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(71) Applicant: **FRED HUTCHINSON CANCER CENTER**
[US/US]; 1100 Fairview Avenue North, Seattle, Washing-
ton 98109 (US).

(72) Inventor: **STEPHAN, Matthias**; 1100 Fairview Avenue
North, Seattle, Washington 98109 (US).

(74) Agent: **WINGER, C. Rachal** et al.; Lee & Hayes PC,
601 W. Riverside Avenue Suite 1400, Spokane, Washing-
ton 99201 (US).

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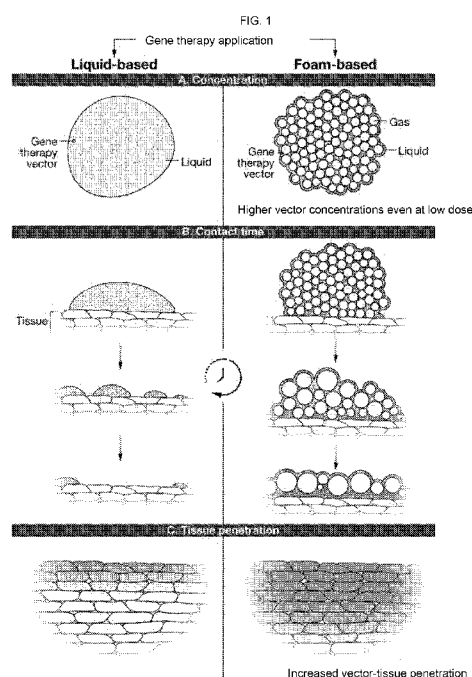
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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: GAS-BUBBLE BASED DEGRADING LIQUID FOAMS TO DELIVER NUCLEIC ACIDS



(57) Abstract: Gas-bubble based degrading liquid foams to deliver nucleic acids are disclosed. The nucleic acids can be delivered for a variety of research, diagnostic, and therapeutic uses, such as in situ CAR T and stem cell engineering. The foams can also be used for administration of therapeutic nucleic acids to anatomical sites, such as cancer sites, through direct application at the site or through the use of edible or drinkable foams in the case of esophageal cancers, the use of rectally applied foams to treat rectal or colorectal cancer, or the use of intraperitoneally injected foam to treat widespread ovarian cancer. The nucleic acids can be delivered in a vector, such as a lipid nanoparticle or a viral vector.

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GAS-BUBBLE BASED DEGRADING LIQUID FOAMS TO DELIVER NUCLEIC ACIDS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/599,438 filed on November 15, 2023, the entire disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE DISCLOSURE

[0002] The current disclosure provides gas-bubble based degrading liquid foams to deliver nucleic acids. The nucleic acids can be delivered for a variety of research, diagnostic, and therapeutic uses, such as in situ CAR T and stem cell engineering. The foams can also be used for administration of therapeutic nucleic acids to anatomical sites, such as cancer sites, through direct application at the site or through the use of edible or drinkable foams in the case of esophageal cancers, the use of rectally applied foams to treat rectal or colorectal cancer, or the use of intraperitoneally injected foam to treat widespread ovarian cancer.

BACKGROUND OF THE DISCLOSURE

[0003] There are numerous conditions and diseases that can be treated by administering nucleic acids. However, therapies based on administering nucleic acids are costly to manufacture and rely on high dosing parameters at target tissues to achieve efficient nucleic acid transfer and therapeutic benefit.

SUMMARY OF THE DISCLOSURE

[0004] The current disclosure provides gas-bubble based degrading liquid foams to deliver nucleic acids. The disclosed foams ensure high-density exposure of target tissue to the nucleic acid. Because foam remains at application sites longer, delivery of nucleic acids to the intended cells is enhanced while minimizing unwanted off-target effects. The higher nucleic acid density combined with longer contact time at an intended delivery site results in higher transfection rates and deeper tissue penetration. The disclosed foam-based nucleic acid delivery can be used for a variety of research, diagnostic, and therapeutic purposes.

BRIEF DESCRIPTION OF THE FIGURES

[0005] Some of the drawings submitted herein may be better understood in color. Applicant considers the color versions of the drawings as part of the original submission and reserves the right to present color images of the drawings in later proceedings.

[0006] FIG. 1. Schematic explaining several advantages of foam as a nucleic acid delivery system in comparison to conventional liquid formulations. (Panel A, Concentration) Foam is mostly gas, so embedded nucleic acid particles become heavily concentrated in the liquid

component, which ensures high-density exposure of target tissue to the nucleic acid vector. (Panel B, Contact Time) Foam remains at application sites longer, thereby enhancing delivery of nucleic acids to the intended cells and minimizing unwanted off-target effects. (Panel C, Tissue Penetration) Higher nucleic acid density combined with longer contact time results in higher transfection rates and deeper tissue penetration.

[0007] FIGs. 2A-2D. Microstructural characterization of LNP-loaded methylcellulose foam. (FIG. 2A) High-resolution foam structure analysis using the Dynamic Foam Analyzer DFA100FSM. Representative images of three independently manufactured foam batches are shown at time points 0 hours, 5 hours and 10 hours. Histograms of bubble size distribution are shown below. Scale bars: 1 mm. (FIG. 2B) Confocal microscopy image of a liquid-filled foam lamella separating two gaseous bubbles. To visualize the distribution of LNPs within the lamella, the lipid envelope was tagged with fluorescent 1,1-dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine (DiD). The shown image represents 50 independent acquisitions. (FIG. 2C) DiD intensities in the foam lamella were analyzed and their spatial distribution plotted in 3D. Data are representative of 20 independently characterized lamellae. (FIG. 2D) Boxplots showing the mean fluorescence intensities of each decile (= distance between two bubbles divided by ten equal segments) and a kernel smoothing curve.

[0008] FIGs. 3A, 3B. Self-expanding nucleic acid delivery foams. (FIG. 3A) Expansion of methylcellulose/xanthan gum foam complemented with 3.75% H_2O_2 and 0.015% Potassium Iodide (KI). The decomposition of H_2O_2 into water and oxygen is catalyzed by the KI. Generated oxygen gas causes the methylcellulose/xanthan gum foam precursor to foam up and expand before degrading over time. (FIG. 3B) Screening of various H_2O_2 and KI concentration and associated foam expansion kinetics. The arrow indicates one formulation resulting in a ten-fold expansion of foam within 90 minutes.

[0009] FIGs. 4A-4E Nucleic acid delivering foam boosts transfection rates and permits precise payload delivery to defined locations. Screening of various foam vehicles to enhance topical nucleic acid delivery. Lipid nanoparticles (LNPs) carrying firefly luciferase-encoding mRNA were mixed with foam precursor solutions composed of 0.8 wt % methylcellulose, sodium caseinate (Na caseinate), or albumin. Prepared foam was added on top of cultured HELA cells, or cells were transfected with an equal dose of LNPs suspended in PBS. The culture plates were incubated for 2 hours either horizontally or in a tilted position, before replenishing media and returning dishes into the incubator horizontally. Luciferase signals were quantified 24 hours later. (FIG. 4A) In vitro bioluminescent imaging of cells exposed to LNP suspension or LNP foam. (FIG. 4B) Box plots summarizing luciferase signals from horizontal transfections. (FIG. 4C) Box plots summarizing

luciferase signals from angled transfections. (FIG. 4D) Quantification of viable (trypan blue-excluding) HELA cells following horizontal or angled transfections with PBS or methylcellulose foam. Pairwise differences between the groups were statistically analyzed using the unpaired, two-tailed Student's t-test. $P < 0.05$ was considered significant (*). Ns., nonsignificant. (FIG. 4E) Photos and corresponding bioluminescence images of cell culture dishes seeded with HELA cells. The text "FRED HUTCH" was written onto cells using syringes loaded either with LNP suspension or LNP foam, while holding the culture plates vertically. Bioluminescent signals were visualized 24 hours later. The shown pictures represent four dishes each with similar outcomes.

[0010] FIGs. 5A-5C. Methylcellulose-based nucleic acid delivery foam is biocompatible. These panels summarize a pathology report prepared by a Comparative Pathologist at Fred Hutchinson Cancer Center. Two days after a bolus injection of methylcellulose foam or PBS into the peritoneal cavity of mice, various key organs close to the peritoneum and blood were isolated for a double-blinded analysis. (FIG. 5A) Serum chemistry and blood counts. Data are mean $\pm$ s.d.; $N = 8$ biologically independent samples. (FIG. 5B) Representative hematoxylin and eosin (H&E)-stained sections of the mesenteric fat from mice (4/10) that exhibited mild regional infiltrates of neutrophils and histiocytes in response to foam injection. (FIG. 5C) Representative image of the marginal zone of a spleen 48 hours after intraperitoneal foam injection.

