleotide, which comprises (a) a first region encoding an IL-12 and (b) a second region comprising a spleen detargeting sensor, which is capable of specifically recognizing a miR-142.

III. Replicons

[0179] Some aspects of the present disclosure relates to a replicon comprising any of the polynucleotides described herein. As used herein, the term "**replicon**" refers to a viral nucleic acid that is capable of directing the generation of copies of itself. Unless indicated otherwise, a replicon comprises a RNA or a DNA. Accordingly, in some aspects, a replicon useful for the present disclosure comprises an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising an immune cell detargeting sensor (*e.g.*, a spleen detargeting sensor), wherein the recognition of the marker by the sensor reduces the expression of the payload.

[0180] In some aspects, a replicon useful for the present disclosure is derived from an alpha virus. Alphaviruses comprise a set of genetically, structurally, and serologically related arthropod-borne viruses of the Togaviridae family. Twenty-six known viruses and virus subtypes have been classified within the alphavirus genus, including, Sindbis virus (SIN), Semliki Forest virus (SFV), Ross River virus (RRV), and Venezuelan equine encephalitis virus (VEE). In some aspects, the replicon is derived from VEE. Accordingly, some aspects of the present disclosure relates to a VEE replicon comprising any of the polynucleotides described herein. For instance, in some aspects, a VEE replicon provided herein comprises a polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising a spleen detargeting sensor, wherein the recognition of the marker by the sensor reduces the expression of the payload.

[0181] The VEE virus is a viral pathogen typically carried by mosquitos that causes VEE or encephalomyelitis predominately in equine species. Humans, however, can also contract VEE, and people with weakened immune systems are especially at risk of having severe complications if infected with VEE. The virion of VEE is spherical and possesses a lipid membrane with glycoprotein surface proteins spread around the outer surface. VEE has a genome of approximately 11.45 kb, excluding the 5′-terminal cap and 3′-terminal poly(A) tract, and comprises four nonstructural proteins (nsPs) and five structural proteins. The non-structural proteins include nsP1, nsP2, nsP3, and nsP4, while the structural region encodes proteins C, E3, E2, 6K, and E1. In some aspects, the self-amplifying replicon RNA is a WT replicon RNA derived from VEE. Exemplary VEE replicon sequences are provided in Table 4.

[0182] In some aspects, the VEE replicon useful for the present disclosure includes any VEE replicons known in the art. In some aspects, the VEE replicon comprises one or more mutations. For instance, in some aspects, the VEE replicon comprises mutations with the non-structural proteins nsP2 and nsP3, wherein the mutations promote subgenomic expression in

human cells. In some aspects, the VEE replicon comprises mutations that allow for greater expression of the encoded payload compared to a corresponding VEE replicon without the mutations. In some aspects, the VEE replicon comprises at least one point mutation in a nucleic acid position 3936 and/or 4758 of any one of the sequences provided in Table 4. In some aspects, the VEE replicon comprises at least one of the following point mutations: guanine to cytosine at position 3936 (G3936C) and adenine to guanine at position 4758 (A4758G) of any one of the sequences provided in Table 4. The G3936C mutation would result in a glycine to arginine change at amino acid residue 1309 (G1309R). The A4758G mutation would result in a serine to glycine change at amino acid residue 1583 (S1583G). Additional disclosure regarding such VEE replicons are provided, *e.g.*, in US20200281994A1, which is incorporated herein by reference in its entirety.

IV. Nanoparticles

[0183] In some aspects, the present disclosure relates to the delivery of a polynucleotide described herein (*e.g.*, encoding a payload and comprising an immune cell detargeting sensor), such that the expression of the payload is reduced or inhibited in an immune cell (*e.g.*, present within the spleen). In some aspects, the delivery can occur *in vivo* (*e.g.*, by administering a polynucleotide described herein to a subject) or *ex vivo* (*e.g.*, by culturing a polynucleotide described herein with the cells *in vitro*). In some aspects, delivery of a polynucleotide described herein can be performed using any suitable delivery system known in the art. In certain aspects, the delivery system is a vector. Accordingly, in some aspects, the present disclosure provides a vector comprising any of the polynucleotides described herein. Suitable vectors that can be used are known in the art. *See, e.g.*, Sung *et al.*, *Biomater Res* 23(8) (2019) which is incorporated herein by reference in its entirety.

[0184] In some aspects, a polynucleotide described herein is delivered using a nanoparticle (*e.g.*, lipid nanoparticle or lipid like nanoparticle). Accordingly, in some aspects, the present disclosure relates to a polynucleotide (*e.g.*, described herein) encapsulated within a nanoparticle, a composition comprising such a nanoparticle, and the use of such a nanoparticle. More specifically, in some aspects, provided herein is a nanoparticle comprising (i) any of the polynucleotides described herein and (ii) one or more types of nanoparticle components.

IV.A. Nanoparticle (NP)

[0185] A "**nanoparticle**" (NP), as used herein, refers to a particle, such as a vesicle, having characteristic dimensions measured in nanometers (nm). Nanoparticles can be used in methods by which pharmaceutical therapies are delivered to targeted locations. Non-limiting examples of NPs

include lipid nanoparticles (LNPs), lipid-like nanoparticles (LLNs), polymeric nanoparticles (PNPs), and inorganic nanoparticles.

IV.B. Lipid Nanoparticle (LNP)

[0186] A "**lipid nanoparticle**" (LNP), as used herein, refers to a nanoparticle composed of lipids. Lipid nanoparticles can be used in methods by which pharmaceutical therapies are delivered to targeted locations. Non-limiting examples of LNPs include cationic lipid nanoparticles, ionizable lipid nanoparticles, liposomes, bolaamphihiles, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and monolayer membrane structures (*e.g.,* archaeosomes and micelles).

[0187] As used herein a "**cationic lipid nanoparticle**" refers to a nanoparticle comprising a cationic lipid. As used herein an "**ionizable lipid nanoparticle**" refers to a nanoparticle comprising an ionizable lipid. In aspects of the disclosure, LNPs comprise one or more of the following lipids: a "**non-cationic helper lipid**," a "**phospholipid**," a "**sterol other structural lipid**," and a "**PEG/pegylated lipid**."

[0188] Exemplary LNPs comprise one or more of the following components:

[0189] (i) an ionizable/cationic lipid;

[0190] (ii) phospholipid or a non-cationic helper lipid;

[0191] (iii) a sterol or other structural lipid;

[0192] (iv) a PEG/PEGylated lipid and

[0193] (v) a targeted delivery molecule (lipid composition/targeting ligand).

IV.C. Lipid Like Nanoparticle (LLN)

[0194] A "**lipid like nanoparticle**" (LLN), as used herein, refers to a nanoparticle comprising a lipid, and a lipid-like material or a lipidoid, as described herein. In aspects of the disclosure, LLNs comprise one or more of the following lipids: a "**non-cationic helper lipid**," a "**phospholipid**," a "**sterol other structural lipid**", and a "**PEG/PEGylated lipid**." Lipid like nanoparticles can be used in methods by which pharmaceutical therapies are delivered to targeted locations.

[0195] Exemplary LLNs comprise one or more of the following components:

[0196] (i) an ionizable/cationic lipid-like material or lipidoid;

[0197] (ii) phospholipid or a non-cationic helper lipid;

[0198] (iii) a sterol or other structural lipid;

[0199] (iv) a PEG/PEGylated lipid; and

[0200] (v) a targeted delivery molecule (lipid composition/targeting ligand).

IV.D. Lipids or Lipid-Like Material

Ionizable lipid

[0201] Non-limiting examples of ionizable lipids include: Non-limiting examples of ionizable lipids include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315), heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (SM-102), heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Lipid 5), di((*Z*)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), 3-(didodecylamino)-N1,N1,4 tridodecyl-1-piperazineethanamine (KL10), Nl-[2 (didodecylamino)ethyl]-N1,N4,N4-tridodecyl 1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2 dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), or any combination thereof.

Cationic lipid

[0202] Non-limiting examples of a cationic lipid include: l,2-dioleoyl-3 -trimethylammonium-propane (DOTAP), lipofectamine, N-[l-(2,3- dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), l-[2- (oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTEVI), 2,3- dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l ,2-dimyristyloxyprop-3 -yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DMRIE), N-(l,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DORIE), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), l,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), l,2-distearoyl-3-trimethylammonium-propane (DSTAP), l,2-dipalmitoyl-3 -trimethylammonium-propane (DPTAP), l,2-dilinoleoyl-3 -trimethylammonium-propane (DLTAP), l,2-dimyristoyl-3-

trimethylammonium-propane (DMTAP), 1,2-distearoyl -sn-glycero-3- ethylphosphocholine (DSePC), 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC), 1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC), 1,2-dioleoyl-sn- glycero-3-ethylphosphocholine (DOePC), 1,2-di-(9Z-tetradecenoyl)-sn-glycero-3- ethylphosphocholine (14: 1 EPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18: 1 EPC), or any combination thereof.

[0203] In some aspects of the disclosure, LNPs primarily comprise cationic lipids along with other lipid ingredients. These typically include other lipid molecules belonging but not limited to the phophatidylcholine (PC) class (*e.g.,* 1,s-Distearoyl-sn-glycero-3-phophocholine (DSPC), and 1,2-Dioleoyl-sn-glycero-3-phophoethanolamine (DOPE), sterols (*e.g.,* cholesterol) and Polyethylene glycol (PEG)-lipid conjugates (*e.g.,* 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-2000 (DSPE-PEG2000), and C14-PEG2000.

DOTAP

[0204] In aspects of the disclosure, the catonic lipid is DOTAP. DOTAP can be used for the highly efficient transfection of DNA including yeast artificial chromosomes (YACs) into eukaryotic cells for transient or stable gene expression, and is also suitable for the efficient transfer of other negatively charged molecules, such as RNA, oligonucleotides, nucleotides, ribonucleoprotein (RNP) complexes, and proteins into research samples of mammalian cells.

Lipofectamine

[0205] In aspects of the disclosure, the catonic lipid is lipofectamine. Lipofectamine, as used herein, is a common transfection reagent, produced and sold by Invitrogen, used in molecular and cellular biology. It is used to increase the transfection efficiency of RNA (including mRNA and siRNA) or plasmid DNA into in vitro cell cultures by lipofection. Lipofectamine contains lipid subunits that can form liposomes or lipid nanoparticles in an aqueous environment, which entrap the transfection payload, *e.g.,* modRNA. The RNA-containing liposomes (positively charged on their surface) can fuse with the negatively charged plasma membrane of living cells, due to the neutral co-lipid mediating fusion of the liposome with the cell membrane, allowing nucleic acid cargo molecules to cross into the cytoplasm for replication or expression.

Lipid-like material or lipidoid

[0206] Unless indicated otherwise, "**lipid-like material**" and "**lipidoid**" can be used interchangeably. Non-limiting examples of lipid-like materials and/or lipidoids include: 1,1′-((2-(4-(2-((2-(bis(2-hydroxydodecyl) amino)ethyl) (2- hydroxydodecyl)amino)ethyl) piperazin-1-yl)ethyl)azanediyl) bis(dodecan-2-ol) (C12-200), 3,6-bis(4-(bis(2-

hydroxydodecyl)amino)butyl)piperazine2,5-dione (cKK-E12), tetrakis(8-methylnonyl) 3,3′,3″,3‴-(((methylazanediyl) bis(propane-3,1 diyl))bis (azanetriyl))tetrapropionate (306Oi$_{10}$), G0-C14, 5A2-SC8, 3,6-bis(4-(bis((9Z,12Z)-2-hydroxyoctadeca9,12-dien-1-yl)amino)butyl)piperazine-2,5-dione (OF-02), (((3,6-dioxopiperazine-2,5-diyl)bis (butane-4,1-diyl))bis(azanetriyl))tetrakis(ethane2,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-Deg-Lin), (((3,6-dioxopiperazine-2,5-diyl)bis(butane-4,1-diyl)) bis(azanetriyl))tetrakis (butane-4,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-C4-Deg-Lin), N1,N3,N5-tris(3-(didodecylamino)propyl)benzene1,3,5-tricarboxamide (TT3), Hexa(octan-3-yl) 9,9′,9″,9‴,9⁗,9⁗′-((((benzene-1,3,5-tricarbonyl)ris(azanediyl)) tris (propane-3,1-diyl))tris(azanetriyl))hexanonanoate (FTT5), PL-1 [disclosed in Nature Communications, 12-7264 (2021), which is is hereby incorporated by reference], 98N12-5 [disclosed in Molecular Therapy vol. 17 no. 5 May 2009, which is hereby incorporated by reference], ethyl 5,5-di((Z)-heptadec-8-en-1-yl)-1-(3-(pyrrolidin-1-yl)propyl)-2,5-dihydro-1H-imidazole-2-carboxylate (A2-Iso5-2DC18 (A2)) and A12-Iso5−2DC18 (A12), or any combination thereof.

[0207] TT3, as used herein, is capable of forming nanoparticles for delivery of various biologic active agents into the cells. In addition, the present disclosure also demonstrates that an unloaded TT3-LLN can induce immunogenic cell death (ICD) in cancer cells *in vivo* and *in vitro*. Immunogenic cell death, as described herein, refers to a form of cell death that can induce an effective immune response through activation of dendritic cells (DCs) and consequent activation of specific T cell response. In some aspects of the disclosure, the cells that undergo immunogenic cell death are tumor cells. Immunogenic tumor cell death can trigger an effective anti-tumor immune response.

[0208] In some aspects, the lipidoid is TT3.

Phospholipid, or other non-cationic helper lipid

[0209] Unless indicated otherwise, "**phospholipid**" and "**other non-cationic helper lipid**" can be used interchangeably and non-limiting examples include: 1,2-dilinoleoyl-sn-glycero-3 phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-

dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, and any combinations thereof.

[0210] In some aspects, the phospholipid is selected from the group consisting of 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (14:0-16:0 PC, MPPC), 1-myristoyl-2 stearoyl-sn-glycero-3-phosphocholine (14:0-18:0 PC, MSPC), 1-palmitoyl 2-acetyl-sn-glycero-3-phosphocholine (16:0-02:0 PC), 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine (16:0-14:0 PC, PMPC), 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (16:0-18:0 PC, PSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18: 1 PC, POPC), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (16:0-18:2 PC, PLPC), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (16:0-20:4 PC), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (14:0-22:6 PC), 1-stearoyl-2-myristoyl-sn-glycero-3-phosphocholine (18:0-14:0 PC, SMPC), 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18:0-16:0 PC, SPPC), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (18:0-18: 1 PC, SOPC), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (18:0-18:2 PC), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (18:0-20:4 PC), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (18:0-22:6 PC), 1-oleoyl-2-myristoyl-sn-glycero-3-phosphocholine (18: 1-14:0 PC, OMPC), 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18: 1-16:0 PC, OPPC), 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (18: 1-18:0 PC, OSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (16:0- 18: 1 PE, POPE), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (16:0-18:2 PE), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (16:0-20:4 PE), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (16:0-22:6 PE), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (18:0-18: 1 PE), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (18:0-18:2 PE), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (18:0-20:4 PE), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (18:0-22:6 PE), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), and any combination thereof.

[0211] In some aspects, the phospholipid is DSPC. In some aspects, the phospholipid is DOPE.

Sterol or other structural lipid

[0212] As used herein, a "**sterol or other structural lipid**," refers to cholesterol or cholesterol analogs that could be used to fill lipid membrane packing defects and provide structural integrity.

[0213] Non-limiting examples of sterols include: a cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and combinations thereof. In some aspects, the sterol is cholesterol.

PEG/Pegylated lipid

[0214] As used herein, a "**PEG lipid**" and a "**pegylated lipid**" are used interchangeably.

[0215] Non-limiting examples of PEG lipids include: 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159), 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (PEG-DSPE), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA). In some aspects, the lipid moiety of the PEG-lipids includes those having lengths of from about C14 to about C22.

[0216] In some aspects, the PEG moiety has a size of about 1000, 2000, 5000, 10,000, 15,000 or 20,000 daltons. In certain aspects, C14-PEG2000 comprises 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DMPE-PEG2000), or both.

[0217] In some aspects, the PEG-lipids can be embedded in the LNP prior to the encapsulation of the polynucleotide. In some aspects, the PEG lipids (or other lipid ingredients disclosed herein) can be added to the LNP after the encapsulation of the polynucleotide. For example, in some aspects, a polynucleotide described herein is encapsulated in the LNP, and then the PEG lipid (other lipid ingredients disclosed herein) is attached to the LNP using, *e.g.*, micelles.

[0218] In some aspects, the nanoparticle does not comprise any pegylated lipid. In some aspects, the lipid nanoparticle does not comprise any pegylated lipid.

Targeted delivery molecule (lipid composition/targeting ligand)

[0219] In aspects of the disclosure, nanoparticles, (*e.g.*, LNPs and LLNs), as described herein, comprise a targeted delivery molecule (lipid composition/targeting ligand). As used herein, a "**targeted delivery molecule (lipid composition/targeting ligand)**," and a "**targeted delivery**

molecule" are used interchangeably and in some aspects, the targeted delivery molecule could be an additional lipid or lipid like component, as described herein. In some aspects, the targeted delivery molecule could change the overall charge of the nanoparticles. In some aspects, the targeted delivery molecule could be a non-covalently or covalently bound ligand to the nanoparticle. In some aspects, the targeted delivery ligand could be a small molecule or a large molecule.

[0220] Non-limiting examples of targeted delivery molecules are disclosed in Pharmaceuticals (Basel). Jul 20;15(7):897. (2022), Nat Rev Drug Discov 20, 101–124 (2021), and Advanced Drug Delivery Reviews, Volume 188, (2022), which are hereby incorporated by reference. Non-limiting examples of targeted delivery molecules include: 1,2-dioleoyl-3 - trimethylammonium-propane (DOTAP), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), 1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt) (18:1 PA), 1,2-dimyristoyl-sn-glycero-3-phosphate (sodium salt) (14:0 PA), bis(monooleoylglycero)phosphate (S,R Isomer) (ammonium salt) (18BMP), 2-((2,3-bis(oleoyloxy)propyl)dimethylammonio)ethyl ethyl phosphate (DOCPe), folic acid, N-acetylgalactosamine (GalNAc), anti-CD3 antibodies.

Molar Ratios

[0221] In some aspects, a nanoparticle described herein comprises a lipid (*e.g.*, an ionizable lipid, a cationic lipid, a non-cationic helper lipid, a phospholipid, a sterol or other structural lipid, or PEG lipid) and/or lipidoid, as described herein at a molar ratio of about 10% to about 50% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 10%, about 20%, about 30%, about 40%, or about 50% in the lipid composition.

[0222] In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 10% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 20% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 30% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 40% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 40% in the lipid and/or lipid like composition.

[0223] In some aspects, a nanoparticle described herein comprises a pegylated lipid at a molar ratio of about 0% to about 10% in the lipid and/or lipid like composition. In some aspects, a lipid nanoparticle comprises a pegylated lipid at a molar ratio of about 0%, about 0.25%, about 0.5%, about 0.75%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% in the lipid and/or lipid like composition.

[0224] In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 0.25% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 0.5% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 0.75% in lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 1.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 2.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 3.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 4.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 5.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 6.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 7.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 8.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 9.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 10.0% in the lipid and/or lipid like composition.

[0225] For example, in some aspects, the pegylated lipid comprises C14-PEG2000. In some aspects, the C14-PEG2000 is present in the lipid nanoparticle at a molar ratio of about 0.25% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 0.5% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 0.75% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 1% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 2% in the lipid and/or lipid

like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 3% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 4% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 5% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 6% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 7% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 8% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 9% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 10% in the lipid and/or lipid like composition.