[0011] FIGs. 6A, 6B. Foam as a carrier system for nucleic acid vectors can improve in situ nucleic acid transfer. Immunocompetent C57BL/6 albino mice were injected intraperitoneally with a single dose of LNPs suspended in PBS or incorporated into methylcellulose foam. To noninvasively track gene expression in vivo, LNPs were loaded with luciferase-encoding mRNA. (FIG. 6A) Representative sequential bioluminescence imaging of gene expression. (FIG. 6B) Box plots showing photon counts from luciferase activity. The boxes represent the mean values and the line in the box represents the median. The bars across the boxes show the minimum and maximum values. Whiskers represent 95% confidence intervals. $N = 10$ biologically independent samples. $P < 0.05$ was considered significant (*).

[0012] FIGs. 7A, 7B. Foam mediates homogeneous tissue transfection. (FIG. 7A) IVIS images of organs and tissues isolated from mice 24 hours following intraperitoneal administration of LNPs containing Luciferase mRNA. Injected LNPs were either suspended in PBS or incorporated into freshly prepared methylcellulose foam. (FIG. 7B) Luciferase signals from each organ. $N = 10$ biologically independent samples. $P < 0.05$ was considered significant (*). Ns., non-significant.

[0013] FIGs. 8A-8C. Foam amplifies lentivirus-mediated nucleic acid transfer while limiting undesirable systemic exposure. C57BL/6 albino mice were injected intraperitoneally with a single dose of 5.25×10^6 lentiviral particles (LV) suspended in PBS or incorporated into methylcellulose

foam. To noninvasively track gene expression in vivo, LV expressed luciferase. (FIG. 8A) Representative sequential bioluminescence imaging of gene expression. (FIG. 8B) Box plots showing photon counts from luciferase activity. N = 10 biologically independent samples. P < 0.05 was considered significant (*). (FIG. 8C) Lentivirus vector concentrations in the peritoneal fluid and peripheral blood 3 hours post injections. N = 10 biologically independent samples. P < 0.05 was considered significant (*)

[0014] FIGs. 9A, 9B. Foam boosts nucleic acid delivery into skin tissue. Following mechanical dermabrasion, 0.5 mL LNP suspension or LNP foam was topically applied to a shaved square area (1.5 × 1.5 cm) of the dorsal skin of C57BL/6 albino mice. To noninvasively track gene expression in vivo, LNPs were loaded with luciferase-encoding mRNA. All mice were single housed to prevent them from licking each other's dorsal skin. (FIG. 9A) Bioluminescence imaging of gene expression after 24 hours. (FIG. 9B) Box plots showing photon counts from luciferase activity. N = 10 biologically independent samples. P < 0.05 was considered significant (*).

[0015] FIG. 10. Schematic depicting targeted nucleic acid delivery foam. To direct foam to specific cell types, a component of the foam starting solution (here, Xanthan Gum) can be functionalized with antigen-specific antibody binding domains or other binding molecules. This can be accomplished using various conjugation techniques, including EDC/NHS coupling of antibody amine and carboxyl groups to xanthan gum carboxyl and amine groups. Targeted vectors can also be used.

[0016] FIGs. 11A, 11B. Schematic illustrations depicting how foams can be freshly prepared and applied to supply new genetic material or change existing DNA in cells. (FIG. 11A) Nonviral or viral vector can be added to a foam precursor in a syringe connected to a second syringe filled with air. The air and foam precursor can be mixed by vigorously drawing the syringe plungers back and forth, for example, 30 times, creating a microfoam consisting of gas bubbles separated by a network of interconnected lamellae. Nucleic acid vectors are concentrated in this liquid phase as the foam matures. (FIG. 11B) Once applied to tissue, the foam gradually degrades and deploys its cargo and either supplies new genetic material or changes the endogenous DNA in the target cell.

[0017] FIGs. 12A-12C. Schematic illustrations depicting an exemplary workflow for engineering cells using nucleic acid delivery foam. (FIG. 12B) Nonviral or viral vector is added to foam precursor in a syringe connected to a second syringe filled with air. The air and foam precursor are mixed by vigorously drawing the syringe plungers back and forth, for example, at least 30 times, creating a microfoam consisting of gas bubbles separated by a network of interconnected lamellae which contain highly concentrated vector. (FIG. 12C) Cultured cells are harvested,

resuspended in complete cell culture media, and externally introduced into foam lamellae using a short and gentle syringe mixing. Foam, now containing vector and cells, is returned to a cell culture device, where it matures and gravity slowly drains the lamellae, resulting in a continuous liquid flow through the capillary network of lamellae. After 24-48 hours (depending on the vector type used), genetically engineered cells can either be directly harvested in the media that drained from the foam and accumulated at the bottom of the culture vessel, or cells can be processed further.

[0018] FIG. 13. Schematic illustration of an exemplary clinical workflow for generating and applying gene therapy foam to ovarian cancer patients.

[0019] FIGs. 14A-14E. Embedding cells into lamellae of methylcellulose-based foam does not compromise viability or yield. (FIG. 14A) Light microscopy image of freshly prepared methylcellulose-based foam with inserted human effector T cells. High magnification is shown on the left, lower magnification on the right. T cells are floating in the liquid-filled foam lamellae separating gaseous bubbles. (FIG. 14B) Dot plot summarizing size differences between foam bubbles and embedded T cells. N = 100 analyzed bubble/cells from 10 independently acquired images. Central marks indicate the mean. (FIG. 14C) Quantification of viable (trypan blue-excluding) T cells harvested after 24 hours from nucleic acid delivery foam versus suspension culture. Pairwise differences between the groups were statistically analyzed using the unpaired, two-tailed Student's t-test. $P < 0.05$ was considered significant. Ns., non-significant. N = 6 biologically independent samples. (FIG. 14E) Representative frames of 24-hour time-lapse videos showing freshly prepared nucleic acid delivery foam with embedded lymphocytes maturing in a G-Rex® cell culture flask. Videos were acquired in an illuminated CO₂ cell culture incubator.

[0020] FIGs. 15A-15F. Engineering human T cells in methylcellulose foam boosts nucleic acid transfer. Isolated human CD3⁺ T cells were stimulated with beads that were coated with antibodies against TCR/CD3 and co-stimulatory CD28 receptors. Twenty-four hours later, beads were removed, and T cells were mixed into methylcellulose-based foam containing GFP-expressing mRNA lipid nanoparticles (mRNA LNPs; FIG. 15A-FIG. 15D) or lentivirus (FIG. 15E-FIG. 15F). Alternatively, cells were transfected/transduced with equal doses of vector in suspension. Nucleic acid transfer was measured after 24 hours (mRNA LNPs) or 48 hours (lentivirus) by flow cytometry. (FIG. 15A, FIG. 15C, FIG. 15E) Representative flow cytometry plots showing nucleic acid transfer. The graphs display CD3[±] gated lymphocyte populations. The numbers within the graphs show the percentage of GFP⁺ T cells. (FIG. 15A, FIG. 15C, FIG. 15F) Comparison of T-cell transfection/transduction efficiencies achieved with foam versus suspension, based on 6 independent experiments. Central marks indicate the mean (FIG. 15B,

FIG. 15D, FIG. 15F).

[0021] FIGs 16A-16C. Testing foams made from methylcellulose derivatives for their ability to boost gene transfer into human T cells. Isolated human CD3+ T cells were stimulated with beads that were coated with antibodies against TCR/CD3 and co-stimulatory CD28 receptors. Twenty-four hours later, beads were removed and T cells were mixed into foam containing GFP-expressing mRNA lipid nanoparticles (mRNA LNPs) or left untransfected. Foams, containing mRNA LNPs were generated using syringe mixing, as illustrated in FIG. 12A. T cells were added to the gene therapy foams during the final 3 mixes, as illustrated in FIG 12B. More specifically, 1.6% solutions of methylcellulose or derivatives were prepared in CTS OpTmizer (Gibco) T cell expansion serum free media containing IL-2 (5 ng/mL) to make the foam precursor. LNPs containing 50 µg GFP-encoding mRNA were added to 1 mL foam precursor and then mixed with 9 mL of air to produce foam. 5 million activated T cells resuspended in 500 µL OpTmizer were then mixed into the foam and immediately dispensed into G-rex 24-well plates. The following starting materials were purchased (cP = centipoise = measure of viscosity): Methylcellulose, 8000 cP, Fisher AA4348322; Methylcellulose, 4000 cP, Sigma M0512; Hydroxypropyl Methylcellulose, Mn 120000, Sigma 423173; Hydroxypropyl Methylcellulose, 4000 cP, Sigma H3785; and Carboxymethyl cellulose, 2300 cP, Thermo Sci AC332631000.

[0022] (FIG. 16A) Representative flow cytometry plots showing gene transfer. Mean fluorescence intensities (MFI) for GFP expression (gene transfer) are shown on top. (FIG. 16B) Summary bar graphs comparing T-cell transfection efficiencies achieved with various foams, based on 2 independent experiments. T-cell viabilities 24-hours post transfection are summarized on the right. (FIG. 16C) Chemical structures of Methylcellulose and Carboxymethyl cellulose are shown to illustrate a working hypothesis. Without being bound by theory, carboxymethyl cellulose can be more efficient in boosting gene transfer into cells compared to Methylcellulose, as its functional groups offer more potential for H-bonding, which could bring gene therapy vector (LNPs) and the cell membrane of the target cell close together.

[0023] FIGs. 17A-17F. Methylcellulose foam increases nucleic acid transfer into stem cells. Human hematopoietic CD34+ stem cells (HSCs) isolated from mobilized peripheral blood were mixed into foam containing GFP-expressing mRNA lipid nanoparticles (mRNA LNPs; FIG. 17A-FIG. 17C) or lentivirus (FIG. 17D-FIG. 17F). Alternatively, cells were transfected/transduced with equal doses of vector in suspension. Negative controls were left unmodified. (FIG. 17A, FIG. 17D) Representative flow cytometry plots from three independent experiments showing nucleic acid transfer. (17B, 17E) Comparison of HSC transfection/transduction efficiencies achieved with foam versus suspension, based on three independent experiments. (FIG. 17C, FIG. 17F) Quantification

of viable (DAPI-negative) HSCs cells harvested after 24 hours (LNP transfection) or 48 hours (LV transduction) from nucleic acid delivery foam versus suspension culture. Central marks indicate the mean. Pairwise differences in nucleic acid transfer between the groups were statistically analyzed using the unpaired, two-tailed Student's t-test. $P < 0.05$ was considered significant.

[0024] FIGs. 18A, 18B. CAR T-cells transduced in nucleic acid delivery foam are fully functional. (FIG. 18A) In vitro cell-killing assays conducted by adding escalating doses of untransduced (T cells exposed to vector-free suspension or foam) or CAR-transduced (T cells exposed to lentiviral vector in suspension or foam) into a culture of bioluminescent tumor targets. To generate tumor-specific CAR-T cells, a lentiviral vector encoding the CAR specific for the tumor antigen ROR1 (Receptor tyrosine kinase-like orphan receptor 1) was used. Forty-eight hours after incubation in foam or suspension, T cells were co-cultured with luciferase expressing ROR1+ breast tumor target cells and bioluminescence signals were quantitated by IVIS imaging 24 hours later and summarized as bar graphs in (FIG. 18B). Graphs show mean $\pm$ s.d. with individual data points. Pairwise differences in bioluminescence signal between the groups were statistically analyzed using the unpaired, two-tailed Student's t-test. $P < 0.05$ was considered significant. Ns., non-significant. $N = 9$ biologically independent samples.

[0025] FIGs. 19A-19E. Nucleic acid delivery foam does not affect viability and function of HSCs or trigger differentiation. (FIG. 19A) Expansion of human CD34+ HSCs after transfection with mRNA LNPs in suspension versus methylcellulose foam. Negative control cells were left untransfected. Fold expansion was calculated based on absolute counts of viable (trypan blue-excluding) cells combined with flow cytometry analysis measuring percentages of CD34+ populations. Experiments were conducted in triplicate. (FIG. 19B) Representative flow cytometry plots from three independent experiments showing phenotypical characterization of HSC-derived subpopulations after 2 days in culture. Cells were either transfected with GFP-expressing mRNA LNP in suspension or foam on day 1 or left unmodified. Gating is indicated in brackets on top of each column. (FIG. 19C) Summary bar graph showing mean frequencies $\pm$ s.d. of Early Myeloid Progenitors (EMPs), Lymphoid-primed Multipotent Progenitors (LMPs), Multipotent Progenitors (MPPs), and primitive Hematopoietic Stem Cells (HSCs). HSCs from three independent donors were analyzed. (FIG. 19D) Colony output of sort purified CD34+ HSCs transfected with mRNA LNPs in suspension versus foam from day 13 cultures ($N = 3$ independent donors). Bar graphs showing mean $\pm$ s.d. are shown in (FIG. 19E).

[0026] FIG. 20. Schematic of an exemplary clinical workflow for programming CAR T-cells in situ using an injectable nucleic acid delivery foam. A small volume of whole blood is isolated from the patient and peripheral blood mononuclear cells (PBMCs) are isolated (using density gradient

centrifugation, such as Lymphoprep™ or Ficoll-Paque™ or alternative methods). The PBMC suspension is then co-embedded with CAR-encoding nucleic acid vector (e.g., lentivirus) within methylcellulose-based foam (prepared at bedside). To boost T-cell activation, stimulatory antibodies (such as anti-CD3/CD28) and/or cytokines (such as IL-2, IL-7, or IL-15) could be added to the formulation. Once prepared, this foam can then be directly re-injected into the patient, using subcutaneous, intraperitoneal or intratumoral routes. Foam depots mature and gradually release genetically modified CAR+ T cells, which enter the patient circulation through the lymphatic system or tissue blood vessels. If medically necessary, this procedure could be repeated as it can be performed within one hour in an outpatient setting (no need for hospitalization or lymphodepletion) and only requires a small dose of CAR-encoding nucleic acid vector.

[0027] FIG. 21. Schematic of an exemplary clinical workflow for bedside manufacturing of HSC-based gene therapy products. Bone marrow aspirate (liquid) is removed from a large bone (e.g., pelvic bone). The liquid is used to make a concentrate, which is co-embedded with therapeutic nucleic acid vector within methylcellulose-based foam. This foam is then directly re-injected into the marrow of the patient, where it matures and gradually releases genetically modified CD34+ HSCs. If medically necessary, this procedure could be repeated as it can be performed within one hour in an outpatient setting (no need for hospitalization) and only requires a small dose of nucleic acid vector.

[0028] An alternative version of the proposed strategy (more involved and not bedside) would be to harvest purified CD34+ HSCs from mobilized peripheral blood instead of using BMAC. This would require patient preconditioning to mobilize stem cells, apheresis to collect cells, and cell processing (magnetic isolation of CD34+ cells, which are then frozen). The second part of the procedure would be identical (mixing CD34+ cells with methylcellulose nucleic acid delivery foam containing nucleic acid vector, followed by direct intra-marrow injection).

[0029] FIG. 22. A three-dimensional model of perfused bone marrow.

[0030] FIG. 23. Schematic of an exemplary clinical workflow for applying nucleic acid delivery foam in esophageal cancer patients. Lipid nanoparticles (LNPs) containing mRNA encoding genes that trigger tumor apoptosis can be embedded in freshly prepared methylcellulose/xanthan gum-based foam using a simple syringe mixing technique that creates a microfoam nucleic acid vector. This medication is then orally administered to the patient to coat the esophagus and form a local reservoir that slowly releases the gene therapy drug at the tumorous esophageal stricture. Nucleic acid delivery foam treatments could be repeated as medically necessary and combined with standard-of-care radiotherapy to further boost the cancer-killing effect.

[0031] FIG. 24. Gene therapy with mRNA LNPs encoding pro-apoptotic genes effectively inhibits

esophageal cancer growth in vitro. Growth of OACM5.1C esophageal cancer cells after bi-weekly transfection with mRNA LNPs encoding combinations of various tumor-suppressor genes and anti-apoptotic genes.

[0032] FIG. 25. Experimental setup of ex vivo esophageal tumor model inside a tissue culture CO₂ incubator.

[0033] FIG. 26. Foam efficiently accumulates at the esophageal tumor constriction. Representative frames of 2-hour time-lapse videos showing 3 mL of liquid or foam entering an esophageal tube narrowed at the bottom by esophageal tumor (OACM5.1C tumor cells established in 3D collagen plugs for 4 weeks). Videos were acquired under a black light, and fluorescent UV dye was added to the liquid and foam for easy detection.

[0034] FIG. 27. Schematic of an exemplary clinical workflow for rectally applying nucleic acid delivery foam in patients with locally advanced rectal cancer.

DETAILED DESCRIPTION

[0035] There are numerous conditions and diseases that can be treated by administering nucleic acids. However, therapies based on administering nucleic acids are costly to manufacture and rely on high dosing parameters at target tissues to achieve efficient nucleic acid transfer and therapeutic benefit.

[0036] The current disclosure provides gas-bubble based degrading liquid foams to deliver nucleic acids. Such foam possesses unique qualities (illustrated in FIG. 1) that give them superior potency and safety compared to the liquid formulations currently used as carriers in nucleic acid therapy applications.