Particle Size

[0226] Particle size of nanoparticles can affect drug release rate, bio-distribution, mucoadhesion, cellular uptake of water and buffer exchange to the interior of the nanoparticles, and protein diffusion. In some aspects of the disclosure, the diameter of the NPs ranges from about 30 to about 500 nm. In some aspects of the disclosure, the diameter of the NPs ranges from about 30 to about 500 nm, about 50 to about 400 nm, about 70 to about 300 nm, about 100 to about 200 nm, about 100 to about 175 nm, or about 100 to about 160 nm. In some aspects of the disclosure, the diameter of the NPs ranges from 100-160 nm. In some aspects of the disclosure, the diameter of the NPs can be about 30 nm, about 40 nm, about 50 nm, about 60 nm, about 70 nm, about 80 nm, about 90 nm, about 100 nm, about 101 nm, about 102 nm, about 103 nm, about 104 nm, about 105 nm, about 106 nm, about 107 nm, about 108 nm, about 109 nm, about 110 nm, about 111 nm, about 112 nm, about 113 nm, about 114 nm, about 115 nm, about 116 nm, about 117 nm, about 118 nm, about 119 nm, about 120 nm., about 130 nm, about 140 nm, about 150 nm, or about 160 nm. In certain aspects, the lipid nanoparticle has a diameter of about 140 nm.

Zeta Potential

[0227] As used herein, "**zeta potential**" refers to the measure of the effective electric charge on the nanoparticle surface. The magnitude of the zeta potential provides information about particle stability. In some aspects of the disclosure, the zeta potential of the nanoparticles ranges from about -20 to about 20 mv. In some aspects of the disclosure, the zeta potential of the NPs can be about -6.0 mv, about -5.9 mv, about -5.8 mv, about -5.7 mv, about -5.6 mv, about -5.5 mv, about -5.4 mv, about -5.3 mv, about -5.2 mv, about -5.1 mv, about -5.0 mv, about -4.9 mv, about -4.8 mv, about -4.7 mv, about -4.6 mv, about -4.5 mv, about -4.4 mv, about -4.3 mv, about -4.2 mv, about -4.1 mv, about -4.0 mv, about -3.9 mv, about -3.8 mv, about -3.7 mv, about -3.6 mv, about -

3.5 mv, about -3.4 mv, about -3.3 mv, about -3.2 mv, about -3.1 mv, about -3.0 mv, about -2.9 mv, about -2.8 mv, about -2.7 mv, about -2.6 mv, about -2.5 mv, about -2.4 mv, about -2.3 mv, about -2.2 mv, about -2.1 mv, about -2.0 mv, about -1.9 mv, about -1.8 mv, about -1.7 mv, about -1.6 mv, about -1.5 mv, about -1.4 mv, about -1.3 mv, about -1.2 mv, about -1.1 mv, about -1.0 mv, about -0.9 mv, about -0.8 mv, about -0.7 mv, about -0.6 mv, about -0.5 mv, about -0.4 mv, about -0.3 mv, about -0.2 mv, about -0.1 mv, about 0.0 mv, 0.1 about mv, about 0.2 mv, about 0.3 mv, about 0.4 mv, about 0.5 mv, about 0.6 mv, about 0.7 mv, about 0.8 mv, about 0.9 mv, about 1.0 mv, about 1.1 mv, about 1.2 mv, about 1.3 mv, about 1.4 mv, about 1.5 mv, about 1.6 mv, about 1.7 mv, about 1.8 mv, about 1.9 mv, about 2.0 mv, about 2.1 mv, about 2.2 mv, about 2.3 mv, about 2.4 mv, about 2.5 mv, about 2.6 mv, about 2.7 mv, about 2.8 mv, about 2.9 mv, about 3.0 mv, about 3.1 mv, about 3.2 mv, about 3.3 mv, about 3.4 mv, about 3.5 mv, about 3.6 mv, about 3.7 mv, about 3.8 mv, about 3.9 mv, about 4.0 mv, about 4.1 mv, about 4.2 mv, about 4.3 mv, about 4.4 mv, about 4.5 mv, about 4.6 mv, about 4.7 mv, about 4.8 mv, about 4.9 mv, about 5.0 mv, about 5.1 mv, about 5.2 mv, about 5.3 mv, about 5.4 mv, about 5.5 mv, about 5.6 mv, about 5.7 mv, about 5.8 mv, about 5.9 mv, or about 6.0 mv.

Mass Ratio

[0228] In some aspects, the mass ratio between the lipid of the LNPs or the LLN and the polynucleotide ranges from about 1:2 to about 15:1. In some aspects, the mass ratio between the lipid and the polynucleotide can be about 1:2, about 1:1.9, about 1:1.8, about 1:1.7, about 1:1.6, about 1:1.5, about 1:1.4, about 1:1.3, about 1:1.2, about 1:1.1, about 1:1, about 1.1:1, about 1.2:1, about 1.3:1, about 1.4:1, about 1.5:1, about 1.6:1, about 1.7:1, about 1.8:1, about 1.9:1, about 2:1, about 2.5:1, about 3:1, about 3.5:1, about 4:1, about 4.5:1, about 5:1, about 5.5:1, about 6:1, about 6.5:1, about 7:1, about 7.5:1, about 8:1, about 8.5:1, about 9:1, about 9.5:1, about 10:1, about 10.5:1, about 11:1, about 11.5:1, about 12:1, about 12.5:1, about 13:1, about 13.5:1, about 14:1, about 14.5:1, or about 15:1. In some aspects of the disclosure, the mass ratio between the lipid and the polynucleotide is about 10:1.

V. Pharmaceutical Composition

[0229] In some aspects, the disclosure relates to a pharmaceutical composition comprising any of the polynucleotides, replicons, and/or nanoparticles described herein. In some aspects, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier (excipient). "**Acceptable**", as used herein, means that the carrier must be compatible with the active ingredient of the composition and not deleterious to the subject to be treated. In some aspects, the carrier is

capable of stabilizing the active ingredient. Pharmaceutically acceptable excipients (carriers) include buffers, which are well known in the art. *See, e.g.*, Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkoins, Ed. K. E. Hoover.

[0230] The pharmaceutical compositions to be used for *in vivo* administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. The nanoparticles can be placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0231] In some aspects, the pharmaceutical composition can be formulated for intratumoral, intrathecal, intramuscular, intravenous, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration. In some aspects of the disclosure, the pharmaceutical composition can be formulated for intratumoral injection. Intratumoral injection, as used herein, refers to direct injections into the tumor. A high concentration of composition can be achieved in situ, while using small amounts of drugs. Local delivery of immunotherapies allows multiple combination therapies, while preventing significant system exposure and off-target toxicities.

[0232] In some aspects, the pharmaceutical composition can be formulated for intramuscular injection, intravenous injection, or subcutaneous injection.

[0233] In some aspects, the pharmaceutical composition comprises pharmaceutically acceptable carriers, buffer agents, excipients, salts, or stabilizers in the form of lyophilized formulations or aqueous solutions. *See, e.g.,* Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover. Acceptable carriers and excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used, and comprises buffers such as phosphate, citrate, and other organic acds; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl, or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose

or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.,* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

[0234] In some aspects, the pharmaceutical composition described herein can be prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl. Acad. Sci. USA 77:4030 (1980); and U.S. Pat. Nos 4,485,045 and 4,544,545, which are hereby incorporated by reference in their entirety. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556, which is hereby incorporated by reference in its entirety. In some aspects, liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

[0235] In some aspects, the pharmaceutical composition is formulated in sustained-release format. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the nanoparticles which matrices are in the form of shaped articles, *e.g.*, films or microcapsules. Examples of sustained-release matrices include, but are not limited to, polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPROM DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[0236] In some aspects, suitable surface-active agents include, but are not limited to, non-ionic agents, such as polyoxyethylenesorbitans (*e.g.,* TWEEN™ 20, 40, 60, 80 or 85) and other sorbitans (*e.g.,* SPAN™ 20, 30, 60, 80, or 85). In some aspects, compositions with a surface-active agent comprise between 0.05 and 5% surface-active agent. In some aspects the composition comprises 0.1 and 2.5%. It will be appreciated that other ingredients can be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

[0237] In some aspects, the pharmaceutical composition is in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral, or rectal administration, or administration by inhalation or insufflation.

[0238] For preparing solid compositions such as tablets, the principal active ingredient can be mixed with a pharmaceutical carrier, *e.g.,* conventional tableting ingredients such as corn starch,

lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, *e.g.*, water, to form a solid preformulation composition containing a homogenous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition can be readily subdivided into equally effective unit dosage forms such as tablets, pills, and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from about 0.1 to about 500 mg of the active ingredient of the present disclosure. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[0239] Suitable emulsions can be prepared using commercially available fat emulsions, such as INTRALIPID™, LIPOSYN™, INFONUTROL™, LIPOFUNDIN™, and LIPIPHYSAN™. The active ingredient can be either dissolved in a pre-mixed emulsion composition or alternatively it can be dissolved in an oil (*e.g.,* soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil, or almond oil) and an emulsion formed upon mixing with a phospholipid (*e.g.,* egg phospholipids, soybean phospholipids, or soybean lecithin) and water. It will be appreciated that other ingredients can be added, for example glycerol or glucose, to adjust tonicity of the emulsion. Suitable emulsions will typically contain up to about 20% oil, for example, between about 5 and about 20%. The fat emulsion can comprise fat droplets having a suitable size and can have a pH in the range of about 5.5 to about 8.0.

[0240] Pharmaceutical compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions can contain suitable pharmaceutically acceptable excipients as set out above. In some aspects, the composition is administered by the oral or nasal respiratory route for local or systemic effect.

[0241] Compositions in pharmaceutically acceptable solvents can be nebulized by use of gases. Nebulized solutions can be breathed directly from the nebulizing device or the nebulizing

device can be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions can be administered from devices which deliver the formulation in an appropriate manner.

VI. Therapeutic Applications

VI.A. Selective Expression

[0242] Some aspects of the present disclosure relates to methods of inducing selective expression of a payload by an immune cell (*e.g.*, present within a lymphoid tissue). As described herein, the polynucleotides described herein comprise an immune cell detargeting sensor, such that when the polynucleotides are administered to a subject, the expression of the encoded payload by the immune cell is reduced or inhibited.

[0243] Accordingly, some aspects of the present disclosure are related to a method of reducing or avoiding the expression of a payload within an immune cell of a subject in need thereof, comprising administering to the subject any of the isolated polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein. As described herein, in some aspects, the immune cell is present within a lymphoid tissue, such as the spleen. In some aspects, provided herein is a method of reducing or avoiding the expression of a payload (payload expression) within the spleen of a subject in need thereof, comprising administering to the subject any of the isolated polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein.

[0244] In some aspects, the expression of the payload within the immune cell is avoided entirely (*i.e.*, the payload is not expressed in the immune cell after the administration). In some aspects, the expression of the payload in the immune cell is reduced as compared to a non-immune cell within the subject. For instance, in some aspects, after administration, the expression of the payload in the immune cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding expression in the non-immune cell.

[0245] As demonstrated herein, in some aspects, reduced expression of the payload within an immune cell can be associated with an overall decreased expression of the payload within the subject. Not to be bound by any one theory, in some aspects, by reducing the expression of the payload within the immune cell present within a lymphoid tissue (*e.g.*, spleen), the expression of the payload in non-lymphoid tissues (*e.g.*, other non-spleen tissues) can be reduced. For example,

as further described elsewhere in the present disclosure, in some aspects, a payload comprises an IL-12 protein. IL-12 is a secretory protein that when produced (*e.g.*, by immune cells within the spleen) is secreted into the periphery (*e.g.*, in the blood). Accordingly, it will be apparent to those skilled in the art that a reduced IL-12 expression within the spleen can be associated with a reduced IL-12 expression in other non-spleen tissues (*e.g.*, blood).

[0246] In some aspects, the reduced expression of the payload can be associated with decreased toxicity. Accordingly, in some aspects, the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions allow for greater tolerability with reduced toxicity when administered to a subject. Toxicity and/or tolerability can be assessed by any suitable methods known in the art. In some aspects, toxicity and/or tolerability can be assessed by monitoring a subject's body weight after administration, wherein reduced toxicity and/or increased tolerability is associated with reduced body weight loss.

[0247] As used herein, "**reducing the expression**" refers to (i) a decrease in the total amount of the payload that is expressed by immune cells (*e.g.*, present in a tissue (*e.g.*, spleen)); (ii) a decrease in how long (*i.e.*, duration) the payload is expressed by the immune cells (*e.g.*, present in a tissue (*e.g.*, spleen)); or (iii) both (i) and (ii).

[0248] Accordingly, in some aspects, after administration, the total amount of payload that is expressed by immune cells (*e.g.*, present in the spleen) is reduced as compared to non-immune cells. In some aspects, the total amount of payload that is expressed by the immune cells is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding amount expressed by the non-immune cells. In some aspects, the total amount of payload that is expressed by the immune cells is reduced compared to the total amount of the payload that is expressed by the corresponding immune cells of a reference subject. In some aspects, after the administration, the total amount of payload that is expressed by the immune cells is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding amount expressed by the immune cells of the reference subject. In some aspects, the reference subject is a subject who received a corresponding polynucleotide that lacks an immune cell detargeting sensor described herein.

[0249] As described herein, in some aspects, after administration, the payload is only transiently expressed by the immune cells (*e.g.*, present in the spleen), as compared to the

corresponding expression by non-immune cells and/or as compared to the corresponding expression by the immune cells of the reference subject. Accordingly, in some aspects, administering any of the polynucleotides, replicons, nanoparticles, and/or pharmaceutical compositions to a subject results in reduced duration of payload expression by the immune cells, as compared to non-immune cells. In some aspects, the duration of payload expression by the immune cells is decreased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding duration for non-immune cells. In some aspects, after administration, the duration of payload expression by the immune cells (*e.g.*, present in the spleen) of the subject is reduced, as compared to the duration of the payload expression by the immune cells of the reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell detargeting sensor). In some aspects, the duration of payload expression by the immune cells of the subject is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding duration for the immune cells of the reference subject.

[0250] In some aspects, after administration, both (i) the total amount of the payload that is expressed and (ii) the duration of payload expression is reduced in the immune cells as compared to non-immune cells. In some aspects, both (i) the total amount of the payload that is expressed and (ii) the duration of payload expression is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding value in the non-immune cells. In some aspects, after administration, both (i) the total amount of the payload that is expressed and (ii) the duration of payload expression is reduced in the immune cells as compared to the corresponding value for the immune cells of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell detargeting sensor). In some aspects, both (i) the total amount of the payload that is expressed and (ii) the duration of payload expression is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding value for the immune cells of the reference subject.

[0251] Some aspects of the present disclosure is directed to methods of selectively expressing a payload in a non-immune cell of a subject in need thereof. As described herein,

immune cells are predominantly present in lymphoid tissues (*e.g.*, spleen) and less present in non-lymphoid tissues. Accordingly, some aspects of the present disclosure is directed to methods of selectively expressing a payload in a non-lymphoid tissue of a subject in need thereof. In some aspects, such methods comprise administering to the subject any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein, wherein after the administration, the payload expression in a lymphoid tissue (*e.g.*, the spleen) is reduced, as compared to the corresponding expression in a non-lymphoid tissue. As used herein, "**selective expression**" or "**preferential expression**," or grammatical variants thereof, refers to an increased expression (*e.g.*, of a payload) in a first tissue (*e.g.*, non-lymphoid tissue) as compared to a second tissue (*e.g.*, lymphoid tissue, such as the spleen). In some aspects, the payload is selectively or preferentially expressed in a non-lymphoid tissue, where the expression (*e.g.*, total amount of the payload and/or duration of payload expression) in the non-lymphoid tissue is increased as compared to the corresponding expression in a lymphoid tissue (*e.g.*, the spleen). In some aspects, the expression of the payload in the non-lymphoid tissue is the same as the corresponding expression in other non-lymphoid tissues of the subject. In some aspects, the expression of the payload in the non-lymphoid tissue is increased as compared to the corresponding expression in other non-lymphoid tissues of the subject. In some aspects, the expression of the payload in the non-lymphoid tissue is decreased as compared to other non-lymphoid tissues in the subject but is increased compared to the corresponding expression in a lymphoid tissue (*e.g.*, the spleen).

VI.B. Methods of Treating

[0252] As is apparent from the present disclosure, the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein can be useful in treating a wide range of diseases or disorders. Accordingly, in some aspects, provided herein is a method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions provided herein. As described herein, after the administration, the payload is preferentially expressed by non-immune cells of a subject.

[0253] The polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions provided herein can be used to treat any suitable diseases or disorders known in the art. Non-limiting examples of diseases and disorders that can be treated include cancer, inflammatory disorders, monogenic disorders, neurological disorders, psychiatric disorders, or combinations thereof.

[0254] In some aspects, the disease or disorder comprises a cancer. Non-limiting examples of cancers include: a melanoma, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, large cell carcinoma, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine cancer, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, head and neck cancer, or combinations thereof. In some aspects, the breast cancer is triple-negative breast cancer.

[0255] In some aspects, any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions can be administered to the subject via intravenous, intratumoral, intrathecal, intramuscular, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration.

[0256] In some aspects, any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions can be administered to the subject in combination with one or more additional therapeutic agents. In some aspects, the additional therapeutic agent comprises an anti-cancer agent. Non-limiting examples of anti-cancer agents include: a chemotherapy, a radiation therapy, a surgical therapy, an immunotherapy, or combinations thereof. In some aspects, the chemotherapeutic agent is carboplatin, cisplatin, docetaxel, gemcitabine, nab-paclitaxel, pemetrexed, vinorelbine, or combinations thereof. In some aspects, the radiation therapy is ionizing radiation, gamma-radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, systemic radioactive isotopes, radiosensitizers, or combinations thereof. In some aspects, the surgical therapy is a curative surgery (*e.g.*, tumor removal surgery), a preventative surgery, a laparoscopic surgery, a laser surgery, or combinations thereof. In some aspects, the immunotherapy is adoptive cell transfer, therapeutic cancer vaccines, or combinations thereof.

[0257] In some aspects, the chemotherapeutic agent is platinating agents, such as carboplatin, oxaliplatin, cisplatin, nedaplatin, satraplatin, lobaplatin, triplatin, tetranitrate, picoplatin, prolindac, aroplatin and other derivatives; topoisomerase I inhibitors, such as camptothecin, topotecan, irinotecan/SN38, rubitecan, belotecan, and other derivatives; topoisomerase II inhibitors, such as etoposide (VP-16), daunorubicin, a doxorubicin agent (*e.g.*, doxorubicin, doxorubicin HCl, doxorubicin analogs, or doxorubicin and salts or analogs thereof in liposomes), mitoxantrone, aclarubicin, epirubicin, idarubicin, amrubicin, amsacrine, pirarubicin,

valrubicin, zorubicin, teniposide and other derivatives; antimetabolites, such as folic family (methotrexate, pemetrexed, raltitrexed, aminopterin, and relatives); purine antagonists (thioguanine, fludarabine, cladribine, 6-mercaptopurine, pentostatin, clofarabine and relatives) and pyrimidine antagonists (cytarabine, floxuridine, azacitidine, tegafur, carmofur, capacitabine, gemcitabine, hydroxyurea, 5-fluorouracil (5fu), and relatives); alkylating agents, such as nitrogen mustards (*e.g.,* cyclophosphamide, melphalan, chlorambucil, mechlorethamine, ifosfamide, trofosfamide, prednimustine, bendamustine, uramustine, estramustine, and relatives); nitrosoureas (*e.g.,* carmustine, lomustine, semustine, fotemustine, nimustine, ranimustine, streptozocin, and relatives); triazenes (*e.g.,* dacarbazine, altretamine, temozolomide, and relatives); alkyl sulphonates (*e.g.,* busulfan, mannosulfan, treosulfan, and relatives); procarbazine; mitobronitol, and aziridines (*e.g.,* carboquone, triaziquone, thiotepa, triethylenemalamine, and relatives); antibiotics, such as hydroxyurea, anthracyclines (*e.g.,* doxorubicin agent, daunorubicin, epirubicin and other derivatives); anthracenediones (*e.g.,* mitoxantrone and relatives); streptomyces family (*e.g.,* bleomycin, mitomycin c, actinomycin, plicamycin); ultraviolet light; and combinations thereof.