[0037] Foams, as described herein, are a special kind of colloidal dispersion in which closely packed gas bubbles are separated by thin layers of continuous liquid, called lamellae (FIG. 1, panel A, right panel). In particular embodiments, the gas bubbles occupy greater than 85%, greater than 90%, or greater than 95% of the actual foam volume. The large gas content and low fluid volume highly concentrates the nucleic acid to be delivered in the lamellae. Thus, even with only a small amount of nucleic acid, large areas of tissue or body cavities can still be exposed to high concentrations. This feature is especially relevant for nucleic acid-based therapies, which, as indicated, are costly to manufacture and rely on high dosing numbers at a target tissue to achieve efficient nucleic acid transfer and therapeutic benefit.

[0038] In contrast to liquid formulations that diffuse and drain away from the administration site in an unpredictable manner, foam is stable and remains at the application site (FIG. 1, panel B) for a sufficient amount of time. This ensures that the delivered nucleic acid will stay in contact with the target tissue for an extended duration. Once in place, the foam matures according to a process

referred to as Ostwald ripening (FIG. 1, panel B, right panel; Dreher, et al., Handbook of cosmetic science and technology, Edn. Fifth edition. (CRC Press, Boca Raton; 2022)). Gas from smaller bubbles is transported to larger bubbles, causing the smaller air bubbles to dissolve and the larger bubbles to increase in size. The liquid gradually drains through channels between the bubbles, ultimately forming a supersaturated layer at the foam/tissue interface. These high and localized concentrations of delivered nucleic acid potentiates cellular internalization while avoiding side effects associated with systemic exposure.

[0039] Governed by Fick's law (Dunn, et al., Numerical methods in biomedical engineering. (Elsevier Academic Press, Amsterdam; Boston; 2006)), drug penetration into target tissue is proportional to the concentration gradient. Therefore, the simplest strategy to facilitate deep drug permeation is to sustain high local drug concentrations at the interface of the delivery vehicle and the target tissue. Foams can act as local depots that slowly release a nucleic acid to maintain a sharp concentration gradient over time, ensuring effective saturation of the target tissue (FIG. 1, panel C).

[0040] FIGs. 11A and 11B depict an exemplary clinical workflow for generating and applying nucleic acid delivering foams. A syringe filled with foam precursor solution and nucleic acid vector can be connected to a syringe containing a gas, such as air (FIG. 11A). The foaming liquid and gas can then be moved repeatedly back and forth through the constriction that connects the two syringes to generate a homogenous foam which contains substantially evenly distributed vector. This foam-based medication can then be directly administered to a patient to form local reservoirs of foam that slowly release the nucleic acid (FIG. 11B).

[0041] Particular embodiments can add sugar molecules to the foam precursor formulation to increase stability and delay foam clearance while at the same time prolonging the viability of embedded nucleic acid vectors. Sucrose, trehalose, maltose and maltodextrin, for example, can be used to increase the viscosity of nucleic acid delivering foams, while preserving function and biocompatibility. These sugars are currently used in food processing to add texture or as protective agents to preserve the quality of sensitive compounds. Ozcelik & Kulozik. The Role of Maltodextrin Concentration in Maintaining Storage Stability of Dried Fruit Foams Texturized Using Plant Protein-Polysaccharide Blends. Foods 12 (2023). Sugars are also extensively used as cryoprotectants for nucleic acid vectors. Kim et al., Optimization of storage conditions for lipid nanoparticle-formulated self-replicating RNA vaccines. J Control Release 353, 241-253 (2023); Rieser et al., J Pharm Sci 111, 2288-2298 (2022). Formulating foam with sugars therefore serves a dual purpose by (i) making the foam more stable and (ii) maintaining the biological activity and potency of an embedded vector (e.g., lipid nanoparticle (LNP) or virus). Lentivirus, for instance,

has a half-life of only 8-9 hours at 37°C, so it would be beneficial to formulate a foam carrier that helps vector remain viable for longer periods of time.

[0042] Particular embodiments include targeted nucleic acid foams. Targeted foams can further improve the efficiency and tissue-specificity of foam as a nucleic acid vehicle. In these embodiments, foam precursors can be functionalized with binding ligands (e.g., by covalently coupling antigen-specific antibody binding domains to xanthan gum in a starting solution) to direct nucleic acid transfer to specific cell types (FIG. 10). Targeted vectors (e.g., LNPs and viral vectors) can also be used.

[0043] At first sight, targeting foam might seem redundant as the foam is designed to stay in place at an application site and deliver its nucleic acid to cells in a spatially defined fashion. Also, exemplary methylcellulose-based foam formulations disclosed herein precisely transfect cells around the application site (FIG. 4E). However, many clinical scenarios would benefit from additional tissue-specific targeting. One example includes treating disseminated tumors in a large body cavity (abdominal, pleural, gastrointestinal tract). In these circumstances, it is difficult or impossible to physically apply foam to each malignant lesion (which is often indistinguishable from healthy tissue). Instead, targeted foams disclosed herein allow the entire body cavity to be filled with tumor-targeted foam, which can gradually mature and aggregate around tumor sites. In certain examples, a sugar within a precursor foam solution and/or a vector within a precursor foam solution include a targeting ligands that binds a cancer antigen.

[0044] Foams can also be used to deliver nucleic acids to anatomical sites having a clustered group of infected cells. Such targeted foams can allow more selective distribution and prolonged contact time at therapeutically relevant tissues.

[0045] Targeted foams can also be used in cell engineering. For example, for in situ CAR T cell engineering, T-cell targeted lentivirus or T-cell targeted LNPs can be used within a nucleic acid delivering foam. Exemplary target antigens on T cells include CD3, CD4, CD5, CD7, and CD2. Similarly, for in situ HSC engineering, vectors can include targeting ligands for CD90, CD34, and/or CD117.

[0046] Particular embodiments include self-expanding nucleic acid delivering foams. Although most people are familiar with foams that have expanding properties, for example industrial foams used to seal gaps in homes or as insulation in wall cavities, or foams designed for recreation and science experiments, such as “Elephant Toothpaste”, the therapeutic use for self-expanding foam is currently limited to a single product: ResQFoam®, which was developed by the U.S. military as a liquid foam that is injected into the abdomen (King et al., J Trauma Acute Care Surg (2023)). The foam expands and forms a seal around a bleeding wound, reducing blood loss.

[0047] As described herein, the expanding properties of foam may be harnessed to actively penetrate anatomical sites, such as solid tumors, and deliver therapeutic nucleic acids more quickly and deeper into difficult to transfect tissues. Rationales include that: (i) in situ expanding foam requires a substantially smaller injection volume to deliver nucleic acids to tissue compared to non-expanding foams, (ii) the positive pressure induced in situ by newly created bubbles can drive foam-embedded nucleic acids through the interstices deep into tissue, and (iii) oxygen gas, which is released as a byproduct of foam expansion, can boost, for example, transgene expression in hypoxic tissue. Low oxygen supply is known to decrease the rate of mRNA-to-protein translation in the cell due to decreased ATP availability, which greatly hinders the efficacy of conventional gene therapy (Chee et al., *Mol Cancer* 18, 49 (2019)). For example, the formulation of a self-expanding foam can include supplementing a methylcellulose/xanthan gum-based foam with 3.75% hydrogen peroxide and 0.015% potassium iodide. This formulation achieves a ten-fold expansion of foam within 90 minutes (FIGs. 3A, 3B).

[0048] While foam may not be suited for systemic infusion, its promise lies in the controlled and uniform delivery of nucleic acids to cells lining body cavities (oral, thoracic, abdominal, bladder), the intestinal tract, the female genital lumen, all injectable tissues (especially muscle for vaccine applications, bone marrow or lymph nodes), tumors (with a focus on oncolytic virus-based gene therapy) and the skin, which has emerged as a desirable organ for gene therapy. Thus, this delivery strategy is both facile and broadly applicable in a variety of clinical settings.

[0049] Foams disclosed herein can also be used in ex vivo cell engineering (e.g., lymphocytes, hematopoietic stem cells (HSCs), induced pluripotent stem cells (iPSCs)). (FIG. 12C). As foam lamellae form a network of liquid-filled tubes that contain highly concentrated nucleic acids (e.g., as a result of gas bubbles occupying more than 95% of the actual foam volume; see FIG. 1), this creates the ideal space for cells to meet and be transfected with, for example, a nucleic acid vector. As foam matures, gravity slowly drains lamellae resulting in a continuous liquid flow through the capillary network of lamellae. This liquid flow further increases the likelihood of foam-embedded cells to encounter nucleic acid vector, resulting in a more efficient nucleic acid transfer compared to the conventional static setup (i.e., adding vector on top of cultured cells). To insert cells into foam lamellae, they may either be admixed to foam during the final 2-3 rounds of syringe mixing. Alternatively, cells may be directly vortexed into the foam or gently inserted using a dual-syringe spiral mixing tip.