[0258] In some aspects, the additional therapeutic agent is an antibody. Antibodies (preferably monoclonal antibodies) achieve their therapeutic effect against cancer cells through various mechanisms. They can have direct effects in producing apoptosis or programmed cell death. They can block components of signal transduction pathways such as *e.g.,* growth factor receptors, effectively arresting proliferation of tumor cells. In cells that express monoclonal antibodies, they can bring about anti-idiotype antibody formation. Indirect effects include recruiting cells that have cytotoxicity, such as monocytes and macrophages. This type of antibody-mediated cell kill is called antibody-dependent cell mediated cytotoxicity (ADCC). Antibodies also bind complement, leading to direct cell toxicity, known as complement dependent cytotoxicity (CDC). Combining surgical methods with immunotherapeutic drugs or methods is an successful approach, as *e.g.,* demonstrated in Gadri et al. 2009: Synergistic effect of dendritic cell vaccination and anti-CD20 antibody treatment in the therapy of murine lymphoma. J Immunother. 32(4): 333-40. The following list provides some non-limiting examples of anti-cancer antibodies and potential antibody targets (in brackets) which can be used in combination with the present disclosure: Abagovomab (CA-125), Abciximab (CD41), Adecatumumab (EpCAM), Afutuzumab (CD20), Alacizumab pegol (VEGFR2), Altumomab pentetate (CEA), Amatuximab (MORAb-009), Anatumomab mafenatox (TAG-72), Apolizumab (HLA-DR), Arcitumomab (CEA), Bavituximab (phosphatidylserine), Bectumomab (CD22), Belimumab (BAFF), Bevacizumab (VEGF-A),

Bivatuzumab mertansine (CD44 v6), Blinatumomab (CD19), Brentuximab vedotin (CD30 TNFRSF8), Cantuzumab mertansin (mucin CanAg), Cantuzumab ravtansine (MUC1), Capromab pendetide (prostatic carcinoma cells), Carlumab (CNT0888), Catumaxomab (EpCAM, CD3), Cetuximab (EGFR), Citatuzumab bogatox (EpCAM), Cixutumumab (IGF-1 receptor), Claudiximab (Claudin), Clivatuzumab tetraxetan (MUC1), Conatumumab (TRAIL-R2), Dacetuzumab (CD40), Dalotuzumab (insulin-like growth factor I receptor), Denosumab (RANKL), Detumomab (B-lymphoma cell), Drozitumab (DR5), Ecromeximab (GD3 ganglioside), Edrecolomab (EpCAM), Elotuzumab (SLAMF7), Enavatuzumab (PDL192), Ensituximab (NPC-1C), Epratuzumab (CD22), Ertumaxomab (HER2/neu, CD3), Etaracizumab (integrin αvβ3), Farletuzumab (folate receptor 1), FBTA05 (CD20), Ficlatuzumab (SCH 900105), Figitumumab (IGF-1 receptor), Flanvotumab (glycoprotein 75), Fresolimumab (TGF-β), Galiximab (CD80), Ganitumab (IGF-I), Gemtuzumab ozogamicin (CD33), Gevokizumab (IL-1β), Girentuximab (carbonic anhydrase 9 (CA-IX)), Glembatumumab vedotin (GPNMB), Ibritumomab tiuxetan (CD20), Icrucumab (VEGFR-1), Igovoma (CA-125), Indatuximab ravtansine (SDC1), Intetumumab (CD51), Inotuzumab ozogamicin (CD22), Ipilimumab (CD152), Iratumumab (CD30), Labetuzumab (CEA), Lexatumumab (TRAIL-R2), Libivirumab (hepatitis B surface antigen), Lintuzumab (CD33), Lorvotuzumab mertansine (CD56), Lucatumumab (CD40), Lumiliximab (CD23), Mapatumumab (TRAIL-R1), Matuzumab (EGFR), Mepolizumab (IL-5), Milatuzumab (CD74), Mitumomab (GD3 ganglioside), Mogamulizumab (CCR4), Moxetumomab pasudotox (CD22), Nacolomab tafenatox (C242 antigen), Naptumomab estafenatox (5T4), Narnatumab (RON), Necitumumab (EGFR), Nimotuzumab (EGFR), Nivolumab (IgG4), Ofatumumab (CD20), Olaratumab (PDGF-R α), Onartuzumab (human scatter factor receptor kinase), Oportuzumab monatox (EpCAM), Oregovomab (CA-125), Oxelumab (OX-40), Panitumumab (EGFR), Patritumab (HER3), Pemtumoma (MUC1), Pertuzuma (HER2/neu), Pintumomab (adenocarcinoma antigen), Pritumumab (vimentin), Racotumomab (N-glycolylneuraminic acid), Radretumab (fibronectin extra domain-B), Rafivirumab (rabies virus glycoprotein), Ramucirumab (VEGFR2), Rilotumumab (HGF), Rituximab (CD20), Robatumumab (IGF-1 receptor), Samalizumab (CD200), Sibrotuzumab (FAP), Siltuximab (IL-6), Tabalumab (BAFF), Tacatuzumab tetraxetan (alpha-fetoprotein), Taplitumomab paptox (CD19), Tenatumomab (tenascin C), Teprotumumab (CD221), Ticilimumab (CTLA-4), Tigatuzumab (TRAIL-R2), TNX-650 (IL-13), Tositumomab (CD20), Trastuzumab (HER2/neu), TRBS07 (GD2), Tremelimumab (CTLA-4), Tucotuzumab celmoleukin (EpCAM), Ublituximab (MS4A1),

Urelumab (4-1BB), Volociximab (integrin α5β1), Votumumab (tumor antigen CTAA16.88), Zalutumumab (EGFR), Zanolimumab (CD4).

[0259] In some aspects, the additional therapeutic agent is a cytokine, chemokine, costimulatory molecule, fusion protein, or combinations thereof. Examples of chemokines include, but are not limited to, CCR7 and its ligands CCL19 and CCL21, furthermore CCL2, CCL3, CCL5, and CCL16. Other examples are CXCR4, CXCR7 and CXCL12. Furthermore, costimulatory or regulatory molecules such as *e.g.,* B7 ligands (B7.1 and B7.2) are useful. Also useful are other cytokines such as *e.g.,* interleukins especially (*e.g.,* IL-1 to IL17), interferons (*e.g.,* IFNalpha1 to IFNalpha8, IFNalpha10, IFNalpha13, IFNalpha14, IFNalpha16, IFNalpha17, IFNalpha21, IFNbeta1, IFNW, IFNE1 and IFNK), hematopoietic factors, TGFs (*e.g.,* TGF-α, TGF-β, and other members of the TGF family), finally members of the tumor necrosis factor family of receptors and their ligands as well as other stimulatory molecules, comprising but not limited to 41BB, 41BB-L, CD137, CD137L, CTLA-4GITR, GITRL, Fas, Fas-L, TNFR1, TRAIL-R1, TRAIL-R2, p75NGF-R, DR6, LT.beta.R, RANK, EDAR1, XEDAR, Fn114, Troy/Trade, TAJ, TNFRII, HVEM, CD27, CD30, CD40, 4-1BB, OX40, GITR, GITRL, TACI, BAFF-R, BCMA, RELT, and CD95 (Fas/APO-1), glucocorticoid-induced TNFR-related protein, TNF receptor-related apoptosis-mediating protein (TRAMP) and death receptor-6 (DR6). Especially CD40/CD40L and OX40/OX40L are important targets for combined immunotherapy because of their direct impact on T cell survival and proliferation.

[0260] In some aspects, the additional therapeutic agent is a kinase inhibitor. The growth and survival of cancer cells is closely interlocked with the deregulation of kinase activity. To restore normal kinase activity and therefor reduce tumor growth a broad range of inhibitors is in used. The group of targeted kinases comprises receptor tyrosine kinases *e.g.,* BCR-ABL, B-Raf, EGFR, HER-2/ErbB2, IGF-IR, PDGFR-α, PDGFR-β, c-Kit, Flt-4, Flt3, FGFR1, FGFR3, FGFR4, CSF1R, c-Met, RON, c-Ret, ALK, cytoplasmic tyrosine kinases *e.g.,* c-SRC, c-YES, Abl, JAK-2, serine/threonine kinases *e.g.,* ATM, Aurora A & B, CDKs, mTOR, PKCi, PLKs, b-Raf, S6K, STK11/LKB1 and lipid kinases *e.g.,* PI3K, SK1. Small molecule kinase inhibitors are *e.g.,* PHA-739358, Nilotinib, Dasatinib, and PD166326, NSC 743411, Lapatinib (GW-572016), Canertinib (CI-1033), Semaxinib (SU5416), Vatalanib (PTK787/ZK222584), Sutent (SU11248), Sorafenib (BAY 43-9006) and Leflunomide (SU101). For more information see *e.g.,* Zhang et al. 2009: Targeting cancer with small molecule kinase inhibitors. Nature Reviews Cancer 9, 28-39.

[0261] In some aspects, the additional therapeutic agent is a toll-like receptor. The members of the Toll-like receptor (TLRs) family are an important link between innate and adaptive

immunity and the effect of many adjuvants rely on the activation of TLRs. A large number of established vaccines against cancer incorporate ligands for TLRs for boosting vaccine responses. Besides TLR2, TLR3, TLR4 especially TLR7 and TLR8 have been examined for cancer therapy in passive immunotherapy approaches. The closely related TLR7 and TLR8 contribute to antitumor responses by affecting immune cells, tumor cells, and the tumor microenvironment and can be activated by nucleoside analogue structures. All TLRs have been used as stand-alone immunotherapeutics or cancer vaccine adjuvants and can be synergistically combined with the formulations and methods of the present disclosure.

[0262] In some aspects, the additional therapeutic agent is an angiogenesis inhibitor. Angiogenesis inhibitors prevent the extensive growth of blood vessels (angiogenesis) that tumors require to survive. The angiogenesis promoted by tumor cells to meet their increasing nutrient and oxygen demands for example can be blocked by targeting different molecules. Non-limiting examples of angiogenesis-mediating molecules or angiogenesis inhibitors which can be combined with the present disclosure are soluble VEGF (VEGF isoforms VEGF121 and VEGF165, receptors VEGFR1, VEGFR2 and co-receptors Neuropilin-1 and Neuropilin-2) 1 and NRP-1, angiopoietin 2, TSP-1 and TSP-2, angiostatin and related molecules, endostatin, vasostatin, calreticulin, platelet factor-4, TIMP and CDAI, Meth-1 and Meth-2, IFN-α, -β and -γ, CXCL10, IL-4, -12 and -18, prothrombin (kringle domain-2), antithrombin III fragment, prolactin, VEGI, SPARC, osteopontin, maspin, canstatin, proliferin-related protein, restin and drugs like *e.g.,* bevacizumab, itraconazole, carboxyamidotriazole, TNP-470, CM101, IFN-α, platelet factor-4, suramin, SU5416, thrombospondin, VEGFR antagonists, angiostatic steroids+heparin, cartilage-derived angiogenesis Inhibitory factor, matrix metalloproteinase inhibitors, 2-methoxyestradiol, tecogalan, tetrathiomolybdate, thalidomide, thrombospondin, prolactina Vβ3 inhibitors, linomide, tasquinimod.

[0263] In some aspects, the additional therapeutic agent is a virus-based vaccine. There are a number of virus-based cancer vaccines available or under development which can be used in a combined therapeutic approach together with the formulations of the present disclosure. One advantage of the use of such viral vectors is their intrinsic ability to initiate immune responses, with inflammatory reactions occurring as a result of the viral infection creating the danger signal necessary for immune activation. An ideal viral vector should be safe and should not introduce an anti-vector immune response to allow for boosting anti-tumor specific responses. Recombinant viruses such as vaccinia viruses, herpes simplex viruses, adenoviruses, adeno-associated viruses, retroviruses and avipoxviruses have been used in animal tumor models and based on their

encouraging results, human clinical trials have been initiated. Especially important virus-based vaccines are virus-like particles (VLPs), small particles that contain certain proteins from the outer coat of a virus. Virus-like particles do not contain any genetic material from the virus and cannot cause an infection but they can be constructed to present tumor antigens on their coat. VLPs can be derived from various viruses such as *e.g.,* the hepatitis B virus or other virus families including Parvoviridae (*e.g.,* adeno-associated virus), Retroviridae (*e.g.,* HIV), and Flaviviridae (*e.g.,* Hepatitis C virus).

[0264] In some aspects, the additional therapeutic agent is a peptide-based target therapy. Peptides can bind to cell surface receptors or affected extracellular matrix surrounding the tumor. Radionuclides which are attached to these peptides (*e.g.,* RGDs) eventually kill the cancer cell if the nuclide decays in the vicinity of the cell. Especially oligo- or multimers of these binding motifs are of great interest, since this can lead to enhanced tumor specificity and avidity.

VII. Kits for Use in Therapy

[0265] The present disclosure also provides kits for use in immunotherapy against a disease or disorder, such as a cancer (*e.g.,* melanoma, lung cancer, colorectal cancer, or renal-cell cancer), and/or treating or reducing the risk for the disease or disorder (*e.g.*, cancer). In some aspects, the kit includes one or more containers comprising any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein.

[0266] In some aspects, the kit comprises instructions for use in accordance with any of the methods described herein. For example, the included instructions can comprise a description of administration of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein to treat, delay the onset, or alleviate a target disease. In some aspects, the instructions comprise a description of administering the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein to a subject at risk of the target disease/disorder (*e.g.*, cancer).

[0267] In some aspects, the instructions comprise dosage information, dosing schedule, and route of administration. In some aspects, the containers are unit doses, bulk packages (*e.g.,* multi-dose packages) or sub-unit doses. In some aspects, the instructions are written instructions on a label or package insert (*e.g.,* a paper sheet included in the kit). In some aspects, the instructions are machine-readable instructions (*e.g.,* instructions carried on a magnetic or optical storage disk).

[0268] In some aspects, the label or package insert indicates that the composition disclosed herein is used for treating, delaying the onset, and/or alleviating a disease or disorder associated

with cancer, such as those described herein. Instructions can be provided for practicing any of the methods described herein.

[0269] In some aspects, the kits described herein are in suitable packaging. In some aspects, suitable packing comprises vials, bottles, jars, flexible packaging (*e.g.,* seal Mylar or plastic bags), or combinations thereof. In some aspects, the packaging comprises packages for use in combination with a specific device such as an inhaler, nasal administration device (*e.g.,* an atomizer), or an infusion device such as a minipump. In some aspects, the kit comprises a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some aspects, the container can also have a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some aspects, at least one active agent is a composition as described herein.

[0270] In some aspects, the kits further comprise additional components such as buffers and interpretive information. In some aspects, the kit comprises a container and a label or package insert(s) on or associated with the container. In some aspects, the disclosure provides articles of manufacture comprising the contents of the kits described herein.

General Techniques

[0271] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Molecular Cloning: A Laboratory Manual, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney, ed. 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Giffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; Method of Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel, et al., eds., 1987): PCR: The Polymerase Chain Reaction, (Mullis, et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty, ed., IRL

Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane, Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanette and J.D. Capra, eds., Harwood Academic Publishers, 1995). Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. All publications cited herein (including those listed above and elsewhere in the present disclosure) are incorporated by reference in their entirety.

Examples

EXAMPLE 1: RNA CIRCUIT CONSTRUCTION AND EXPRESSION ANALYSIS

[0272] To assess the ability of the polynucleotides described herein to reduce tissue-specific and/or immune cell-specific payload expression, a self-replicating RNA circuit was designed *in silico* that encodes a payload with an immune cell detargeting sensor (*e.g.*, a spleen detargeting sensor). A schematic of the RNA circuit is provided in FIG. 4. As shown in FIG. 5, this self-replicating RNA circuit design simulated controlled expression of the payload in tumor cells (*i.e.*, low immune cell-specific marker expression) with reduced expression in splenocytes (*i.e.*, higher immune cell-specific marker expression). The results provided in FIG. 5 confirm that the above-described RNA circuit simulates payload expression based on the level of the spleen-specific and/or immune cell-specific marker within a tissue. To confirm these *in silico* predictions, the above-described circuit was loaded into lipid nanoparticles (LNPs) and systematically administered to mice. As shown in FIGs. 6A and 6B, payload expression was specifically reduced in the spleen (which as described herein comprises a large population of immune cells) but not in other non-spleen tissue (*i.e.*, lung which is known to comprise fewer immune cells, *e.g.*, as compared to the spleen).

[0273] To further assess the polynucleotides described herein, lipid nanoparticles (LNPs) comprising one of the following were administered to mice: (a) VEE replicon comprising a mRNA encoding IL-12 alone (*i.e.*, uncircuited mRNA without an immune cell detargeting sensor (e.g., no spleen detargeting sensor, e.g., no miR-142 sensor)) and (b) VEE replicon comprising a mRNA encoding IL-12 and comprising an immune cell detargeting sensor (e.g., spleen detargeting sensor, e.g., miR-142 sensor) (*i.e.*, circuited mRNA). The LNPs were administered at two different doses: 0.1 mg/kg (low dose) and 0.3 mg/kg (high dose). Control animals received PBS alone. Then, the expression of IL-12 and IFN-γ were assessed in the spleen and in the serum at 6 hours, 24 hours,

and 264 hours post-administration. It was expected that reduced expression of IL-12 within the spleen would result in decreased IL-12 secretion and reduced levels within the serum.

[0274] As shown in FIGs. 1A, 7B, and 7C, at 6 hours post-administration, mice that received the LNP without the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, uncircuited mRNA) exhibited significant levels of IL-12 protein within the spleen as well as in the serum. In contrast, in mice that received the LNP with the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA), IL-12 protein level was significantly reduced and was more comparable to that of the PBS control animals. At 24 and 264 hours post-administration, the IL-12 protein expression began to return to basal levels (both in the spleen as well as in the serum) in all the animals (comparable to the PBS treated control animals). As to IFN-γ level (downstream effect of IL-12, *e.g.*, upon IL-12 exposure, immune cells such as dendritic cells produce IFN-γ), the general trend was similar to that described above for IL-12 (*e.g.*, at 6 hours post administration, reduced IFN-γ level in the spleen and serum in animals that received the circuited mRNA as compared to animals that received the uncircuited mRNA). FIGs. 1B, 7E, and 7F. Differences in IFN-γ level were still observed at 24 hours post-administration. In mice that received the LNP without the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor), there was still noticeably higher level of IFN-γ within the spleen.

[0275] Then, miR-142 expression was asssed across different cell types, including both immune and non-immune cells (*i.e.*, A549, BT-20, HeLa, HEK293T, HEK293, CD3+ T, CD4+ T, CD8+ T, CD14+ monocyte, CD19+ B, CD56+ NK, and B16F10). As shown in FIG. 10, miR-142 expression was generally low in cancer cells (*e.g.*, A549) and high in immune cells (*e.g.*, CD19+ B cells).

[0276] Next, to assess whether the above-described polynucleotides have an effect on other immune cells, human Kupffer cells (*i.e.*, liver macrophages) were treated with either a VEE replicon comprising mRNA encoding firefly luciferase without an immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor) ("Rep-Fluc") or a VEE replicon comprising mRNA encoding firefly luciferase with an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) described herein ("Rep-Fluc-miRNA sensor"). Then, luciferease expression was assessed. Consistent with the data described above and as shown in **FIG. 11**, payload expression in human Kupffer cells treated with Rep-Fluc-miRNA sensor was reduced by approximately 35x compared to payload expression in cells treated with Rep-Fluc.

[0277] FIG. 14 similarly shows that the miR-142 sensor detargeted payload (*i.e.*, reporter) expression in T cells compared to a control.

[0278] These results show that the above-described RNA circuit can detarget reporter payload expression in immune cells (*e.g.*, liver macrophages and T cells). Not to be bound by any one theory, the above results suggest that the reduced payload expression observed in the spleen is not necessarily limited to the spleen and that similar effects can occur in other tissues comprising immune cells (*e.g.*, other lymphoid tissues, such as those described herein).