[0050] Referring to FIG. 20, peripheral blood can be collected from a patient and peripheral blood mononuclear cells (PMBCs) maybe isolated. Isolated PMBCs may be mixed with a foam including nucleic acids for delivery to the PMBCs. In certain embodiments, the foams may also include

lymphocyte activating components. Following the mixing of PMBCs with foam, the foam can be administered to a subject, either subcutaneously, intraperitoneally, or intratumorally. The entirety of the described process can occur at a patient's bedside and in under an hour.

[0051] In certain examples, for HSC gene therapy using foam, the foam can be (i) directly injected into the bone marrow of patients, (ii) mixed with bone marrow aspirate and directly injected back into patient marrow (while the needle is still in the patient's bone marrow) (with HSCs and nucleic acid vector trapped within the foam lamellae), or (iii) admixed with ex vivo cultured HSCs, which are then re-infused into patients as part of a conventional stem cell transplantation. Referring to FIG. 21, cells can also be directly harvested from a patient's bone marrow (e.g. as part of a bone marrow aspiration) and immediately mixed with foam containing nucleic acids (e.g. using the syringe method depicted in FIG. 11A, FIG. 12A, and FIG. 12B), before injecting the foam/cell mixture back into the patient's bone marrow, without the need for ex vivo culture.

[0052] The present disclosure harnesses these exceptional qualities of foam to improve the effectiveness and safety of nucleic acid-based therapies, while concomitantly lowering costs to help make these treatments widely accessible.

[0053] Importantly, foams as disclosed herein are distinct from hydrogels. Hydrogels are three-dimensional network structures able to imbibe large amounts of water. Hydrogels do not typically dissolve due to chemical or physical cross-links and/or chain entanglements. They exist naturally in the form of polymer networks such as collagen or gelatin or can be made synthetically. Hydrogels are less likely to, and are not intended to, undergo Ostwald ripening. Within the present disclosure, Ostwald ripening is intended and advantageous because liquid gradually drains through channels between foam bubbles, ultimately forming a supersaturated layer of nucleic acids at a foam/tissue interface.

[0054] Aspects of the disclosure are now described with additional detail and options as follows: (i) Foam Precursors and Characteristics, (ii) Stabilizing Sugars, (iii) Nucleic Acids, (iv) Lipid Nanoparticles, (v) Viral Vectors, (vi) Targeting Ligands, (vii) Foam Expansion Agents, (viii) Methods of Forming Foams, (ix) Methods of Use Using Foam Application to an Anatomical Site, (x) Methods of Use Using Ex Vivo Cell Manufacturing, (xi) Kits, (xii) Exemplary Embodiments, (xiii) Experimental Examples, and (xiv) Closing Paragraphs. These headings are provided for organizational purposes only and do not limit the scope or interpretation of the disclosure.

[0055] (i) Foam Precursors and Characteristics. Foam precursors of the present disclosure have foaming properties that are suitable to deliver nucleic acid vectors to human cells, and are biocompatible with mammalian, and in particular human, tissue. The term "biocompatible" (which also can be referred to as "tissue compatible") generally refers to the inability of a material to

promote an adverse biological response in the body that outweighs the benefit of administration, within the sound judgment of an experienced medical professional. A biocompatible material can be non-toxic, non-mutagenic, non-allergenic, non-carcinogenic, and/or non-irritating or substantially non-toxic, non-mutagenic, non-allergenic, non-carcinogenic, and/or non-irritating.

[0056] In particular embodiments, biocompatible foaming agents may be selected because they are generally regarded as safe (GRAS) by the United States Food and Drug Administration. In particular embodiments, biocompatible foaming agents may be selected because they are manufactured in bulk. These features can ensure that the foams are biocompatible, scalable, and low-cost. Examples of biocompatible foaming agents with these properties include cellulose and cellulose derivatives, sodium caseinate, and albumin.

[0057] Cellulose is a molecule that contains carbon, hydrogen, and oxygen according to the formula $(C_6H_{10}O_5)_n$, creating a chain of linked units. Cellulose derivatives include cellulose with chemically modified hydroxyl groups, for example modified by esterification or etherification. Exemplary cellulose derivatives include cellulose esters (e.g., cellulose acetate), cellulose ethers (e.g., methylcellulose, ethyl cellulose), cellulose sulfate, and cellulose nitrate.

[0058] Methylcellulose is a plant-based food additive that is widely used as a foaming agent in foods like pudding, ice cream, or whipped toppings. Carboxymethyl cellulose is a methylcellulose derivative formed through carboxymethylation. Additional examples of cellulose and methylcellulose derivatives include sodium carboxymethyl cellulose (SCMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), methyl hydroxyethyl cellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), carboxymethylcellulose, hydroxyethyl methyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose succinate (HPMCS), hydroxypropyl methylcellulose trimellitate (HPMCT), hydroxypropyl methylcellulose acetate phthalate (HPMCAP), and hydroxypropyl methylcellulose acetate maleate (HPMCAM).

[0059] In certain embodiments, a methylcellulose derivative with additional hydroxyl or carboxyl groups as compared to a reference methylcellulose is selected. A reference methylcellulose has the same number as repeating units as the derivative to which it is compared. In certain embodiments, a methylcellulose derivative with decreased methyl substitutions as compared to a reference methylcellulose is selected.

[0060] Without being bound by theory, methylcellulose may enhance transfection efficiency by increasing cell—LNP contact through hydrogen bonding. The extensive network of hydroxyl groups in methylcellulose present multiple hydrogen bonding sites which may bind both cell surface molecules and the PEG coating of LNPs, facilitating the interaction between the cell and

LNP. Cellulose derivatives that possess hydrogen bonding capability similar to methylcellulose, such as (hydroxypropyl)methylcellulose, are able to function in the same way. Other cellulose derivatives, such as carboxymethylcellulose, have improved hydrogen bonding potential compared to methylcellulose, which may increase the probability of contact between the cell and LNP.

[0061] Sodium caseinate, the sodium salt of casein (a milk protein), is used in foods and cosmetics for its foaming and thickening properties. Human serum albumin is a globular protein that is routinely administered into patients with acute conditions such as trauma, cardiogenic shock, and sepsis, but it is also being explored by bioengineers for its foaming properties (Caironi et al., N Engl J Med 370, 1412-1421 (2014); Zhang et al., Dermatol Surg 46, 1030-1034 (2020)). In particular embodiments, the biocompatible foaming agent may be selected from, methylcellulose, carboxymethyl cellulose, SCMC, HEC, HPC, MHEC, HPMC cellulose, carboxymethylcellulose, hydroxyethyl methyl cellulose, hydroxypropyl methylcellulose, HPMCAS, HPMCP, HPMCS, HPMCT, HPMCAP, HPMCAM hydroxypropyl methylcellulose, microcrystalline cellulose (cellulose gel), sodium caseinate, albumin (including human serum albumin, bovine serum albumin, and ovalbumin), gelatin, collagen, soybean extract, propylene glycol esters of fatty acids, glycyrrhizin, calcium glycyrrhizinate, disodium glycyrrhizinate, monoammonium glycyrrhizinate, potassium glycyrrhizinate, magnesium glycyrrhizinate, ammonium glycyrrhizinate pentahydrate, ammoniated glycyrrhizin, alginic acid, lecithines (such as phosphatidylcholine (lecithin)), polysorbates (including polyoxyethylene sorbitan monolaurate (Polysorbate 20, Tween 20), polyoxyethylene sorbitan monopalmitate (Polysorbate 40), polyoxyethylene sorbitan monostearate (Polysorbate 60), polyoxyethylene sorbitan monooleate (Polysorbate 80, Tween 80)), propylene glycol monostearate, sucrose esters, sorbitan esters (such as sorbitan monooleate or sorbitan monopalmitate), fatty acid polyglycerol esters, lactic acid esters of mono- and diglycerides of fatty acids (Lactems), quillaia extract, saponin, sodium bicarbonate, alginate, ammonium alginate, calcium alginate, calcium stearoyl lactylate, nitrous oxide, potassium alginate, propylene glycol alginate, sodium alginate, sodium lauryl sulfate, stearoyl lactate, sodium stearoyl lactylate, xanthan gum, serum protein (such as 6-lactoglobulin), whey protein, milk proteins, milk protein hydrolysates, caseino-macro-peptide, and mixtures thereof.

[0062] Biocompatible foaming agents can be used alone, or in combination with others.