EXAMPLE 2: TOXICITY ANALYSIS IN MICE

[0279] To assess whether the reduced expression of IL-12 observed in Example 1 has any effect on toxicity, the above-described LNPs were again administered to mice (as described in Example 1) and then body weight was monitored over a course of about 10 days.

[0280] As shown in FIGs. 2, 7A, and 7D, in mice that received the LNP with the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA), there was much decreased body weight loss, as compared to mice that received the corresponding LNP without the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, uncircuited mRNA). Differences in body weight were much more pronounced at the higher dose as compared to the lower dose (*see, e.g.*, FIGs 7A and 7B). These results confirm that the reduced IL-12 expression observed in Example 1 is associated with reduced overall toxicity.

EXAMPLE 3: ANALYSIS OF ANTI-TUMOR ACTIVITY

[0281] To assess the specificity and potency of the polynucleotides described herein (*e.g.*, RNA circuit with an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA)), mice were implanted subcutaneously with B16-F10 melanoma cells. Once the tumors developed within the animals, the above-described LNPs (*see* Example 2) were administered intravenously. The tumor volume was then measured periodically for 30 days. IL-12 level in the tumor and spleen were also assessed at 6 and 24 hours post-administration.

[0282] As shown in FIG. 8A, mice that received the LNP with the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA) had similar tumor control compared to mice that received the LNP without the immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor) (*i.e.*, uncircuited mRNA), and both LNPs conferred enhanced tumor control compared to the PBS control. As to IL-12 protein level, mice that received the LNP with the immune cell detargeting sensor (*e.g.*, spleen detargeting

sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA) had similar IL-12 level in the tumor as compared to mice that received the corresponding LNP without the immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor) (*i.e.*, uncircuited mRNA). *See* FIG. 8B. This is consistent with the results described earlier and confirms that the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) has minimal activity within the tumor (which expresses low to no miR-142-3p expression). In contrast as shown in FIG. 8C, mice that received the LNP with the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, circuited mRNA) had lower IL-12 protein level in the spleen relative to the corresponding LNP without the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, uncircuited mRNA).

[0283] The above results demonstrate both the specificity and anti-tumor capability of the polynucleotides described herein.

EXAMPLE 4: EXPRESSION ANALYSIS IN NON-HUMAN PRIMATES

[0284] To confirm the results observed in mice described above in Examples 1-3, the four constructs shown in Table 1 (below) were produced. Construct #1 included a modified mRNA (*i.e.*, non-self replicating) encoding IL-12 and lacked any tissue-specific sensors (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)). Construct #2 included a modified mRNA (*i.e.*, non-self replicating) encoding IL-12 and included two different tissue-specific sensors: (i) a sensor recognizing a marker expressed in the spleen (immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor)) and (ii) a sensor recognizing a marker expressed in the liver (liver detargeting sensor). As described in the present disclosure, it was expected that the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) and the liver detargeting sensor would reduce or inhibit the expression of the IL-12 protein within the spleen and liver, respectively. Construct #3 included a VEE replicon (*i.e.*, self-replicating) comprising a mRNA encoding IL-12 and lacked any tissue-specific sensors (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)). Construct #4 included a VEE replicon (*i.e.*, self-replicating) comprising a mRNA encoding IL-12 and included an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor).

Table 1. LNP Constructs

Construct	**Description of Construct**
mod-IL12 (no sensor) ("Construct #1")	modified mRNA encoding IL-12 alone (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)) (uncircuited mRNA)
mod-IL12-circuit (spleen-OFF & liver-OFF miRNA sensors) ("Construct #2")	modified mRNA encoding IL-12 + immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) + liver detargeting sensor (circuited mRNA)
rep-IL12 (no sensor) ("Construct #3")	VEE replicon comprising mRNA encoding IL-12 alone (*i.e.*, no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)) (uncircuited mRNA)
rep-IL12-circuit (spleen-OFF miRNA sensor) ("Construct #4")	VEE replicon comprising mRNA encoding IL-12 + immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (circuited mRNA)

[0285] The above constructs were loaded into separate nanoparticles and administered intravenously to non-human primates. Then, both IL-12 and IFN-γ levels were observed in plasma.

[0286] As shown in FIG. 3A and Table 2, in the animal that received Construct #1 (*i.e.*, modified mRNA encoding IL-12 without any immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor)), a high level of IL-12 protein was observed in the plasma. Interestingly, in the animal that received Construct #2 (*i.e.*, modified mRNA encoding IL-12 with both an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) and a liver marker sensor), there was only a modest decrease in IL-12 level, as compared to the animal that received Construct #1. In fact, the IL-12 level was higher in the animal that received Construct #2, as compared to animals that received Construct #3, which included a VEE replicon but no immune cell detargeting sensor (*e.g.*, no spleen detargeting sensor, *e.g.*, no miR-142 sensor). The greatest reduction in IL-12 level was observed in animals that received Construct #4 (*i.e.*, VEE replicon encoding IL-12 and comprising an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor)). As compared between animals that received Construct #3 or Construct #4, there was a 155-fold reduction in C_{max} and 200-fold reduction in

AUC for IL-12 levels in animals that received the LNP with the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) (*i.e.*, Construct #4).

[0287] As observed in mice, the general trend was similar when IFN-γ level was assessed in the plasma of the non-human primates (*see* FIG. 3B and Table 3). Again, as compared between animals that received Construct #3 or Construct #4, there was a 18-fold reduction in C_{max} and 32-fold reduction in AUC for IFN-γ levels in animals that received the LNP with the spleen detargeting sensor (*i.e.*, Construct #4).

Table 2. IL-12 Pharmacodynamic Properties

Group	**Construct**	**T_{max} (H)**	**C_{max} (pg/mL)**	**AUC (pg * H/mL)**	**% KD in plasma**
1	mod-IL12 (no sensor) "Construct #1"	24	84,319		
2	mod-IL12-circuit (immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) + liver detargeting sensor) "Construct #2"	24	19,115		77.3%
3	rep-IL12 (no sensor) "Construct #3"	12	4,043	133,230	
4	rep-IL12-circuit (immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor))	12	26	665	99.4%

	"Construct 4"				

Table 3. IFN-γ Pharmacodynamic Properties

Group	**Construct**	**T_{max} (H)**	**C_{max} (pg/mL)**	**AUC (pg * H/mL)**	**% KD in plasma**
1	mod-IL12 (no sensor) "Construct #1"	6	11,376		
2	mod-IL12-circuit (immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) + liver detargeting sensor) "Construct #2"	6	4,365		61.6%
3	rep-IL12 (no sensor) "Construct #3"	12	9,902	212,943	
4	rep-IL12-circuit (immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor)) "Construct 4"	12	541	6,667	94.5%

[0288] **FIG. 12** provides a further comparison of spleen, mesenteric lymph nodes (LN), tracheobronchial LN, mandibular LN, axillary LN, and bone marrow IL-12 expression in non-human primates following intravenous delivery of a lipid nanoparticle loaded with one of the following: (a) mod-IL-12 (*i.e.*, Construct #1); (b) mod-IL-12-circuit (*i.e.*, Construct #2); (c) rep-

IL-12 (*i.e.*, Construct #3); and (d) rep-IL-12-circuit (*i.e.*, Construct #4). The results for IL-12 levels for all tested tissues were consistent with the serum results described above. Again, the greatest reduction of IL-12 was observed in animals that received Construct #4 (*i.e.*, VEE replicon encoding IL-12 and comprising an immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor)). These results demonstrate the ability of the immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor), especially combined with the restrictive replication of VEE, to reduce IL-12 levels in immune cells. **FIG. 13** shows miR-142-3p expression within non-human primate spleen; specifically, miR-142-3p expression within the white pulp (containing lymphocytes and macrophages) of non-human primate spleen.

EXAMPLE 5: CIRCUIT EXPRESSION IN CANCER CELLS

[0289] To confirm if the Construct #3 and Construct #4 tested in Example 4 would transfect human cancer cells, LNPs containing Construct #3 (*i.e.*, uncircuited mRNA) and Construct #4 (*i.e.*, circuited mRNA) were administered to patient-derived breast cancer organoids or A549 cells in cell culture.

[0290] As shown in FIG. 9A and FIG. 9B, the LNP containing Construct #4 (*i.e.*, circuited mRNA) resulted in higher expression of hIL-12 in both patient-derived breast cancer organoids and A549 cells (a human non-small cell lung cancer cell line) compared to LNP delivery of Construct #3 (*i.e.*, uncircuited mRNA). This result shows that the circuited construct (*i.e.*, immune cell detargeting sensor (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor)) which had lower expression of circulating hIL-12 and IFN-γ in NHPs after systemic administration (*See* Example 4), likewise yielded robust expression of hIL-12 in cancer organoids or A549 cancer cells. FIGs. 15A and 15B further demonstrate that payload expression is preserved in cancer cells that lack miR-142 expression (*i.e.*, A549 and B16.F10). Specifically, in A549 cells, treatment with a construct comprising a payload-encoding mRNA and a miR-142 sensor (*see* hsa-mir142-3p group in FIG. 15A) did not reduce payload (*i.e.*, luminescence) expression as compared to corresponding cells that were treated with a construct comprising the payload-encoding mRNA but lacking the miR-142 sensor) (*see* no miR group in FIG. 15A). Similarly, in B16.F10 cells (a murine melanoma cell line), mIL-12 expression was not reduced in the cells treated with a construct comprising an IL-12-encoding mRNA and a miR-142 sensor (*see* hsa-miR-142-3p sensor group in FIG. 15B) as compared to the corresponding cells that were treated with a construct comprising the IL-12-encoding mRNA but lacking the miR-142 sensor (*see* no sensor group in FIG. 15B). The above data demonstrate that the miR-142 sensor is capable of downregulating payload expression in

immune cells (which express miR-142) but not in cancer cells (which lack miR-142 expression), highlighting the specificity of the immune cell detargeting sensors described herein (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor).

[0291] Collectively, the above results demonstrate that the polynucleotides described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a spleen detargeting sensor) can selectively reduce the expression of the encoded payload within the spleen and other immune cells. The above results further demonstrate that the observed specificity can be greatly improved through the use of self-replicating replicons, *e.g.*, VEE replicon described herein (*see*, *e.g.*, FIG. 16).

Table 4. Exemplary VEE Replicon Sequences

VEE Replicon Sequence #1 (SEQ ID NO: 3)	ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAAAG TTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCT TCCCGCAGTTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATG CCAGAGCGTTTTCGCATCTGGCTTCAAAACTGATCGAAACGGAGGTGGACCCAT CCGACACGATCCTTGACATTGGAAGTGCGCCCGCCCGCAGAATGTATTCTAAGC ACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGATCCGGACAGATTGT ATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAATAACTGATAAGGAAT TGGACAAGAAAATGAAGGAGCTGGCCGCCGTCATGAGCGACCCTGACCTGGAAA CTGAGACTATGTGCCTCCACGACGACGAGTCGTGTCGCTACGAAGGGCAAGTCG CTGTTTACCAGGATGTATACGCGGTTGACGGACCGACAAGTCTCTATCACCAAG CCAATAAGGGAGTTAGAGTCGCCTACTGGATAGGCTTTGACACCACCCCTTTTA TGTTTAAGAACTTGGCTGGAGCATATCCATCATACTCTACCAACTGGGCCGACG AAACCGTGTTAACGGCTCGTAACATAGGCCTATGCAGCTCTGACGTTATGGAGC GGTCACGTAGAGGGATGTCCATTCTTAGAAAGAAGTATTTGAAACCATCCAACA ATGTTCTATTCTCTGTTGGCTCGACCATCTACCACGAGAAGAGGGACTTACTGA GGAGCTGGCACCTGCCGTCTGTATTTCACTTACGTGGCAAGCAAAATTACACAT GTCGGTGTGAGACTATAGTTAGTTGCGACGGGTACGTCGTTAAAAGAATAGCTA TCAGTCCAGGCCTGTATGGGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCG AGGGATTCTTGTGCTGCAAAGTGACAGACACATTGAACGGGGAGAGGGTCTCTT TTCCCGTGTGCACGTATGTGCCAGCTACATTGTGTGACCAAATGACTGGCATAC TGGCAACAGATGTCAGTGCGGACGACGCGCAAAAACTGCTGGTTGGGCTCAACC AGCGTATAGTCGTCAACGGTCGCACCCAGAGAAACACCAATACCATGAAAAATT ACCTTTTGCCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAAGG AAGATCAAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGG GGTGTTGTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGG ATACCCAAACCATCATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCA GGATAGGCAGTAACACATTGGAGATCGGGCTGAGAACAAGAATCAGGAAAATGT TAGAGGAGCACAAGGAGCCGTCACCTCTCATTACCGCCGAGGACGTACAAGAAG CTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAAGCCGAGGAGTTGCGCG CAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGAGGCAGACG TCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACACCTCGTGGCT TGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCTCTTACGCTGTGC TTTCTCCGCAGGCTGTACTCAAGAGTGAAAAATTATCTTGCATCCACCCTCTCG CTGAACAAGTCATAGTGATAACACACTCTGGCCGAAAAGGGCGTTATGCCGTGG AACCATACCATGGTAAAGTAGTGGTGCCAGAGGGACATGCAATACCCGTCCAGG ACTTTCAAGCTCTGAGTGAAAGTGCCACCATTGTGTACAACGAACGTGAGTTCG TAAACAGGTACCTGCACCATATTGCCACACATGGAGGAGCGCTGAACACTGATG

	AAGAATATTACAAAACTGTCAAGCCCAGCGAGCACGACGGCGAATACCTGTACG
	ACATCGACAGGAAACAGTGCGTCAAGAAAGAACTAGTCACTGGGCTAGGGCTCA
	CAGGCGAGCTGGTGGATCCTCCCTTCCATGAATTCGCCTACGAGAGTCTGAGAA
	CACGACCAGCCGCTCCTTACCAAGTACCAACCATAGGGGTGTATGGCGTGCCAG
	GATCAGGCAAGTCTGGCATCATTAAAAGCGCAGTCACCAAAAAAGATCTAGTGG
	TGAGCGCCAAGAAAGAAAACTGTGCAGAAATTATAAGGGACGTCAAGAAAATGA
	AAGGGCTGGACGTCAATGCCAGAACTGTGGACTCAGTGCTCTTGAATGGATGCA
	AACACCCCGTAGAGACCCTGTATATTGACGAAGCTTTTGCTTGTCATGCAGGTA
	CTCTCAGAGCGCTCATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGCGGGG
	ATCCCAAACAGTGCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACC
	ACGAGATTTGCACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAAT
	CTGTGACTTCGGTCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGA
	ATCCGAAAGAGACTAAGATTGTGATTGACACTACCGGCAGTACCAAACCTAAGC
	AGGACGATCTCATTCTCACTTGTTTCAGAGGGTGGGTGAAGCAGTTGCAAATAG
	ATTACAAAGGCAACGAAATAATGACGGCAGCTGCCTCTCAAGGGCTGACCCGTA
	AAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTGTACGCACCCA
	CCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTGTGGA
	AAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACCCTGGGA
	ATTTCACTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCATCATGAGGC
	ACATCTTGGAGAGACCGGACCCTACCGACGTCTTCCAGAATAAGGCAAACGTGT
	GTTGGGCCAAGGCTTTAGTGCCGGTGCTGAAGACCGCTGGCATAGACATGACCA
	CTGAACAATGGAACACTGTGGATTATTTTGAAACGGACAAAGCTCACTCAGCAG
	AGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGACTCGATCTGGACTCCG
	GTCTATTTTCTGCACCCACTGTTCCGTTATCCATTAGGAATAATCACTGGGATA
	ACTCCCCGTCGCCTAACATGTACGGGCTGAATAAAGAAGTGGTCCGTCAGCTCT
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VEE- Replicon Sequence #2 (SEQ ID NO: 5)	ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAAAG TTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGGAGCT TCCCGCAGTTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATGCTAATG CCAGAGCGTTTTCGCATCTGGCTTCAAAACTGATCGAAACGGAGGTGGACCCAT CCGACACGATCCTTGACATTGGAAGTGCGCCCGCCCGCAGAATGTATTCTAAGC ACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGATCCGGACAGATTGT ATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAATAACTGATAAGGAAT TGGACAAGAAAATGAAGGAGCTCGCCGCCGTCATGAGCGACCCTGACCTGGAAA

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	CAGCGATCCAGAACACGCTCCAGAACGTCCTGGCAGCTGCCACAAAAAGAAATT GCAATGTCACGCAAATGAGAGAATTGCCCGTATTGGATTCGGCGGCCTTTAATG TGGAATGCTTCAAGAAATATGCGTGTAATAATGAATATTGGGAAACGTTTAAAG AAAACCCCATCAGGCTTACTGAAGAAAACGTGGTAAATTACATTACCAAATTAA AAGGACCAAAAGCTGCTGCTCTTTTTGCGAAGACACATAATTTGAATATGTTGC AGGACATACCAATGGACAGGTTTGTAATGGACTTAAAGAGAGACGTGAAAGTGA CTCCAGGAACAAAACATACTGAAGAACGGCCCAAGGTACAGGTGATCCAGGCTG CCGATCCGCTAGCAACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGA GATTAAATGCGGTCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTG AAGACTTTGACGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGG AAACTGACATCGCGTCGTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCG CGTTAATGATTCTGGAAGACTTAGGTGTGGACGCAGAGCTGTTGACGCTGATTG AGGCGGCTTTCGGCGAAATTTCATCAATACATTTGCCCACTAAAACTAAATTTA AATTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACTGTTTGTGAACACAG TCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCGGATCAC CATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCGGACA AATTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGATTATAG ATGCTGTGGTGGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTATTTTGTGTG ACTCCGTGACCGGCACAGCGTGCCGTGTGGCAGACCCCCTAAAAAGGCTGTTTA AGCTTGGCAAACCTCTGGCAGCAGACGATGAACATGATGATGACAGGAGAAGGG CATTGCATGAAGAGTCAACACGCTGGAACCGAGTGGGTATTCTTTCAGAGCTGT GCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACTTCCATCATAGTTATGG CCATGACTACTCTAGCTAGCAGTGTTAAATCATTCAGCTACCTGAGAGGGGCCC CTATAACTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTAGTCCGCCAA GGCCACCGATATCTATGTTACGTGCAAAGGTGATTGTCACCCCCCGAAAGACCA TATTGTGACACACCCTCAGTATCACGCCCAAACATTTACAGCCGCGGTGTCAAA AACCGCGTGGACGTGGTTAACATCCCTGCTGGGAGGATCAGCCGTAATTATTAT AATTGGCTTGGTGCTGGCTACTATTGTGGCCATGTACGTGCTGACCAACCAGAA ACATAATTGAATACAGCAGCAATTGGCAAGCTGCTTACATAGAACTCGCGGCGA TTGGCATGCCGCCTTAAAATTTTTATTTTATTTTTCTTTTCTTTTCCGAATCGG ATTTTGTTTTTAATATTTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAGAAGAGCGCAGCTCTGGCCCGTGTCTCAAAATCTCTGATGTTACA TTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAA ACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGC GATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATA ATGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGC CAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATG AGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGC ATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGAA AAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTG ATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTC CTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATA ACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTG AACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCG TCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAA TAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTG CCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTT TTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGA TGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGA GCATTACGCTGACTTGACGGGACGGCGCAAGCTCATGACCAAAATCCCTTAACG TGAGTTACGCGTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGAT CTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAAC CACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTC CGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGT

	AGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCG CTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTA CCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAA CGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGA GATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGG CGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGC TTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCT GACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAA ACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTC ACATGCTCTTCTAAGTAATACGACTCACTATA
VEE Replicon Sequence #3 (SEQ ID NO: 4)	gctcttctaagTAATACGACTCACTATAATGGGCGGCGCATGAGAGAAGCCCAG ACCAATTACCTACCCAAAATGGAGAAAGTTCACGTTGACATCGAGGAAGACAGC CCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCGCAGTTTGAGGTAGAAGCCAAG CAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTCGCATCTGGCTTCA AAACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGACATTGGAAGT GCGCCCGCCCGCAGAATGTATTCTAAGCACAAGTATCATTGTATCTGTCCGATG AGATGTGCGGAAGATCCGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAA AACTGTAAGGAAATAACTGATAAGGAATTGGACAAGAAAATGAAGGAGCTCGCC GCCGTCATGAGCGACCCTGACCTGGAAACTGAGACTATGTGCCTCCACGACGAC GAGTCGTGTCGCTACGAAGGGCAAGTCGCTGTTTACCAGGATGTATACGCGGTT GACGGACCGACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAGTCGCCTAC TGGATAGGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATAT CCATCATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATA GGCCTATGCAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTT AGAAAGAAGTATTTGAAACCATCCAACAATGTTCTATTCTCTGTTGGCTCGACC ATCTACCACGAGAAGAGGGACTTACTGAGGAGCTGGCACCTGCCGTCTGTATTT CACTTACGTGGCAAGCAAAATTACACATGTCGGTGTGAGACTATAGTTAGTTGC GACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCCTGTATGGGAAGCCT TCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTGCAAAGTGACA GACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACGTATGTGCCAGCT ACATTGTGTGACCAAATGACTGGCATACTGGCAACAGATGTCAGTGCGGACGAC GCGCAAAAACTGCTGGTTGGGCTCAACCAGCGTATAGTCGTCAACGGTCGCACC CAGAGAAACACCAATACCATGAAAAATTACCTTTTGCCCGTAGTGGCCCAGGCA TTTGCTAGGTGGGCAAAGGAATATAAGGAAGATCAAGAAGATGAAAGGCCACTA GGACTACGAGATAGACAGTTAGTCATGGGGTGTTGTTGGGCTTTTAGAAGGCAC AAGATAACATCTATTTATAAGCGCCCGGATACCCAAACCATCATCAAAGTGAAC AGCGATTTCCACTCATTCGTGCTGCCCAGGATAGGCAGTAACACATTGGAGATC GGGCTGAGAACAAGAATCAGGAAAATGTTAGAGGAGCACAAGGAGCCGTCACCT CTCATTACCGCCGAGGACGTACAAGAAGCTAAGTGCGCAGCCGATGAGGCTAAG GAGGTGCGTGAAGCCGAGGAGTTGCGCGCAGCTCTACCACCTTTGGCAGCTGAT GTTGAGGAGCCCACTCTGGAAGCCGATGTCGACTTGATGTTACAAGAGGCTGGG GCCGGCTCAGTGGAGACACCTCGTGGCTTGATAAAGGTTACCAGCTACGATGGC GAGGACAAGATCGGCTCTTACGCTGTGCTTTCTCCGCAGGCTGTACTCAAGAGT GAAAAATTATCTTGCATCCACCCTCTCGCTGAACAAGTCATAGTGATAACACAC TCTGGCCGAAAAGGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGGTG CCAGAGGGACATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCC ACCATTGTGTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCC ACACATGGAGGAGCGCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCC AGCGAGCACGACGGCGAATACCTGTACGACATCGACAGGAAACAGTGCGTCAAG AAAGAACTAGTCACTGGGCTAGGGCTCACAGGCGAGCTGGTGGATCCTCCCTTC CATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGCCGCTCCTTACCAAGTA CCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCATCATTAAA AGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAACTGTGCA GAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATGCCAGAACT

	GTGGACTCAGTGCTCTTGAATGGATGCAAACACCCCGTAGAGACCCTGTATATT GACGAAGCTTTTGCTTGTCATGCAGGTACTCTCAGAGCGCTCATAGCCATTATA AGACCTAAAAAGGCAGTGCTCTGCGGGGATCCCAAACAGTGCGGTTTTTTTAAC ATGATGTGCCTGAAAGTGCATTTTAACCACGAGATTTGCACACAAGTCTTCCAC AAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACTTCGGTCGTCTCAACCTTG TTTTACGACAAAAAAATGAGAACGACGAATCCGAAAGAGACTAAGATTGTGATT GACACTACCGGCAGTACCAAACCTAAGCAGGACGATCTCATTCTCACTTGTTTC AGAGGGTGGGTGAAGCAGTTGCAAATAGATTACAAAGGCAACGAAATAATGACG GCAGCTGCCTCTCAAGGGCTGACCCGTAAAGGTGTGTATGCCGTTCGGTACAAG GTGAATGAAAATCCTCTGTACGCACCCACCTCAGAACATGTGAACGTCCTACTG ACCCGCACGGAGGACCGCATCGTGTGGAAAACACTAGCCGGCGACCCATGGATA AAAACACTGACTGCCAAGTACCCTGGGAATTTCACTGCCACGATAGAGGAGTGG CAAGCAGAGCATGATGCCATCATGAGGCACATCTTGGAGAGACCGGACCCTACC GACGTCTTCCAGAATAAGGCAAACGTGTGTTGGGCCAAGGCTTTAGTGCCGGTG CTGAAGACCGCTGGCATAGACATGACCACTGAACAATGGAACACTGTGGATTAT TTTGAAACGGACAAAGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTG AGGTTCTTTGGACTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCG TTATCCATTAGGAATAATCACTGGGATAACTCCCCGTCGCCTAACATGTACGGG CTGAATAAAGAAGTGGTCCGTCAGCTCTCTCGCAGGTACCCACAACTGCCTCGG GCAGTTGCCACTGGAAGAGTCTATGACATGAACACTGGTACACTGCGCAATTAT GATCCGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCATGCTTTAGTC CTCCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAATTG AAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAATG GTTGACTGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGATTTA GGCATCCCAGGTGATGTGCCCAAATATGACATAATATTTGTTAATGTGAGGACC CCATATAAATACCATCACTATCAGCAGTGTGAAGACCATGCCATTAAGCTTAGC ATGTTGACCAAGAAAGCTTGTCTGCATCTGAATCCCGGCGGAACCTGTGTCAGC ATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCATCATTGGTGCTATAGCG CGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCTCACTTGAAGAGACG GAAGTTCTGTTTGTATTCATTGGGTACGATCGCAAGGCCCGTACGCACAATCCT TACAAGCTTTCATCAACCTTGACCAACATTTATACAGGTTCCAGACTCCACGAA GCCGGATGTGCACCCTCATATCATGTGGTGCGAGGGGATATTGCCACGGCCACC GAAGGAGTGATTATAAATGCTGCTAACAGCAAAGGACAACCTGGCGGAGGGGTG TGCGGAGCGCTGTATAAGAAATTCCCGGAAAGCTTCGATTTACAGCCGATCGAA GTAGGAAAAGCGCGACTGGTCAAAGGTGCAGCTAAACATATCATTCATGCCGTA GGACCAAACTTCAACAAAGTTTCGGAGGTTGAAGGTGACAAACAGTTGGCAGAG GCTTATGAGTCCATCGCTAAGATTGTCAACGATAACAATTACAAGTCAGTAGCG ATTCCACTGTTGTCCACCGGCATCTTTTCCGGGAACAAAGATCGACTAACCCAA TCATTGAACCATTTGCTGACAGCTTTAGACACCACTGATGCAGATGTAGCCATA TACTGCAGGGACAAGAAATGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGA GAAGCAGTGGAGGAGATATGCATATCCGACGACTCTTCAGTGACAGAACCTGAT GCAGAGCTGGTGAGGGTGCATCCGAAGAGTTCTTTGGCTGGAAGGAAGGGCTAC AGCACAAGCGATGGCAAAACTTTCTCATATTTGGAAGGGACCAAGTTTCACCAG GCGGCCAAGGATATAGCAGAAATTAATGCCATGTGGCCCGTTGCAACGGAGGCC AATGAGCAGGTATGCATGTATATCCTCGGAGAAAGCATGAGCAGTATTAGGTCG AAATGCCCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGC TTGTGCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGT CCAGAACAAATTACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACT GGTGTGCAGAAGATCCAATGCTCCCAGCCTATATTGTTCTCACCGAAAGTGCCT GCGTATATTCATCCAAGGAAGTATCTCGTGGAAACACCACCGGTAGACGAGACT CCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGGACACCTGAACAACCACCA CTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGATCATCATCGAA GAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGACCCACCAGGTG CTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCTAGCTCATCCTGG

	TCCATTCCTCATGCATCCGACTTTGATGTGGACAGTTTATCCATACTTGACACC CTGGAGGGAGCTAGCGTGACCAGCGGGGCAACGTCAGCCGAGACTAACTCTTAC TTCGCAAAGAGTATGGAGTTTCTGGCGCGACCGGTGCCTGCGCCTCGAACAGTA TTCAGGAACCCTCCACATCCCGCTCCGCGCACAAGAACACCGTCACTTGCACCC AGCAGGGCCTGCTCGAGAACCAGCCTAGTTTCCACCCCGCCAGGCGTGAATAGG GTGATCACTAGAGAGGAGCTCGAGGCGCTTACCCCGTCACGCACTCCTAGCAGG TCGGTCTCGAGAACCAGCCTGGTCTCCAACCCGCCAGGCGTAAATAGGGTGATT ACAAGAGAGGAGTTTGAGGCGTTCGTAGCACAACAACAATGACGGTTTGATGCG GGTGCATACATCTTTTCCTCCGACACCGGTCAAGGGCATTTACAACAAAAATCA GTAAGGCAAACGGTGCTATCCGAAGTGGTGTTGGAGAGGACCGAATTGGAGATT TCGTATGCCCCGCGCCTCGACCAAGAAAAAGAAGAATTACTACGCAAGAAATTA CAGTTAAATCCCACACCTGCTAACAGAAGCAGATACCAGTCCAGGAAGGTGGAG AACATGAAAGCCATAACAGCTAGACGTATTCTGCAAGGCCTAGGGCATTATTTG AAGGCAGAAGGAAAAGTGGAGTGCTACCGAACCCTGCATCCTGTTCCTTTGTAT TCATCTAGTGTGAACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGT AACGCCATGTTGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCA GAGTACGATGCCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACT GCCAGTTTTTGCCCTGCAAAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTG GAACCCACAATACGATCGGCAGTGCCTTCAGCGATCCAGAACACGCTCCAGAAC GTCCTGGCAGCTGCCACAAAAAGAAATTGCAATGTCACGCAAATGAGAGAATTG CCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAGAAATATGCGTGT AATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGAAGAA AACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCTCTTTTT GCGAAGACACATAATTTGAATATGTTGCAGGACATACCAATGGACAGGTTTGTA ATGGACTTAAAGAGAGACGTGAAAGTGACTCCAGGAACAAAACATACTGAAGAA CGGCCCAAGGTACAGGTGATCCAGGCTGCCGATCCGCTAGCAACAGCGTATCTG TGCGGAATCCACCGAGAGCTGGTTAGGAGATTAAATGCGGTCCTGCTTCCGAAC ATTCATACACTGTTTGATATGTCGGCTGAAGACTTTGACGCTATTATAGCCGAG CACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGACATCGCGTCGTTTGATAAA AGTGAGGACGACGCCATGGCTCTGACCGCGTTAATGATTCTGGAAGACTTAGGT GTGGACGCAGAGCTGTTGACGCTGATTGAGGCGGCTTTCGGCGAAATTTCATCA ATACATTTGCCCACTAAAACTAAATTTAAATTCGGAGCCATGATGAAATCTGGA ATGTTCCTCACACTGTTTGTGAACACAGTCATTAACATTGTAATCGCAAGCAGA GTGTTGAGAGAACGGCTAACCGGATCACCATGTGCAGCATTCATTGGAGATGAC AATATCGTGAAAGGAGTCAAATCGGACAAATTAATGGCAGACAGGTGCGCCACC TGGTTGAATATGGAAGTCAAGATTATAGATGCTGTGGTGGGCGAGAAAGCGCCT TATTTCTGTGGAGGGTTTATTTTGTGTGACTCCGTGACCGGCACAGCGTGCCGT GTGGCAGACCCCCTAAAAAGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGAC GATGAACATGATGATGACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGG AACCGAGTGGGTATTCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAA ACCGTAGGAACTTCCATCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTT AAATCATTCAGCTACCTGAGAGGGGCCCCTATAACTCTCTACGGCTAACCTGAA TGGACTACGACATAGTCTAGTCCGCCAAGGCCACCGATATCTATGTTACGTGCA AAGGTGATTGTCACCCCCCGAAAGACCATATTGTGACACACCCTCAGTATCACG CCCAAACATTTACAGCCGCGGTGTCAAAAACCGCGTGGACGTGGTTAACATCCC TGCTGGGAGGATCAGCCGTAATTATTATAATTGGCTTGGTGCTGGCTACTATTG TGGCCATGTACGTGCTGACCAACCAGAAACATAATTGAATACAGCAGCAATTGG CAAGCTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAATTTTTAT TTTATTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTCAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAaaagaagagcGCAGCTCTGG CCCGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCA TGAACAATAAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGC CATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTCCAACATGGATGCTGAT TTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATC

	TATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAA GGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACG GAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCA TGGTTACTCACCACTGCGATCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAA TATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGG TTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGT CTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTT GATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAA CTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGAT AACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTC GGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAG TTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCT GATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAA TTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGC GCAAGCTCATGACCAAAATCCCTTAACGTGAGTTACGCGTCGTTCCACTGAGCG TCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGC GTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTG CCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCG CAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAG AACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCT GCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTA CCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGC TTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAA AGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTT TATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGC TCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGG TTCCTGGCCTTTTGCTGGCCTTTTGCTCACAT

Table 5. Additional Exemplary Sequences

miR-142-3p Sensor Sequence (SEQ ID NO: 6)	TCCATAAAGTAGGAAACACTACA
Spacer Sequence (SEQ ID NO: 7)	GCGGCCGCTAAA

EXAMPLE 6: MIR-142 ACTIVITY IN MACROPHAGE CELLS PLUS SPECIFICITY CONTROL WITH MIR-INHIBITORS

[0292] To further assess the specificity of the immune detargeting sensors provided herein (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor), miRNA inhibitors were used to regulate miR-142-3p expression in cells. Briefly, a VEE replicon (Rep) comprising a firefly luciferase (Fluc)-encoding mRNA and a miR-142 sensor (Rep with Sensor) or without such a sensor (Rep without Sensor) were transfected into RAW264.7 macrophages, which express high levels of miR-142-3p. Some of the cells were co-transfected with a miRNA inhibitor targeting miR-142-3p (anti-

miR-142-3p) or a negative control miRNA inhibitor (anti-cel-miR-67). In the absence of either miRNA inhibitor (0 pmol/well), the miR-142 sensor repressed expression of the Fluc payload (*see* second and fourth black bars in FIG. 17). However, co-transfection with any of three doses of anti-miR-142-3p (*see* second bar in each of 0.125, 1.25, and 12.5 pmol/well groups in FIG. 17) abrogated this repression, while anti-cel-miR-67 did not (*see* fourth bar in each of 0.125, 1.25, and 12.5 pmol/well groups in FIG. 17).

[0293] These results further demonstrate that the immune cell detargeting sensor provided herein (*e.g.*, spleen detargeting sensor, *e.g.*, miR-142 sensor) is specifically responsive to miR-142-3p activity

[0294] It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections can set forth one or more but not all exemplary aspects of the present disclosure as contemplated by the inventor(s), and thus, are not intended to limit the present disclosure and the appended claims in any way.

[0295] The present disclosure has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

[0296] The foregoing description of the specific aspects will so fully reveal the general nature of the disclosure that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific aspects, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed aspects, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0297] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary aspects, but should be defined only in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising (a) a first region encoding a payload and (b) a second region comprising a sensor that is capable of specifically recognizing a marker expressed by an immune cell (immune cell detargeting sensor), wherein the recognition of the marker by the immune cell detargeting sensor results in reduced expression of the payload as compared to a reference cell (*e.g.*, non-immune cell).

2. The isolated polynucleotide of claim 1, wherein the immune cell comprises hematopoietic stem cells and/or derivatives thereof.

3. The isolated polynucleotide of claim 1 or 2, wherein the immune cell comprises T cells, B cells, natural killer (NK) cells, macrophages, monocytes, dendritic cells, neutrophils, basophils, eosinophils, or combinations thereof.

4. The isolated polynucleotide of any one of claims 1 to 3, wherein the immune cell is present within a lymphoid tissue at a greater quantity as compared to a non-lymphoid tissue.

5. The isolated polynucleotide of claim 4, wherein the number of the immune cell present within the lymphoid tissue is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold or more, as compared to the corresponding value for the non-lymphoid tissue.

6. The isolated polynucleotide of claim 4 or 5, wherein the lymphoid tissue comprises a spleen, lymph node, or both.

7. The isolated polynucleotide of any one of claims 1 to 6, wherein the second region comprises multiple immune cell detargeting sensors.

8. The isolated polynucleotide of claim 7, wherein each of the multiple immune cell detargeting sensors is the same.

9. The isolated polynucleotide of claim 7, wherein one or more of the multiple immune cell detargeting sensors are different.

10. The isolated polynucleotide of any one of claims 1 to 9 wherein the marker comprises a microRNA.

11. The isolated polynucleotide of claim 10, wherein the microRNA comprises a miR-142.

12. The isolated polynucleotide of any one of claims 1 to 11, wherein the payload comprises a cytokine, a ligand-binding protein, or both.

13. The isolated polynucleotide of claim 12, wherein the cytokine comprises an interleukin (IL)-12 protein.

14. The isolated polynucleotide of any one of claims 1 to 13, which further comprises: (1) an Internal Ribosome Entry Site (IRES), (2) an untranslated region (UTR), (3) a sequence encoding a signal peptide, (4) a translation initiation sequence, (5) a polyA sequence, (6) a sequence encoding a RNA binding protein, (7) a 5'-cap, (8) a sequence encoding a 2A ribosome skip peptide, (9) a translation enhancer element, or (10) any combination of (1) to (9).

15. An isolated polynucleotide comprising (a) a first region encoding a payload and (b) a second region comprising a sensor that is capable of specifically recognizing a marker expressed within a spleen (spleen detargeting sensor), and wherein the recognition of the marker by the spleen detargeting sensor reduces the expression of the payload.

16. The isolated polynucleotide of claim 15, wherein the marker comprises a microRNA.

17. The isolated polynucleotide of claim 16, wherein the microRNA comprises a miR-142.

18. The isolated polynucleotide of any one of claims 15 to 17, wherein the payload comprises a cytokine, a ligand-binding protein, or both.

19. The isolated polynucleotide of claim 18, wherein the cytokine comprises an interleukin (IL)-12 protein.

20. The isolated polynucleotide of any one of claims 15 to 19, wherein the second region comprises multiple spleen detargeting sensors.

21. The isolated polynucleotide of claim 20, wherein each of the multiple spleen detargeting sensors are the same.

22. The isolated polynucleotide of claim 20, wherein one or more of the multiple spleen detargeting sensors are different.

23. The isolated polynucleotide of any one of claims 1 to 22, which further comprises: (1) an Internal Ribosome Entry Site (IRES), (2) an untranslated region (UTR), (3) a sequence encoding a signal peptide, (4) a translation initiation sequence, (5) a polyA sequence, (6) a sequence encoding a RNA binding protein, (7) a 5'-cap, (8) a sequence encoding a 2A ribosome skip peptide, (9) a translation enhancer element, or (10) any combination of (1) to (9).

24. A synthetic circuit comprising the isolated polynucleotide of any one of claims 1 to 23.

25. A synthetic circuit consisting essentially of the isolated polynucleotide of any one of claims 1 to 23.

26. A synthetic circuit consisting of the isolated polynucleotide of any one of claims 1 to 23.

27. A replicon comprising the isolated polynucleotide of any one of claims 1 to 23 or the synthetic circuit of any one of claims 24 to 26, wherein the replicon is self-replicating.

28. The replicon of claim 27, which is derived from an alpha virus.

29. The replicon of claim 28, wherein the alpha virus comprises a Venezuelan equine encephalitis (VEE) virus.

30. A nanoparticle comprising (a) the isolated polynucleotide of any one of claims 1 to 23, the synthetic circuit of any one of claims 24 to 26, or the replicon of any one of claims 27 to 29, and (b) a lipid and/or lipid-like material.