[0063] Gasses that can be used for generating foams include any biocompatible gas. Examples include air, oxygen (O₂), nitrogen (N₂), and inert gasses such as Argon (Ar), Neon (Ne), Radon (Ra), Helium (He), Krypton (Kr), Nitric oxide (NO), and mixtures thereof.

[0064] The composition of ambient environmental air is 78% nitrogen, 21% oxygen, 1% argon, with, in certain instances, trace percentages of other gases, such as carbon dioxide, neon, methane, helium, krypton, hydrogen, xenon, ozone, nitrogen dioxide, iodine, carbon monoxide, and ammonia.

[0065] Particular embodiments select a gas with particular characteristics for an intended use. For example, at a hypoxic tumor site, one may select a gas with a higher than average oxygen content. A gas with a higher than average oxygen content has more than 21% oxygen.

For stem cell related applications, one may select a gas with a lower than average oxygen content. A gas with a lower than average oxygen content has less than 21% oxygen. Certain embodiments select a gas with a 1-5% oxygen concentration. For example, bone marrow has a 1-5% oxygen concentration, so a gas with a 1-5% oxygen concentration could be selected when injecting a foam into the bone marrow and during ex vivo stem cell engineering.

[0066] In particular embodiments, the foam may have an initial mean bubble count of 10/mm² to 200/mm², 20/mm² to 180/mm², 50/mm² to 150/mm², 60/mm² to 130/mm², 70/mm² to 120/mm², 80/mm² to 110/mm², 90/mm² to 100/mm², or 95/mm².

[0067] In particular embodiments, the foam may have an initial mean bubble area of 500 µm² to 20,000 µm², 2,000 µm² to 18,000 µm², 5,000 µm² to 15,000 µm², 7,000 µm² to 14,000 µm², 8,000 µm² to 13,000 µm², 9,000 µm² to 12,000 µm², 10,000 µm² to 11,000 µm², or 10,528 µm².

[0068] In particular embodiments, after 10 hours at room temperature the foam may have a diffusion of air from smaller bubbles to larger bubbles resulting in a bubble count of 1/mm² to 100/mm², 1/mm² to 50/mm², 5/mm² to 45/mm², 10/mm² to 40/mm², 12/mm² to 38/mm², 15/mm² to 35/mm², 20/mm² to 32/mm², or 26/mm² (25.7 ± 6.7 /mm²).

[0069] In particular embodiments, after 10 hours at room temperature the foam may have a mean bubble area of 5,000 µm² to 80,000 µm², 10,000 µm² to 75,000 µm², 15,000 µm² to 70,000 µm², 20,000 µm² to 65,000 µm², 25,000 µm² to 60,000 µm², 30,000 µm² to 55,000 µm², 32,000 µm² to 52,000 µm², or 41,597 µm².

[0070] In particular embodiments, after 10 hours at room temperature foam height (total height – liquid height) may decay to an average of 25% to 100%, 50% to 100%, 60% to 95%, 65% to 90%, 70% to 88%, or 78% of the maximum value.

[0071] In particular embodiments, homogenous dispersion of particles, such as LNPs or viral vectors, throughout the liquid lamellae include a 1.1 fold, 1 to 2 fold, 0.5 to 3 fold, or 0.1 to 5 fold increased concentration in centers compared to edges facing gas bubbles.

[0072] Edible or drinkable foams can include, for example, flavoring agents, sweeteners, nutritional additives, preservatives, pH modifiers, coloring agents, taste-masking agents, viscosity

modifiers, and thickeners. Foams that are edible require chewing before comfortable swallowing while foams that are drinkable can be comfortably swallowed without chewing.

[0073] Flavoring agents useful herein include any material or mixture of materials operable to enhance the taste of a nucleic acid delivering foam. Any orally acceptable natural or synthetic flavoring agents can be used, such as essential oils, various flavoring aldehydes, flavoring oils, esters, alcohols, similar materials, as well as sweeteners such as sodium saccharin, xylitol, D-mannose, and combinations thereof. Essential oils can include: Ylang Ylang (*Cananga odorata*); Yarrow (*Achillea millefolium*); Violet (*Viola odorata*); Vetiver (*Vetiveria zizanoides*); Vanilla (*Vanilla plantifolia*); Tuberose (*Polianthes tuberosa*); Thyme (*Thymus vulgaris* L.); Tea Tree (*Melaleuca altemifolia*); Tangerine (*Citrus reticulata*); Spruce, Black (*Picea mariana*); Spruce (*Tsuga Canadensis*); Spikenard (*Nardostachys jatamansi*); Spearmint (*Mentha spicata*); Sandalwood (*Santalum spicatum*); Rosewood (*Aniba rosaeodora*); Rosemary Verbenone (*Rosmarinus officinalis*); Rosemary (*Rosmarinus officinalis*); Rose (*Rosa damascena*); Rose Geranium (*Pelargonium roseum*); Ravensara (*Ravensara aromatica*); Plai (*Zingiber cassumunar*); Pine Needle (*Pinus sylvestris* L.); Petitgrain (*Citrus aurantium*); Peppermint (*Mentha piperita*); Pepper, Black (*Piper nigrum* L.); Patchouli (*Pogostemon cablin*); Palo Santo (*Bursera graveolens*); Palmarosa (*Cymbopogon martini*); Osmanthus (*Osmanthus fragrans*); Oregano (*Origanum vulgare*); Orange, Sweet (*Citrus sinensis*); Oak Moss (*Evernia prunastri*); Nutmeg (*Myristica fragrans*); Niaouli (*Melaleuca viridiflora*); Neroli (aka Orange Blossom) (*Citrus aurantium*); Myrtle (*Myrtus communis*); Myrrh (*Commiphora myrrha*); Mimosa (*Acacia decurrens*); Melissa (*Melissa officinalis* L.); Marjoram, Sweet (*Origanum majorana*); Manuka (*Leptospermum scoparium*); Mandarin, Red (*Citrus deliciosa*); Mandarin (*Citrus deliciosa*); Lotus, White (*Nelumbo nucifera*); Lotus, Pink (*Nelumbo nucifera*); Lotus, Blue (*Nelumbo nucifera*); Lime (*Citrus aurantifolia*); Lily (*Lilium aurantium*); Lemongrass (*Cymbopogon citratus*); Lemon (*Citrus limonum*); Lavender (*Lavandula angustifolium*); Lavandin (*Lavandula hybrida* grosso); Kanuka (*Kunzea ericoides*); Juniper Berry (*Juniperus communis*); Jasmine (*Jasminum officinale*); Jasmine Abs (*Jasminum sambac*); Helichrysum (*Helichrysum italicum*); Grapefruit, White (*Citrus x paradisi*); Grapefruit, Pink (*Citrus paradisi*); Ginger (*Zingiber officinalis*); Geranium (*Pelargonium graveolens*); Geranium, Bourbon (*Pelargonium graveolens*, 'Herit'); Gardenia (*Gardenia jasminoides*); Galbanum (*Ferula galbaniflua*); Frankincense (*Boswellia carterii*); Frangipani (*Plumeria alba*); Fir Needle White (*Abies alba*); Fir Needle Siberia (*Abies sibirica*); Fir Needle Canada (*Abies balsamea*); Fennel, Sweet (*Foeniculum vulgare*); Eucalyptus Smithii. Eucalyptus Radiata, Eucalyptus Globulus, Eucalyptus Citriodora, Eucalyptus Blue Mallee (*Eucalyptus polybractea*); Elemi (*Canarium luzonicum*); Dill (*Anethum graveolens*); Cypress (*Cupressus sempervirens*);

Cumin (*Cuminum cyminum*); Coriander (*Coriandum sativum*); Cocoa (*Theobroma cacao*); Clove (*Eugenia caryophyllata*); Clary Sage (*Salvia sclarea*); Cistus (aka Labdanum) (*Cistus ladaniferus* L.); Cinnamon (*Cinnamomum zeylanicum*); Chamomile, Roman (*Anthemis nobilis*); Chamomile, Blue (*Matricaria chamomilla*); Celery Seed (*Apium graveolins*); Cedarwood, Western Red (*Thuja plicata*); Cedarwood, Blood (*Juniperus virginiana*); Cedarwood Atlas (*Cedrus atlantica*); Carrot Seed (*Daucus carota*); Cardamon (*Elettaria cardamomum*); Caraway Seed (*Carum carvi*); Cajeput (*Melaleuca cajuputi*); Cade (*Juniperus oxycedrus*); Birch, White (*Betula alba*); Birch, Sweet (*Betula lenta*); Bergamot (*Citrus bergamia*); Bay Laurel (*Laurus nobilis*); Basil (*Ocimum basilicum*); Basil, Holy (*Ocimum sanctum*); Basil (*Ocimum basilicum*); Balsam Poplar (*Populus balsamifera*); Balsam Peru (*Myroxylon balsamum*); Angelica (*Angelica archangelica* L.); and combinations thereof.