31. The nanoparticle of claim 30, wherein the lipid comprises an ionizable lipid, cationic lipid, lipidoid, non-cationic helper lipid, phospholipid, sterol or other structural lipids, or combinations thereof.

32. The nanoparticle of claim 31, wherein the ionizable lipid comprises ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315), heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (SM-102), heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Lipid 5), di((*Z*)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), 3-(didodecylamino)-N1,N1,4

tridodecyl-1-piperazineethanamine (KL10), Nl-[2 (didodecylamino)ethyl]-N1,N4,N4-tridodecyl 1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2 dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), or combinations thereof.

33. The nanoparticle of claim 31, wherein the cationic lipid comprises l,2-dioleoyl-3 -trimethylammonium-propane (DOTAP), lipofectamine, N-[l-(2,3- dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), l-[2- (oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTEVI), 2,3- dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l ,2-dimyristyloxyprop-3 -yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DMRIE), N-(l,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DORIE), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), l,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), l,2-distearoyl-3-trimethylammonium-propane (DSTAP), l,2-dipalmitoyl-3 -trimethylammonium-propane (DPTAP), l,2-dilinoleoyl-3 -trimethylammonium-propane (DLTAP), l,2-dimyristoyl-3-trimethylammonium-propane (DMTAP), 1,2-distearoyl -sn-glycero-3- ethylphosphocholine (DSePC), l,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC), 1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC), 1,2-dioleoyl-sn- glycero-3-ethylphosphocholine (DOePC), l,2-di-(9Z-tetradecenoyl)-sn-glycero-3- ethylphosphocholine (14: 1 EPC), l-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18: 1 EPC), or any combination thereof.

34. The nanoparticle of claim 31, wherein the lipidoid comprises 1,1′-((2-(4-(2-((2-(bis(2-hydroxydodecyl) amino)ethyl) (2- hydroxydodecyl)amino)ethyl) piperazin-1-yl)ethyl)azanediyl) bis(dodecan-2-ol) (C12-200), 3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine2,5-dione (cKK-E12), tetrakis(8-methylnonyl) 3,3′,3″,3‴- (((methylazanediyl) bis(propane-3,1 diyl))bis (azanetriyl))tetrapropionate ($306Oi_{10}$), G0-C14, 5A2-SC8, 3,6-bis(4-(bis((9Z,12Z)-2-

hydroxyoctadeca9,12-dien-1-yl)amino)butyl)piperazine-2,5-dione (OF-02), (((3,6-dioxopiperazine-2,5-diyl)bis (butane-4,1-diyl))bis(azanetriyl))tetrakis(ethane2,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-Deg-Lin), (((3,6-dioxopiperazine-2,5-diyl)bis(butane-4,1-diyl)) bis(azanetriyl))tetrakis (butane-4,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-C4-Deg-Lin), N1,N3,N5-tris(3-(didodecylamino)propyl)benzene1,3,5-tricarboxamide (TT3), Hexa(octan-3-yl) 9,9′,9″,9‴,9⁗,9⁗′- ((((benzene-1,3,5-tricarbonyl)ris(azanediyl)) tris (propane-3,1-diyl))tris(azanetriyl))hexanonanoate (FTT5), PL-1, 98N12-5, ethyl 5,5-di((Z)-heptadec-8-en-1-yl)-1-(3-(pyrrolidin-1-yl)propyl)-2,5-dihydro-1H-imidazole-2-carboxylate (A2-Iso5-2DC18 (A2)), A12-Iso5−2DC18 (A12), or any combination thereof.

35. The nanoparticle of claim 31, wherein the phospholipid is selected from the group consisting of 1,2-dilinoleoyl-sn-glycero-3 phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanola mine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (14:0-16:0 PC, MPPC), 1-myristoyl-2 stearoyl-sn-glycero-3-phosphocholine (14:0-18:0 PC, MSPC), 1-palmitoyl 2-acetyl-sn-glycero-3-phosphocholine (16:0-02:0 PC), 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine (16:0-14:0 PC, PMPC), 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (16:0-18:0 PC, PSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18: 1 PC, POPC), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (16:0-18:2 PC, PLPC), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (16:0-20:4 PC), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (14:0-22:6 PC), 1-stearoyl-2-

myristoyl-sn-glycero-3-phosphocholine (18:0-14:0 PC, SMPC), 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18:0-16:0 PC, SPPC), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (18:0-18: 1 PC, SOPC), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (18:0-18:2 PC), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (18:0-20:4 PC), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (18:0-22:6 PC), 1-oleoyl-2-myristoyl-sn-glycero-3-phosphocholine (18: 1-14:0 PC, OMPC), 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18: 1-16:0 PC, OPPC), 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (18: 1-18:0 PC, OSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (16:0- 18: 1 PE, POPE), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (16:0-18:2 PE), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (16:0-20:4 PE), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (16:0-22:6 PE), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (18:0-18: 1 PE), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (18:0-18:2 PE), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (18:0-20:4 PE), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (18:0-22:6 PE), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), and any combination thereof.

36. The nanoparticle of claim 31, wherein the sterol comprises a cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and combinations thereof.

37. The nanoparticle of claim 31, wherein the lipid and/or lipid-like material is pegylated.

38. The nanoparticle of any one of claims 30 to 37, which further comprises a targeting ligand.

39. A pharmaceutical composition comprising (a) the isolated polynucleotide of any one of claims 1 to 23, the synthetic circuit of any one of claims 24 to 26, the replicon of any one of claims 27 to 29, the nanoparticle of any one of claims 30 to 38, and (b) a pharmaceutically acceptable carrier.

40. A method of reducing an expression of a payload (payload expression) within an immune cell of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 23, (b) the synthetic circuit of any one of claims 24 to 26, (c) the replicon of any one of claims 27 to 29, (d) the nanoparticle of any one of claims 30 to 38, (e) the pharmaceutical composition of claim 39, or (f) any combination of (a) to (e).

41. The method of claim 40, wherein reducing the payload expression comprises (a) reducing the amount of payload that is expressed by the immune cell, (b) reducing the duration of payload expression by the immune cell, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell detargeting sensor).

42. The method of claim 41, wherein after the administration, the amount of payload that is expressed by the immune cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

43. The method of claim 41 or 42, wherein after the administration, the duration of the payload expression by the immune cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

44. The method of any one of claims 40 to 43, wherein the immune cell comprises a T cell, macrophage, or both.

45. A method of selectively expressing a payload in a non-immune cell of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 23, (b) the synthetic circuit of any one of claims 24 to 26, (c) the replicon of any one of claims 27 to 29, (d) the nanoparticle of any one of claims 30 to 38, (e) the pharmaceutical composition of claim 39, or (f) any combination of (a) to (e).

46. The method of claim 45, wherein after the administration, (a) an amount of the payload that is expressed in the non-immune cell, (b) a duration of the expression of the payload in the non-immune cell, or (c) both (a) and (b) are increased, as compared to the corresponding amount and/or duration in the immune cell of the subject.

47. A method of reducing an expression of a payload (payload expression) within a lymphoid tissue of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 23, (b) the synthetic circuit of any one of claims 24 to 26,

(c) the replicon of any one of claims 27 to 29, (d) the nanoparticle of any one of claims 30 to 38, (e) the pharmaceutical composition of claim 39, or (f) any combination of (a) to (e).

48. The method of claim 47, wherein the lymphoid tissue comprises a spleen, lymph node, or both.

49. The method of claim 47 or 48, wherein reducing the payload expression comprises (a) reducing the amount of payload that is expressed in the lymphoid tissue, (b) reducing the duration of payload expression in the lymphoid tissue, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell detargeting sensor).

50. The method of claim 49, wherein after the administration, the amount of payload that is expressed in the lymphoid tissue is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

51. The method of claim 49 or 50, wherein after the administration, the duration of the payload expression in the lymphoid tissue is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

52. A method of reducing an expression of a payload (payload expression) within the spleen of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 23, (b) the synthetic circuit of any one of claims 24 to 26, (c) the replicon of any one of claims 27 to 29, (d) the nanoparticle of any one of claims 30 to 38, (e) the pharmaceutical composition of claim 39, or (f) any combination of (a) to (e).

53. The method of claim 52, wherein reducing the payload expression comprises (a) reducing the amount of payload that is expressed in the spleen, (b) reducing the duration of payload expression in the spleen, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the spleen detargeting sensor).

54. The method of claim 53, wherein after the administration, the amount of payload that is expressed in the spleen is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

55. The method of claim 54, wherein after the administration, the payload is not expressed in the spleen.

56. The method of claim 53 or 54, wherein after the administration, the duration of the payload expression in the spleen is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

57. A method of selectively expressing a payload in a non-lymphoid tissue of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 23, (b) the synthetic circuit of any one of claims 24 to 26, (c) the replicon of any one of claims 27 to 29, (d) the nanoparticle of any one of claims 30 to 38, (e) the pharmaceutical composition of claim 39, or (f) any combination of (a) to (e).

58. The method of claim 57, wherein after the administration, (a) an amount of the payload that is expressed in the non-lymphoid tissue, (b) a duration of the expression of the payload in the non-spleen tissue, or (c) both (a) and (b) are increased, as compared to the corresponding amount and/or duration in the spleen of the subject.

59. A method of selectively expressing a payload in a non-spleen tissue of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 23, (b) the synthetic circuit of any one of claims 24 to 26, (c) the replicon of any one of claims 27 to 29, (d) the nanoparticle of any one of claims 30 to 38, (e) the pharmaceutical composition of claim 25, or (f) any combination of (a) to (e).

60. The method of claim 59, wherein after the administration, (a) an amount of the payload that is expressed in the non-spleen tissue, (b) a duration of the expression of the payload in the non-spleen tissue, or (c) both (a) and (b) are increased, as compared to the corresponding amount and/or duration in the spleen of the subject.

61. A method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 23, (b) the synthetic circuit of any one of claims 24 to 26, (c) the replicon of any one of claims 27 to 29, (d) the nanoparticle of any one of claims 30 to 38, (e) the pharmaceutical composition of claim 39, or (f) any combination of (a) to (e).

62. The method of claim 61, wherein the disease or disorder comprises a cancer, inflammatory disorders, monogenic disorders, neurological disorders, psychiatric disorders, or combinations thereof.

63. The method of claim 62, wherein the cancer comprises a melanoma, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine cancer, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, head and neck cancer, or combinations thereof.

64. The method of any one of claims 40 to 63, wherein the isolated polynucleotide, the synthetic circuit, the replicon, the nanoparticle, and/or the pharmaceutical composition is administered to the subject via intravenous, intratumoral, intrathecal, intramuscular, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration.

65. The method of any one of claims 40 to 64, further comprising administering to the subject an additional therapeutic agent.

FIG. 1A

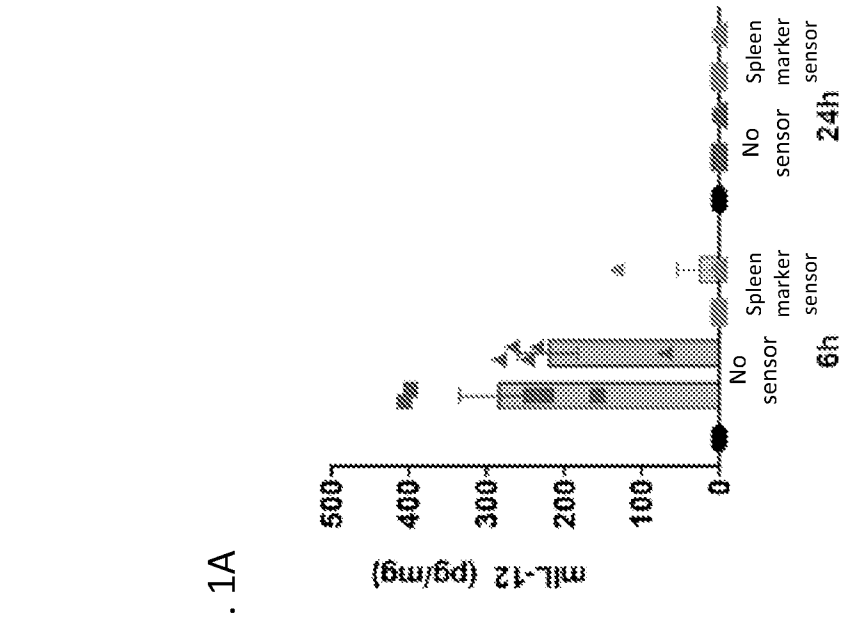
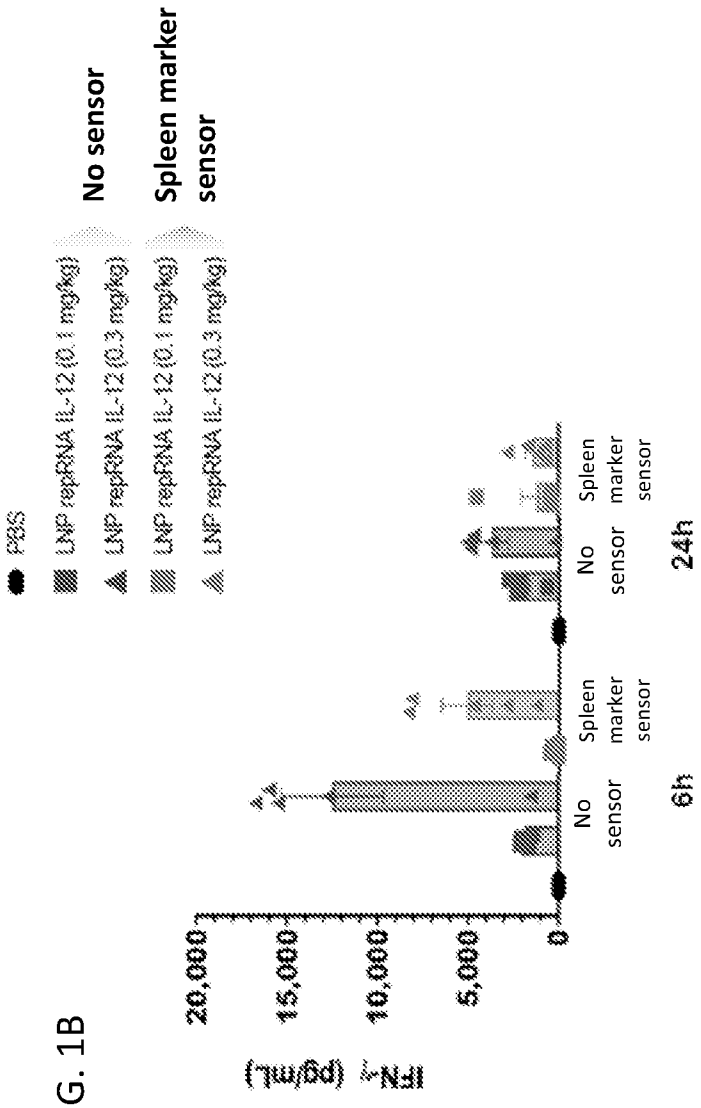

IL-12 (pg/mg)
500
400
300
200
100
0
No sensor
Spleen marker sensor
No sensor
Spleen marker sensor
6h
24h

FIG. 1B


PBS
LNP repRNA IL-12 (0.1 mg/kg)
LNP repRNA IL-12 (0.3 mg/kg)
LNP repRNA IL-12 (0.1 mg/kg)
LNP repRNA IL-12 (0.3 mg/kg)
No sensor
Spleen marker sensor
IFN-γ (pg/mL)
20,000
15,000
10,000
5,000
0
No sensor
Spleen marker sensor
No sensor
Spleen marker sensor
6h
24h

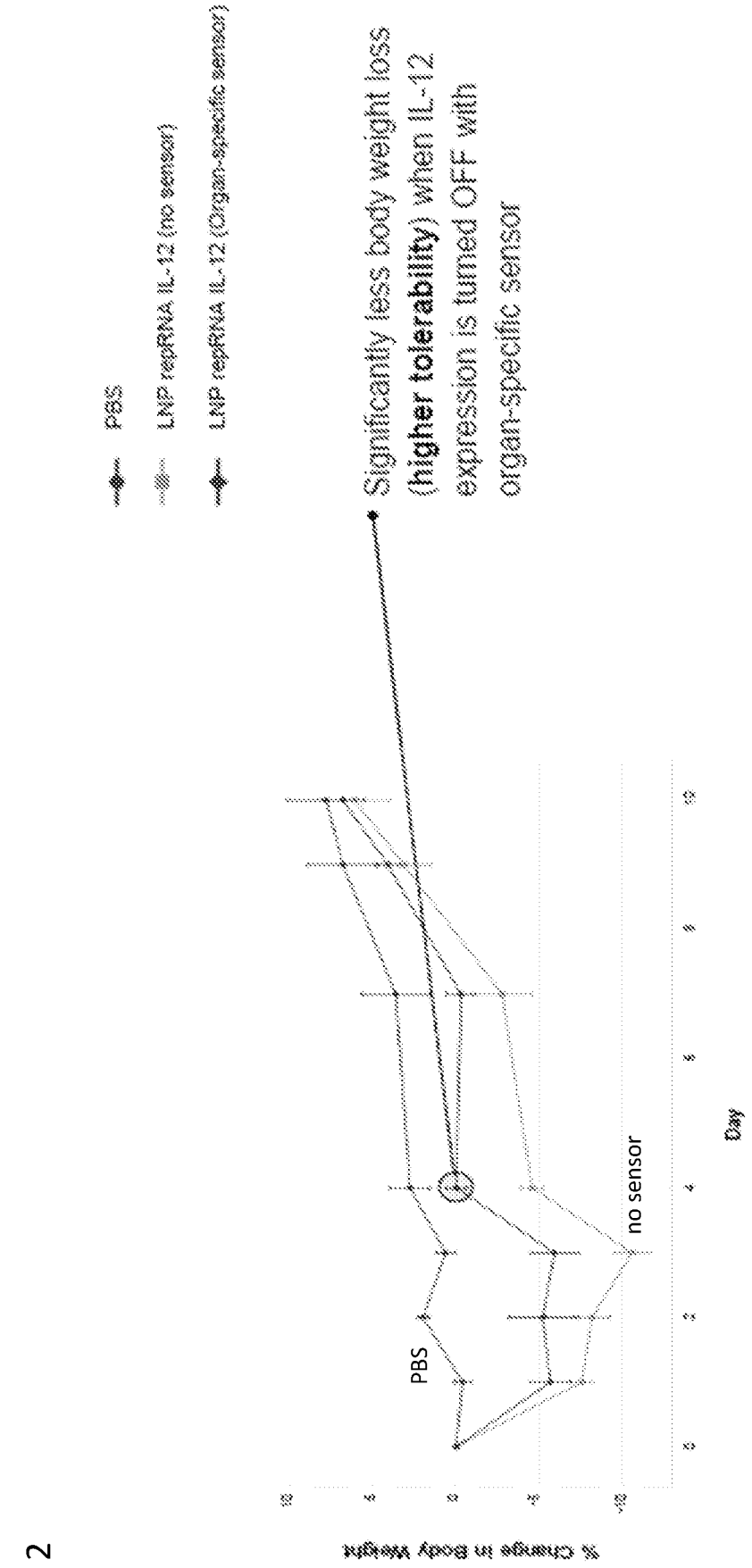

PBS
LNP repRNA IL-12 (no sensor)
LNP repRNA IL-12 (Organ-specific sensor)
Significantly less body weight loss (higher tolerability) when IL-12 expression is turned OFF with organ-specific sensor
PBS
no sensor
% Change in Body Weight
Day

FIG. 2

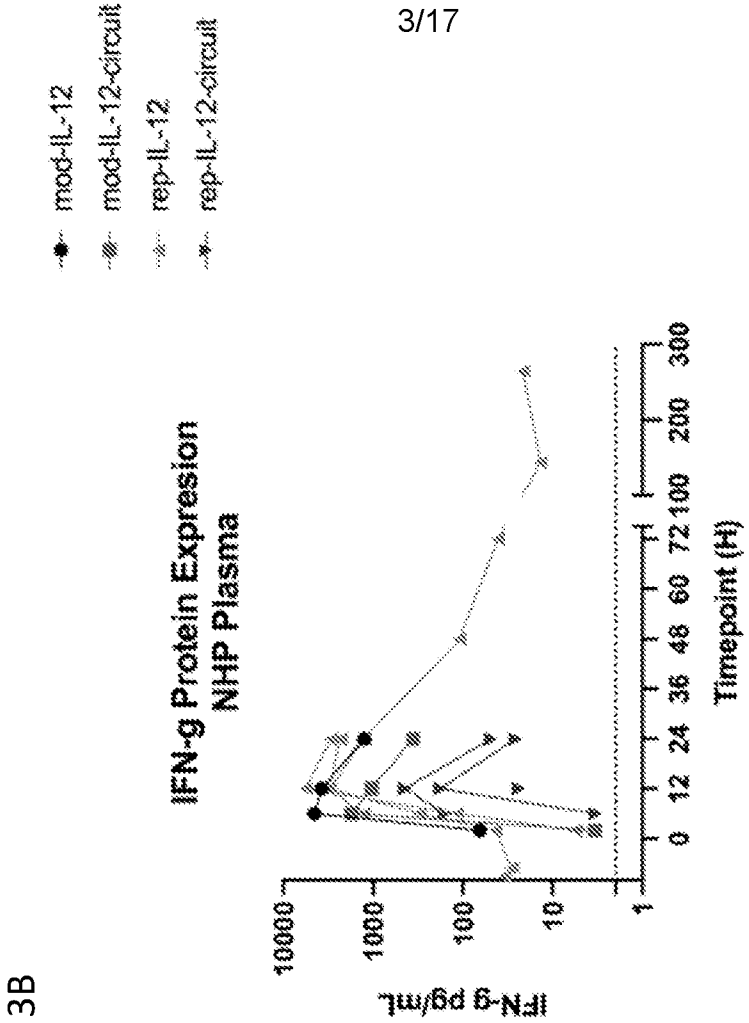

mod-IL-12
mod-IL-12-circuit
rep-IL-12
rep-IL-12-circuit
IFN-g Protein Expresion
NHP Plasma
IFN-g pg/mL
10000
1000
100
10
1
0 12 24 36 48 60 72 100 200 300
Timepoint (H)

FIG. 3B

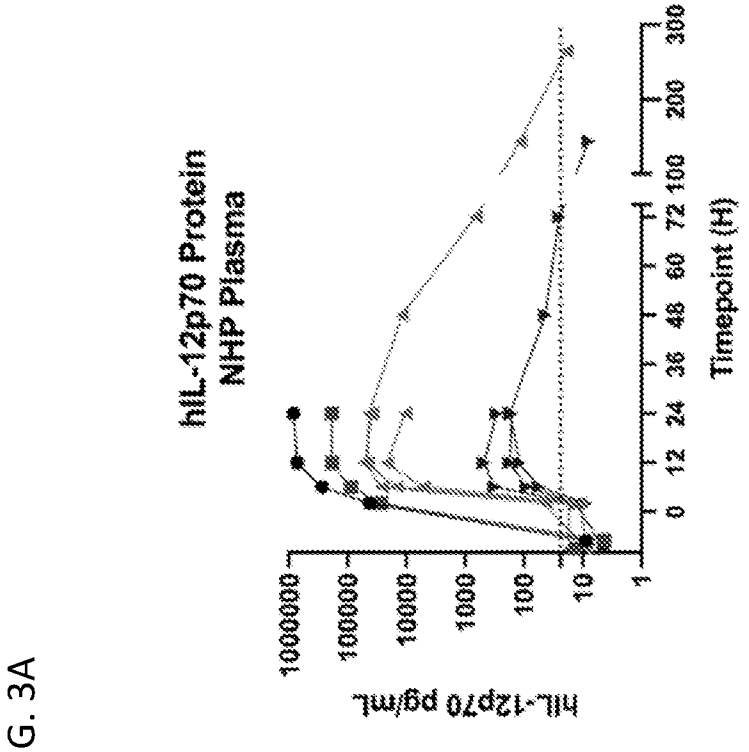

hIL-12p70 Protein
NHP Plasma
hIL-12p70 pg/mL
1000000
100000
10000
1000
100
10
1
0 12 24 36 48 60 72 100 200 300
Timepoint (H)

FIG. 3A

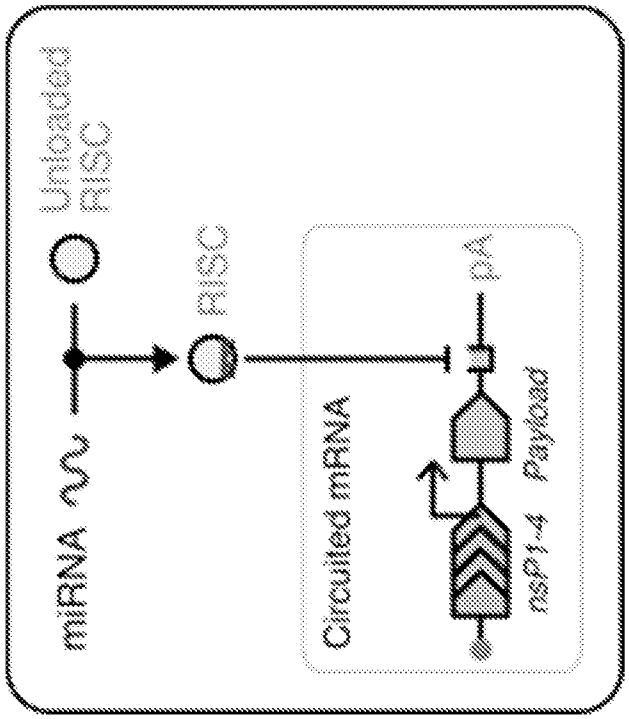


FIG. 4

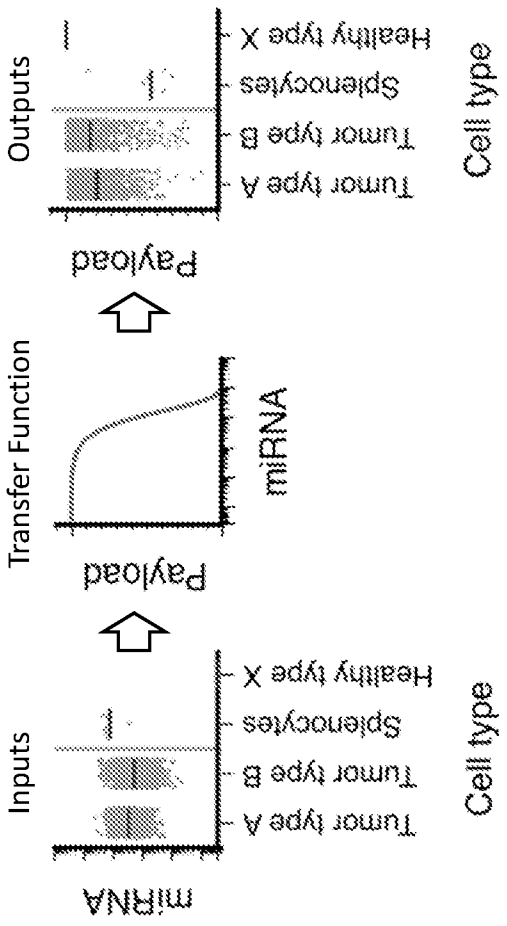

miRNA
Tumor type A
Tumor type B
Splenocytes
Healthy type X
Cell type
Inputs
Payload
Transfer Function
miRNA
Payload
Tumor type A
Tumor type B
Splenocytes
Healthy type X
Cell type
Outputs

FIG. 5

FIG. 6B

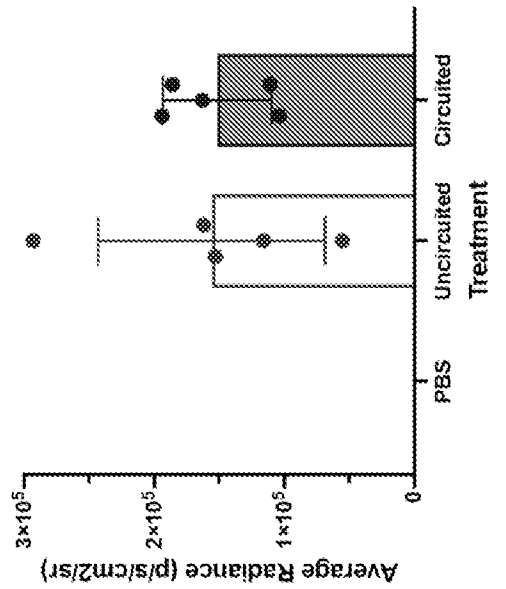

Payload Expression in Non-Spleen
Average Radiance (p/s/cm2/sr)
3×10^5
2×10^5
1×10^5
0
PBS
Uncirculted
Circulted
Treatment

FIG. 6A

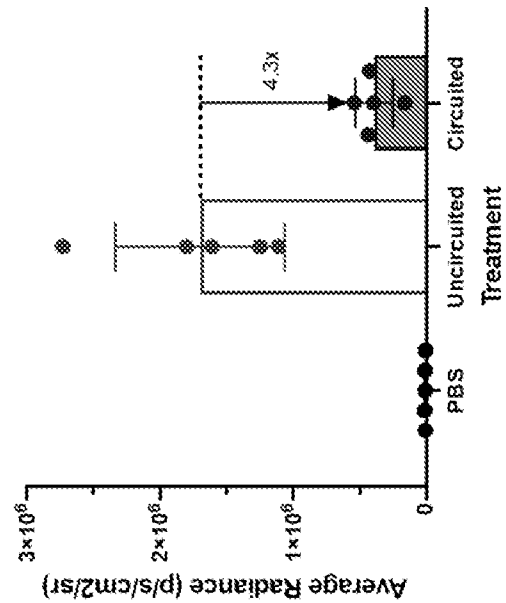

Payload Expression in Spleen
Average Radiance (p/s/cm2/sr)
3×10^6
2×10^6
1×10^6
0
4.3x
PBS
Uncirculted
Circulted
Treatment

FIG. 7A

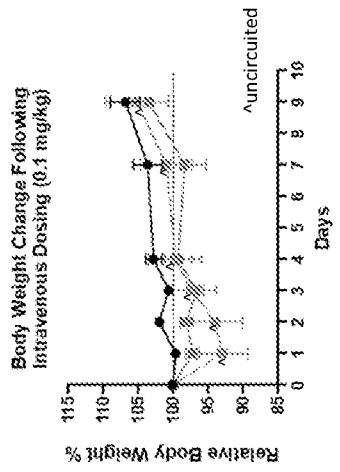


FIG. 7B

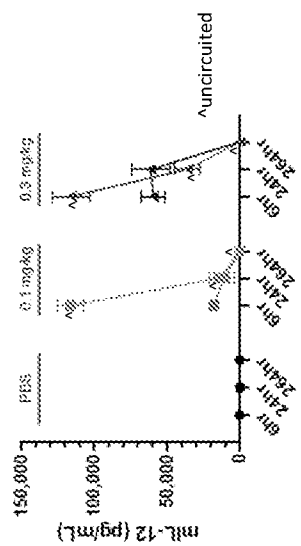


FIG. 7C

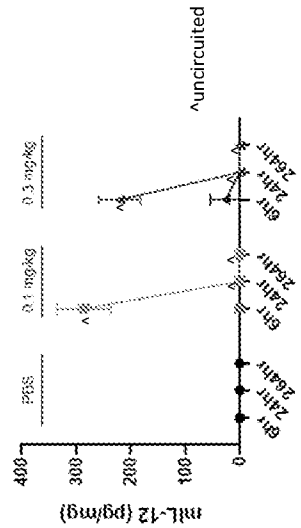


FIG. 7D

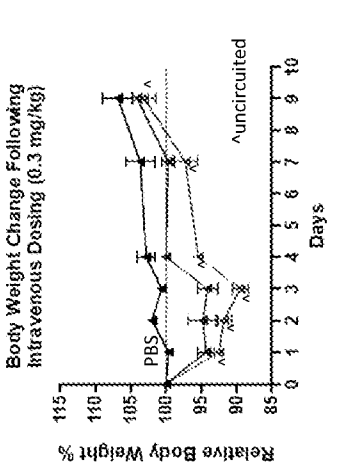


FIG. 7E

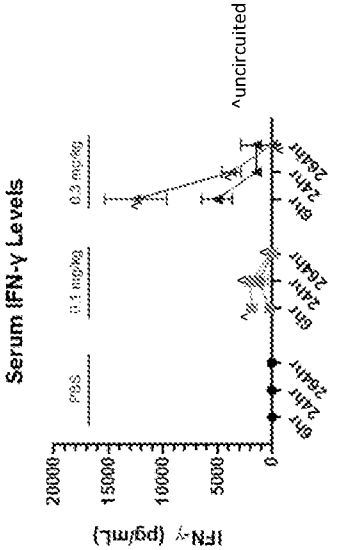
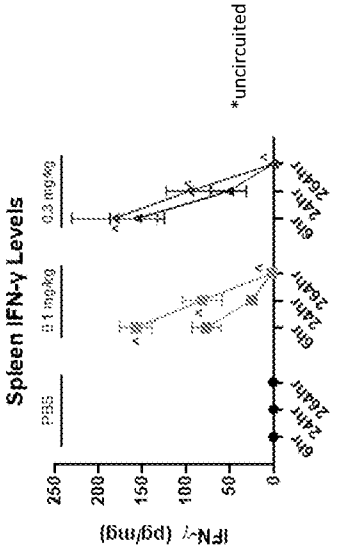


FIG. 7F



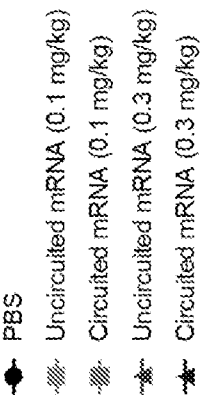


FIG. 8A

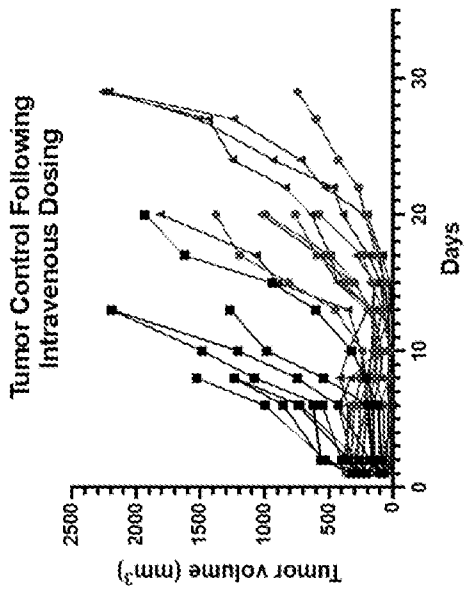


FIG. 8B

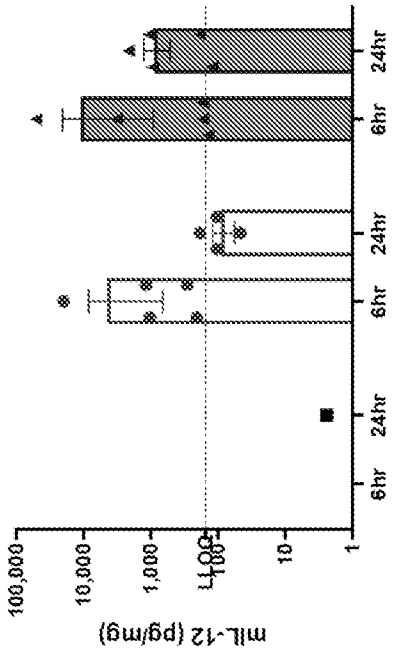
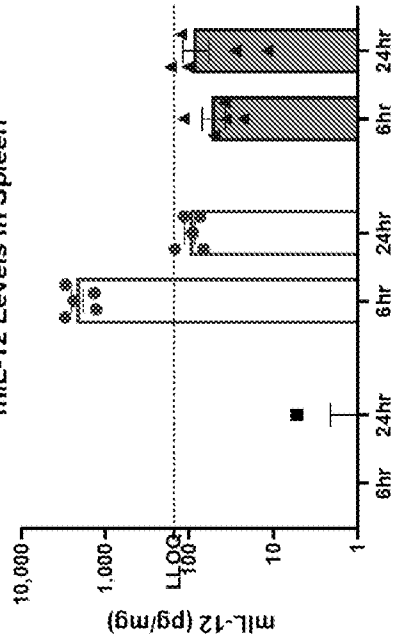


FIG. 8C



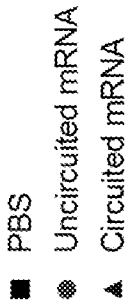


FIG. 9A

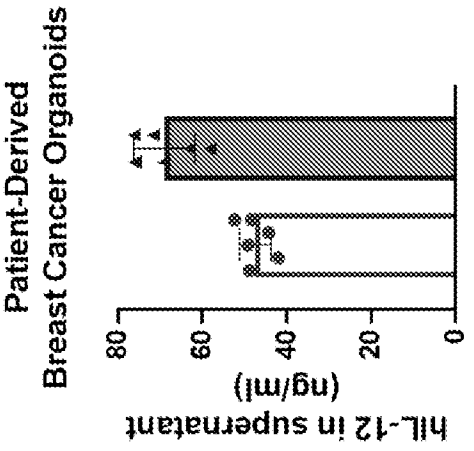
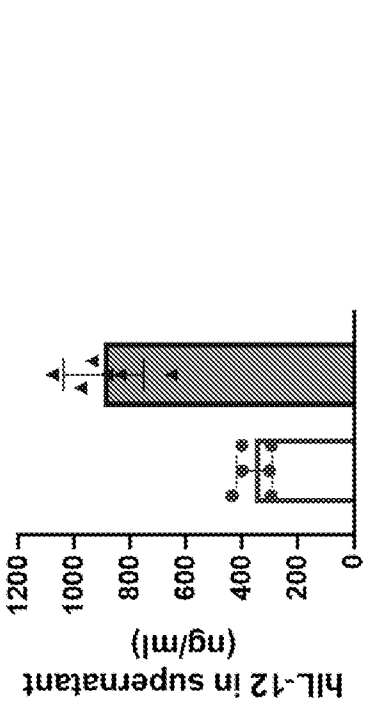

Patient-Derived
Breast Cancer Organoids
hIL-12 in supernatant
(ng/ml)
0
20
40
60
80

FIG. 9B


Uncircuited mRNA
Circuited mRNA
A549 Cells
hIL-12 in supernatant
(ng/ml)
0
200
400
600
800
1000
1200