[0074] Additional flavoring agents include, for example, vanillin, sage, marjoram, parsley oil, spearmint oil, cinnamon oil, oil of wintergreen (methylsalicylate), peppermint oil, clove oil, bay oil, anise oil, eucalyptus oil, citrus oils, fruit oils, and essences including those derived from lemon, orange, lime, grapefruit, apricot, banana, grape, apple, strawberry, cherry, pineapple, etc., bean- and nut-derived flavors such as coffee, cocoa, cola, peanut, almond, etc., adsorbed and encapsulated flavoring agents, and mixtures thereof. Flavoring agents also include ingredients that provide fragrance and/or other sensory effect in the mouth, including cooling or warming effects. Such ingredients include menthol, menthyl acetate, menthyl lactate, camphor, eucalyptus oil, eucalyptol, anethole, eugenol, cassia, oxanone, [α]-irisone, propenyl guaiethol, thymol, linalool, benzaldehyde, cinnamaldehyde, N-ethyl-p-menthan-3-carboxamide, N,2,3-trimethyl-2-isopropylbutanamide, 3-l-menthoxypropane-1,2-diol, cinnamaldehyde glycerol acetal (CGA), methane glycerol acetal (MGA) and mixtures thereof. In particular embodiments, the foam includes a lipophilic flavoring agent including menthol, vanillin or an essential oil (e.g., orange oil, lemon oil, clove oil, peppermint oil, spearmint oil or aniseed oil).

[0075] In particular embodiments, flavoring agents include lemon juice concentrate, tangerine flavor, tangerine concentrate, and/or apple juice concentrate. In particular embodiments, flavoring agents include jasmine, rooibos, and/or peppermint tea.

[0076] Sweeteners include maltose, sucrose, glucose, fructose, invert sugars and mixtures thereof. Fructose can be obtained or provided as liquid fructose, high fructose corn syrup (HFCS), dry fructose or fructose syrup. High fructose corn syrup is commercially available as HFCS-42, HFCS-55 and HFCS-90, which include 42%, 55% and 90%, respectively, by weight of the sugar solids therein of fructose. In particular embodiments, sweeteners can be provided to some extent by other components of the foam, such as by fruit juice, flavorants, and so forth.

[0077] Optional artificial or noncaloric sweeteners can be used alone or in combination with carbohydrate sweeteners in foams. They include, for example, saccharin, cyclamates, acetosulfam, L-aspartyl-L-phenylalanine lower alkyl ester sweeteners (e.g., aspartame). L-aspartyl-D-alanine amides disclosed in U.S. Pat. No. 4,411,930 to Brennan et al., L-aspartyl-D-serine amides disclosed in U.S. Pat. No. 4,399,163 to Brennan et al., L-aspartyl-L-1-hydroxymethyl-alkaneamide sweeteners disclosed in U.S. Pat. No. 4,338,346 to Brand, L-aspartyl-1-hydroxyethylakaneamide sweeteners disclosed in U.S. Pat. No. 4,423,029 to Rizzi, L-aspartyl-D-phenylglycine ester and amide sweeteners disclosed in European Patent Application 168,112 to J. M. Janusz, published Jan. 15, 1986, and the like.

[0078] Nutritional additives include essential oils, vitamins, minerals, and amino acids. Vitamins include Vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B12, vitamin C, vitamin E, vitamin D, niacin (vitamin PP), biotin (vitamin H), menadione (vitamin K), folic acid, and pyridoxine (B6). Minerals include iron, zinc, calcium, phosphorus, potassium, magnesium, and fluoride. Amino acids include lysine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine. Exemplary nutritional additives can be found in Roberts et al., *Nutraceuticals: The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods* (American Nutraceutical Association, 2001); in *Physicians' Desk Reference for Nutritional Supplements*, 1st Ed. (2001); and in *The Physicians' Desk Reference for Herbal Medicines*, 1st Ed. (2001).

[0079] Taste-masking agents include sodium bicarbonate, ion-exchange resins, cyclodextrin inclusion compounds, adsorbates, and the like.

[0080] In additional embodiments, foams can include transduction enhancers. Transduction enhancers are compounds that increase the efficiency of nucleic acid transfer into cells. They are particularly useful in gene therapy, where efficient delivery of nucleic acids is crucial for achieving desired outcomes. Examples of transduction enhancers include dimethyl sulfoxide (DMSO), polybrene, and protamine sulfate.

[0081] (ii) Stabilizing Sugars. As described previously, inclusion of sugars in a foam formulated to deliver nucleic acids to an anatomical site serves two beneficial purposes. The sugars (i) make the foam more stable and (ii) maintain the biological activity and potency of an embedded vector.

[0082] Sugars include saccharides, such as monosaccharides, disaccharides, and oligosaccharides. Suitable sugars also include sugar alcohols, such as erythritol, maltitol, mannitol, sorbitol, xylitol, hydrogenated starch hydrolysates, isomalt, and glycosides, such as steviol glycosides, rebaudiosides, and mogrosides. Additional examples include sucrose, maltose, glucose, raffinose, maltodextrin, trehalose, gum arabic, capsul, lactose, levulose,

fructose, ribose, dextrose, isomalt, erythritol, sorbitol, mannitol, xylitol, lactitol, maltitol, pentitol, arabinose, pentose, xylose, galactose, leucrose, stevia, stevioside, rebaudioside A, rubusoside, and mogroside V.

[0083] Particular embodiments of the foams disclosed herein include sucrose, maltose, maltodextrin, and/or trehalose. Trehalose is a disaccharide that also has anti-cancer effects (Del Bello, et al., Cancer Cell Int, 22, 232, 2022, Takashi Kudo, et al., International Scholarly Research Notices, vol. 2012, Article ID 968493, 9 pages, 2012), so in particular embodiments, a trehalose-functionalized foam could have multiple desirable effects such as stabilization of the foam, protection of the nucleic acid vector, and weakening of the tumor.

[0084] Particular embodiments include xanthum gum and/or guar gum.

[0085] (iii) Nucleic Acids. According to the current disclosure, foams are used to deliver nucleic acids. Nucleic acids and their equivalents refer to molecules that include a nitrogenous base, a sugar, and a phosphate group. A nucleotide is a monomer of DNA or RNA. A nucleotide, for instance, is a chemical structure. Nucleic acids refer to linked monomers of DNA or RNA.

[0086] DNA refers to a polymer of nucleotides (also referred to as “nucleobases”) including deoxyribose. The nucleotides in DNA include cytosine (C), guanine (G), adenine (A), and thymine (T). Each DNA nucleotide includes a deoxyribose and a phosphate group. An example single-stranded DNA (ssDNA) molecule includes a chain of covalently bonded DNA nucleotides. In the example ssDNA molecule, the phosphate group of the m th nucleotide is covalently bonded to the deoxyribose of the $(m-1)$ th nucleotide, wherein m is a positive integer greater than 2 and less than or equal to the number of DNA nucleotides in the chain. In various examples, DNA is double-stranded and includes two ssDNA molecules that are complementary to one another and coiled around each other in a double helix form. The nucleotides of one ssDNA molecule are hydrogen bonded to the nucleotides of the other ssDNA molecule. In particular, the pyrimidines (A and T) hydrogen bond to each other, and the purines (C and G) hydrogen bond to each other.

[0087] RNA refers to a polymer of nucleotides containing ribose. The nucleotides in RNA include cytosine (C), guanine (G), adenine (A), and uracil (U). Each RNA nucleotide includes a ribose and a phosphate group. In an example RNA molecule, the phosphate group of the n th nucleotide is covalently bonded to the ribose of the $(n-1)$ th nucleotide, wherein n is a positive integer greater than 2 and less than or equal to the number of RNA nucleotides in the chain. Messenger RNA (mRNA) is a type of RNA molecule that is synthesized (or “transcribed”) by RNA polymerase to be complementary to a gene encoded in a DNA sequence and is also used by a ribosome to synthesize a polypeptide or protein. An mRNA is therefore an example of a “coding RNA.” MicroRNA (“miRNA”) are single-stranded RNA molecules that perform post-transcriptional gene

expression regulation. For instance, a miRNA may bind to a complementary mRNA molecule, thereby cleaving, destabilizing, or otherwise preventing the mRNA molecule from being translated into a polypeptide or protein by a ribosome. In various examples, a miRNA has a length in a range of 21 to 23 RNA nucleotides. As used herein, the terms “non-coding RNA” may refer to a type of RNA that is not translated into a protein. Examples of non-coding RNA include miRNA, transfer RNA (tRNA), and ribosomal RNA (rRNA). The term “functional RNA,” and its equivalents, may refer to any RNA molecule that impacts a biological process. For instance, functional RNA may include mRNA, miRNA, tRNA, and rRNA.