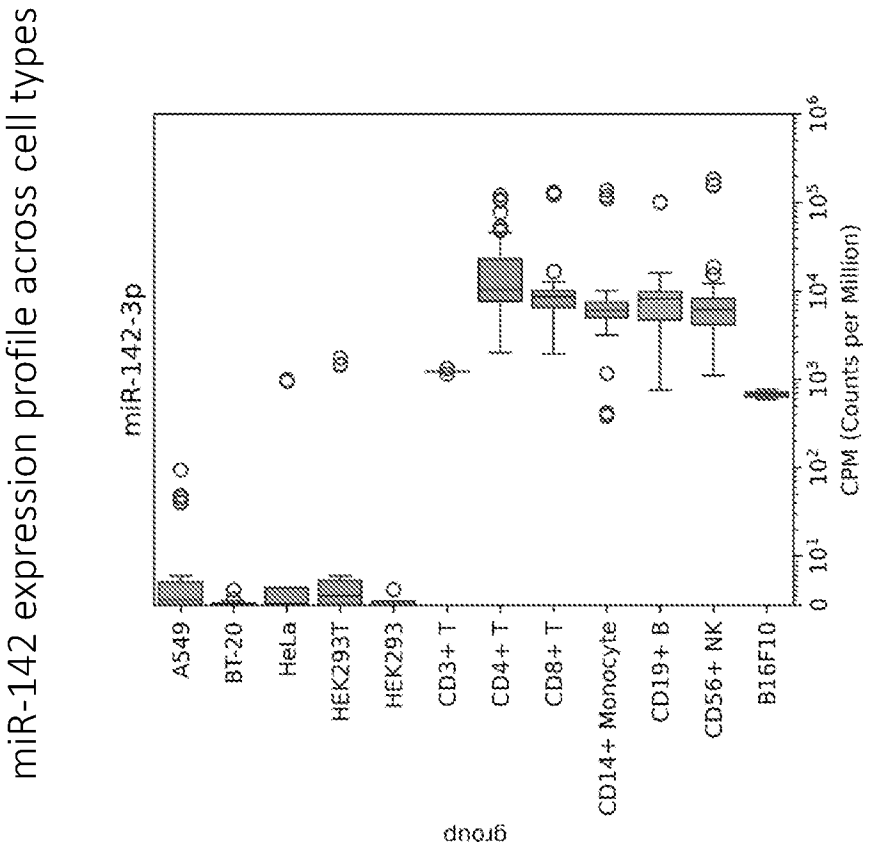

miR-142 expression profile across cell types
miR-142-3p
A549
BT-20
HeLa
HEK293T
HEK293
CD3+ T
CD4+ T
CD8+ T
CD14+ Monocyte
CD19+ B
CD56+ NK
B16F10
group
0
10^1
10^2
10^3
10^4
10^5
10^6
CPM (Counts per Million)

FIG. 10

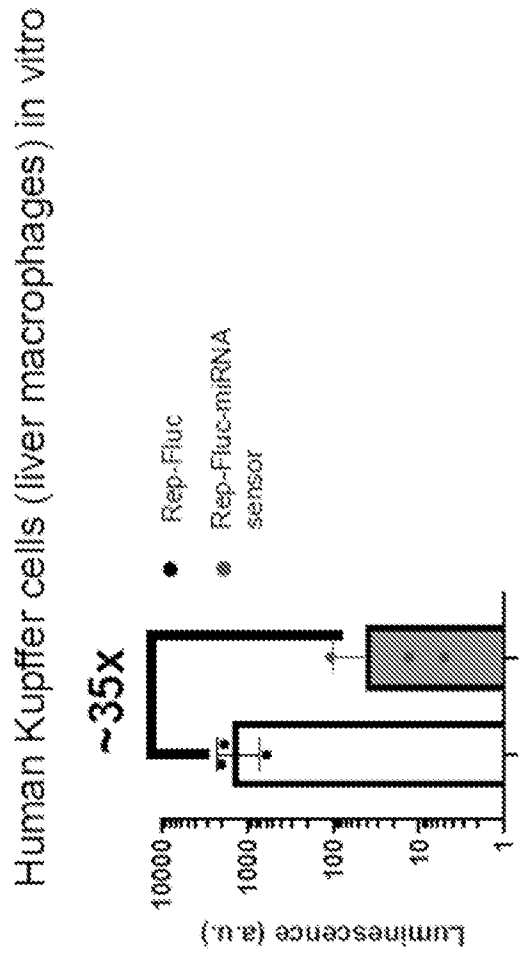

Human Kupffer cells (liver macrophages) in vitro
Rep-Fluc
Rep-Fluc-miRNA sensor
~35x
Luminescence (a.u.)
10000
1000
100
10
1

FIG. 11

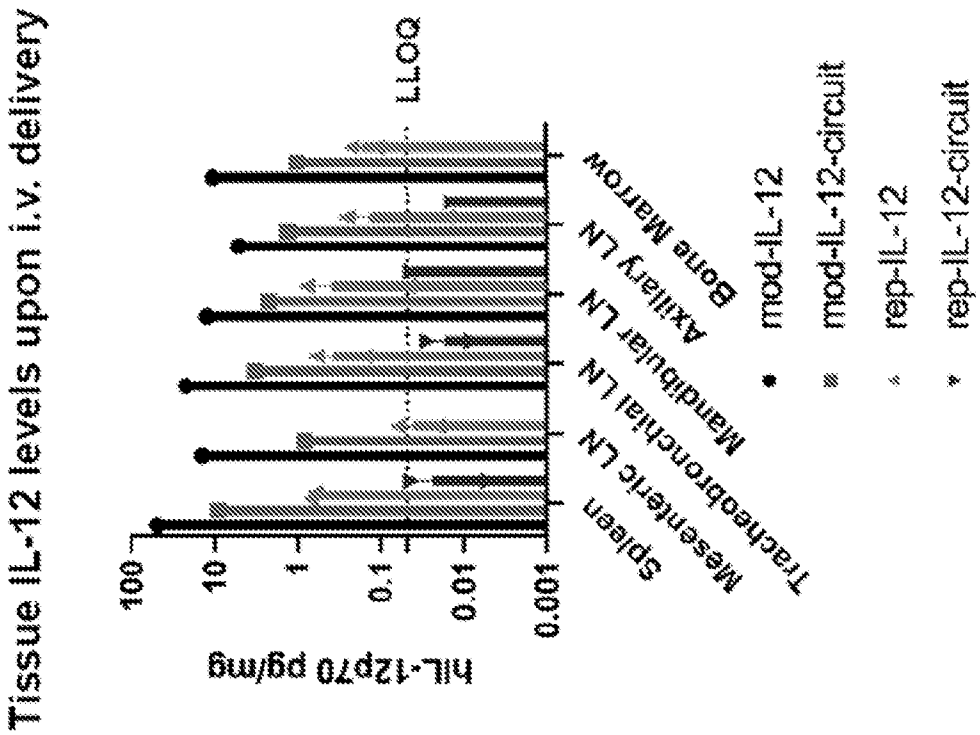

Tissue IL-12 levels upon i.v. delivery
hIL-12p70 pg/mg
100
10
1
0.1
0.01
0.001
LLOQ
Spleen
Mesenteric LN
Tracheobronchial LN
Mandibular LN
Axillary LN
Bone Marrow
mod-IL-12
mod-IL-12-circuit
rep-IL-12
rep-IL-12-circuit

FIG. 12

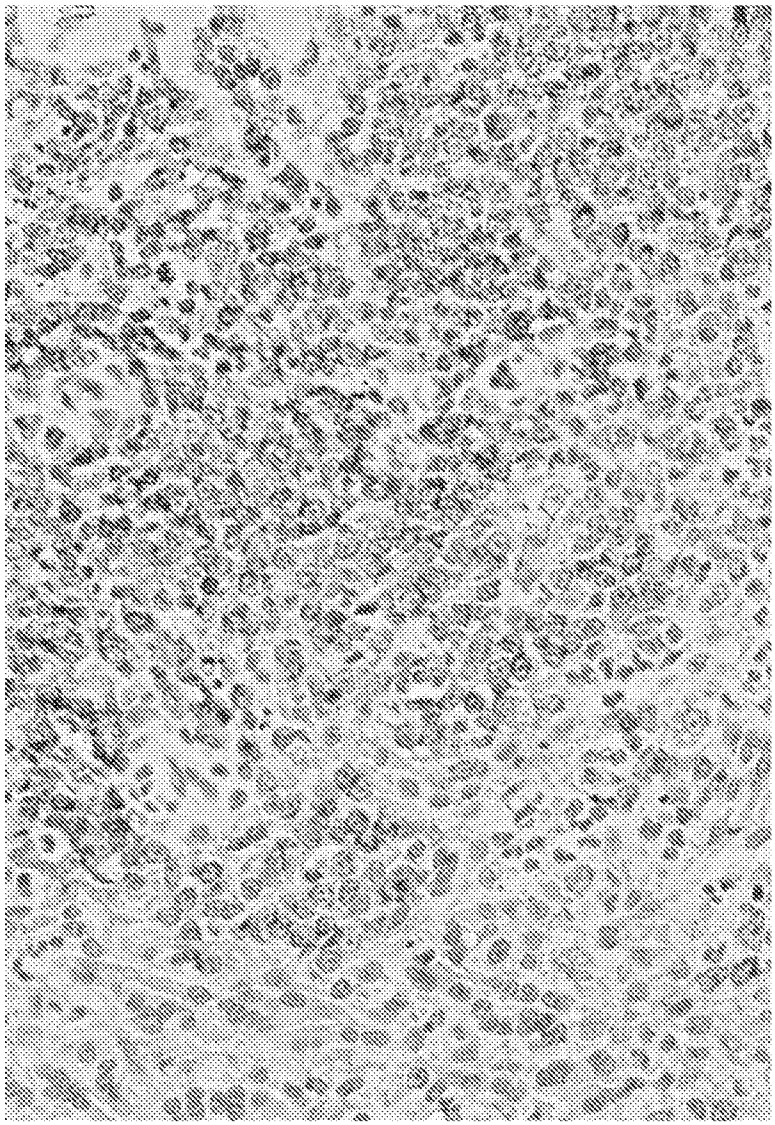

miR-142-3p expression within NHP spleen showing staining from macrophages and lymphocytes

FIG. 13

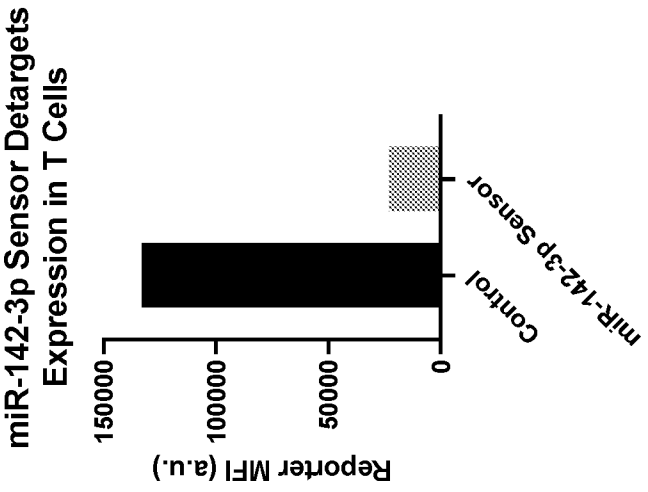

miR-142-3p Sensor Detargets Expression in T Cells
Reporter MFI (a.u.)
150000
100000
50000
0
Control
miR-142-3p Sensor

FIG. 14

FIG. 15A

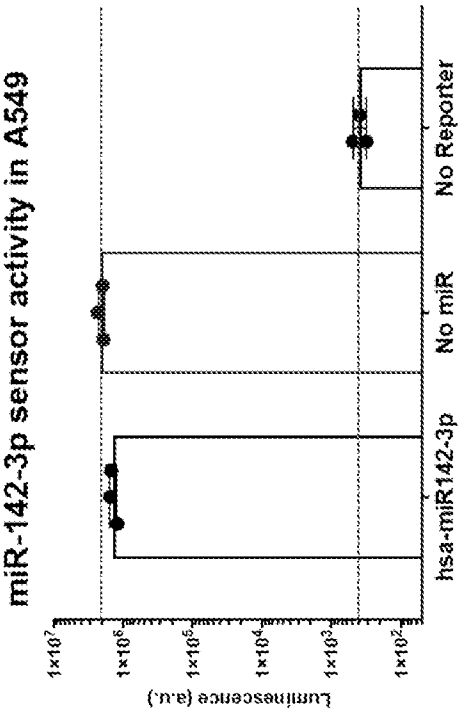
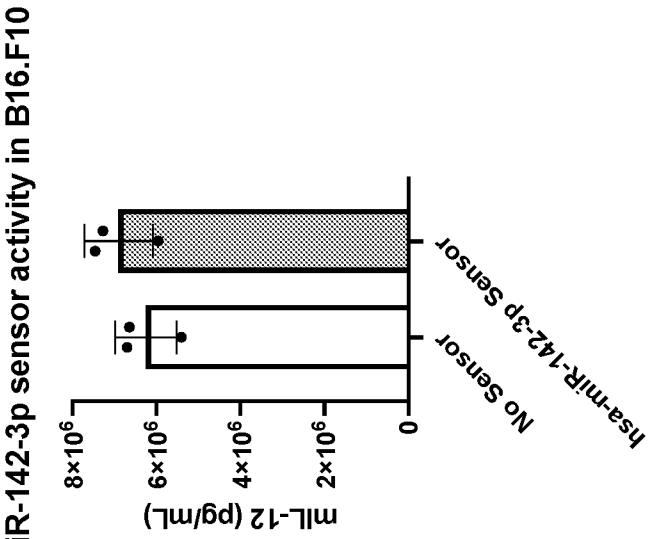


FIG. 15B



Schematic of plasmid encoding VEE-derived replicon

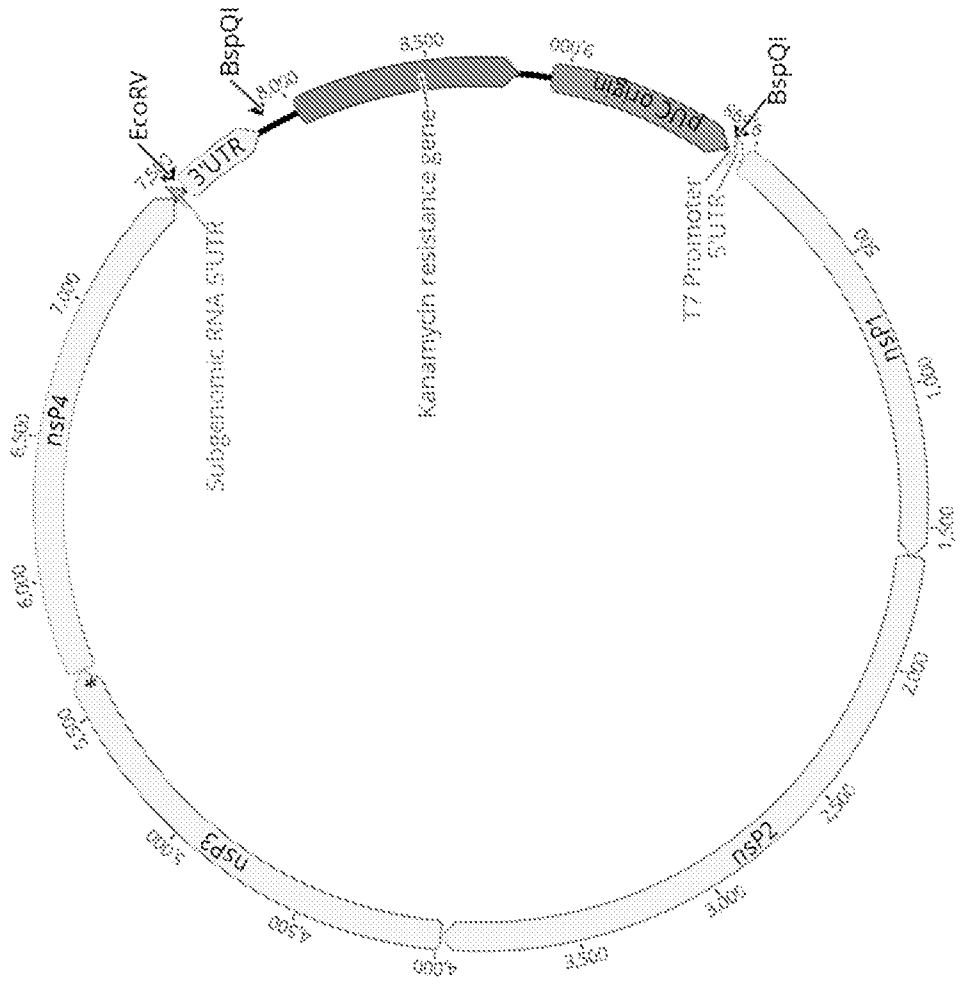


FIG. 16

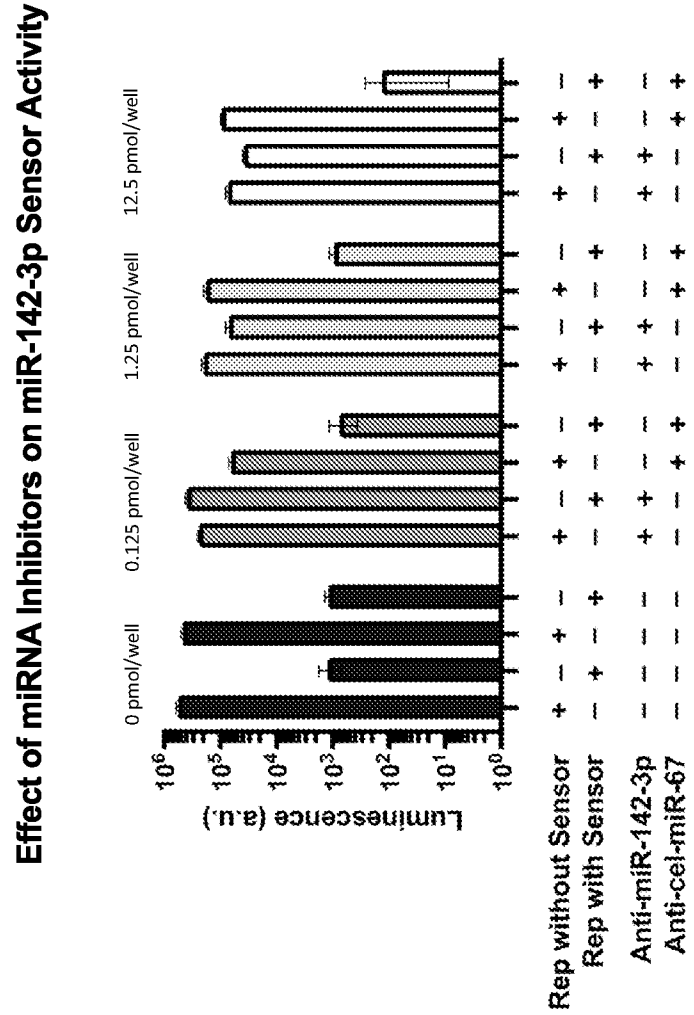

Effect of miRNA Inhibitors on miR-142-3p Sensor Activity
miRNA Inhibitor
0 pmol/well
0.125 pmol/well
1.25 pmol/well
12.5 pmol/well
Luminescence (a.u.)
Rep without Sensor
Rep with Sensor
Anti-miR-142-3p
Anti-cel-miR-67

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/039618

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K48/00 C12Q1/6897 C12N15/63
ADD. C12N15/85 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2022/150712 A1 (STRAND THERAPEUTICS INC [US]) 14 July 2022 (2022-07-14) cited in the application paragraphs [0057], [0067], [0124], [0137], [0140] - [0142], [0144] - [0145], [0311]; claims 88-91,93-110,115,117-119	1-65
X	WO 2022/266083 A2 (MODERNATX INC [US]) 22 December 2022 (2022-12-22)	1-11, 14-17, 20-65
A	PG 24,105,106,109,115; claims 1,10,12,17; figure 1; example 6; table 5; sequence 76	12,13, 18,19

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 September 2024	04/10/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kosten, Jonas

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/039618

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/039618

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 7 047002 B2 (MODERNATX INC) 4 April 2022 (2022-04-04) cited in the application	1-26, 30-65
A	paragraphs [0127] - [0133], [0149], [0661], [0694], [0853]; claims 1,9	27-29

X	WO 2016/040395 A1 (MASSACHUSETTS INST TECHNOLOGY [US]) 17 March 2016 (2016-03-17) page 117; claims 1,9,25,33,40,77,187,202, 274	1-65

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/039618

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022150712 A1	14-07-2022	AR 124599 A1	12-04-2023
		AU 2022205679 A1	06-07-2023
		CA 3204373 A1	14-07-2022
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		CO 2023009063 A2	21-07-2023
		EP 4274846 A1	15-11-2023
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		LT 2922554 T	27-06-2022
		PL 2922554 T3	20-06-2022
		PT 2922554 T	28-06-2022
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		SI 2922554 T1	30-06-2022
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		WO 2014081507 A1	30-05-2014
WO 2016040395 A1	17-03-2016	US 2018296702 A1	18-10-2018
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		WO 2016040395 A1	17-03-2016