[0088] Particular embodiments utilize as a nucleic acid plasmid DNA, minicircle DNA, self-replicating RNA, in vitro transcribed RNA, circular RNA, doggybone DNA (dbDNA), siRNA, or any kind of genome editing sequence (examples described elsewhere herein).

[0089] In preferred embodiments of the disclosure, nucleic acids are delivered to cells through the use of a lipid nanoparticle or a viral vector. Also in preferred embodiments, the nucleic acids provide a therapeutic effect. In particular embodiments, the nucleic acid provides a direct effect to the cell. In particular embodiments, the nucleic acid encodes a molecule that, when expressed, has an effect on the cell.

[0090] In particular embodiments, a delivered nucleic acid provides a beneficial effect to a cell to which it is delivered. For example, the cell may lack expression of a particular molecule or have reduced expression of a particular molecule. The delivered nucleic acid can increase the expression of the particular molecule, providing a therapeutic effect to the cell. In these embodiments, the nucleic acid could encode a functioning receptor, a functioning ion channel, a functioning enzyme, or a functioning transcription factor.

[0091] In particular embodiments, a delivered nucleic acid encodes a chimeric antigen receptor (CAR), engineered T cell receptor (eTCR), cytokine, or cytokine receptor.

[0092] A CAR refers to a recombinant protein including several distinct subcomponents that allow a genetically modified immune cell (e.g., T cell) to recognize and kill unwanted cell types, such as cancer cells, infected cells, or autoimmune cells. The subcomponents include at least an extracellular component and an intracellular component. The extracellular component includes a binding domain that specifically binds a marker (e.g., an antigen) that is preferentially present on the surface of unwanted cells. When the binding domain binds such markers, the intracellular component signals the immune cell (e.g., T cell) to destroy the bound cell. CAR can additionally include a transmembrane domain that can link the extracellular component to the intracellular component. Binding domains generally include scFv that bind a target antigen, as described elsewhere herein.

[0093] Other subcomponents that can increase a CAR's function can also be included. For example, spacers provide CAR with additional conformational flexibility, often increasing the binding domain's ability to bind the targeted cell marker, leading to enhanced cytolytic effects. The appropriate length of a spacer within a particular CAR can depend on numerous factors including how close or far a targeted marker is located from the surface of an unwanted cell's membrane.

[0094] An eTCR refers to a recombinant protein that includes an engineered binding domain linked to the C α and/or C β chains of a T cell receptor (TCR). A TCR is a heterodimeric fusion protein that typically includes an α and β chain. Each chain includes a variable region (V α and V β) and a constant region (C α and C β). In particular embodiments, an eTCR does not include the native TCR variable region but does include the native TCR constant region. In particular embodiments, eTCR include a C α and/or C β chain sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to an amino acid sequence of a known or identified TCR C α or C β . For additional information regarding recombinant proteins, see Mansilla-Soto et al., Nat. Med. 2022 Feb; 28(2):345-352.

[0095] Cytokines are small proteins that are important in cell signaling. Cytokines can be, for example, chemokines, interferons, interleukins, lymphokines, and/or tumor necrosis factor.

[0096] Exemplary cytokines include Acylation stimulating protein, Adipokine, Albinterferon, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL5, CCL6, CCL7, CCL8, CCL9, Colony-stimulating factor, CX3CL1, CX3CR1, CXCL1, CXCL10, CXCL11, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL9, Erythropoietin, Gc-MAF, Granulocyte colony-stimulating factor, Granulocyte macrophage colony-stimulating factor, Hepatocyte growth factor, interleukin (IL)-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, and IL-36.

[0097] In particular embodiments, a delivered nucleic acid provides a cytotoxic effect to a cell.

[0098] In particular embodiments, a delivered nucleic acid encodes a vaccine antigen.

[0099] Vaccines include compounds and preparations that are capable of providing immunity against one or more conditions related to infectious diseases and so may include nucleic acids encoding infectious disease-derived antigens and/or epitopes. Vaccines also include compounds

and preparations that direct an immune response against cancer cells and can include nucleic acids encoding tumor cell derived antigens, epitopes, and/or neoepitopes.

[0100] The antigenic peptides or proteins may be pathogenic antigens, tumor antigens, allergenic antigens or autoimmune self-antigens. Such pathogenic antigens may be those derived from pathogenic organisms, in particular bacterial, viral or protozoological (multicellular) pathogenic organisms, which evoke an immunological reaction in a mammalian subject, such as a human. Pathogenic antigens may be surface antigens, for example proteins or fragments thereof, located at the surface of the virus or the bacterial or protozoological organism.

[0101] In embodiments, the nucleic acids encode for an antigen from an infectious agent.

[0102] Pathogenic antigens of interest may include those derived from one or more of: *Acinetobacter baumannii*, *Anaplasma* genus, *Anaplasma phagocytophilum*, *Ancylo stoma braziliense*, *Ancylo stoma duodenale*, *Area nobacteri urn haemo lyticum*, *Ascaris lumbricoides*, *Aspergillus* genus, *Astroviridae*, *Babesia* genus, *Bacillus anthracis*, *Bacillus cereus*, *Bartonella henselae*, BK virus, *Blastocysts hominis*, *Blastomyces dermatitidis*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Borrelia* genus, *Borrelia* spp, *Brucella* genus, *Brugia malayi*, *Bunyaviridae* family, *Burkholderia cepacia* and other *Burkholderia* species, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Caliciviridae* family, *Campylobacter* genus, *Candida albicans*, *Candida* spp, *Chlamydia trachomatis*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, QD prion, *Clonorchis sinensis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium perfringens*, *Clostridium* spp, *Clostridium tetani*, *Coccidioides* spp, coronavi ruses, *Corynebacterium diphtheriae*, *Coxiella burnetii*, *Crimean-Congo hemorrhagic fever virus*, *Cryptococcus neoformans*, *Cryptosporidium* genus, *Cytomegalovirus* (CMV), *Dengue viruses* (DEN-1, DEN-2, DEN-3 and DEN-4), *Dientamoeba fragilis*, *Ebolavirus* (EBOV), *Echinococcus* genus, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Ehrlichia* genus, *Entamoeba histolytica*, *Enterococcus* genus, *Enterovirus* genus, *Enteroviruses*, mainly *Coxsackie A virus* and *Enterovirus 71* (EV71), *Epidermophyton* spp, *Epstein-Barr Virus* (EBV), *Escherichia coli* 0157:H7, 011 1 and 0104: H4, *Fasciola hepatica* and *Fasciola gigantica*, FFI prion, *Filarioidea* superfamily, *Flaviviruses*, *Francisella tularensis*, *Fusobacterium* genus, *Geotrichum candidum*, *Giardia intestinalis*, *Gnathostoma* spp, GSS prion, *Guanarito virus*, *Haemophilus ducreyi*, *Haemophilus influenzae*, *Helicobacter pylori*, *Henipavirus* (*Hendra virus* *Nipah virus*), *Hepatitis A Virus*, *Hepatitis B Virus* (HBV), *Hepatitis C Virus* (HCV), *Hepatitis D Virus*, *Hepatitis E Virus*, *Herpes simplex virus 1 and 2* (HSV-1 and HSV-2), *Histoplasma capsulatum*, *HIV* (*Human immunodeficiency virus*), *Hortaea werneckii*, *Human bocavirus* (HBoV), *Human herpesvirus 6* (HHV-6) and *Human herpesvirus 7* (HHV-7), *Human metapneumo virus* (hMPV), *Human*

papillomavirus (HPV), Human parainfluenza viruses (HPIV), Japanese encephalitis virus, JC virus, Junin virus, *Kingella kingae*, *Klebsiella granulomatis*, Kuru prion, Lassa virus, *Legionella pneumophila*, *Leishmania* genus, *Leptospira* genus, *Listeria monocytogenes*, Lymphocytic choriomeningitis virus (LCMV), Machupo virus, *Malassezia* spp, Marburg virus, Measles virus, *Metagonimus yokagawai*, Microsporidia phylum, *Molluscum contagiosum* virus (MCV), Mumps virus, *Mycobacterium leprae* and *Mycobacterium lepromatosis*, *Mycobacterium tuberculosis*, *Mycobacterium ulcerans*, *Mycoplasma pneumoniae*, *Naegleria fowleri*, *Necator americanus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Nocardia asteroides*, *Nocardia* spp, *Onchocerca volvulus*, *Orientia tsutsugamushi*, Orthomyxoviridae family (Influenza), *Paracoccidioides brasiliensis*, *Paragonimus* spp, *Paragonimus westermani*, Parvovirus B19, *Pasteurella* genus, *Plasmodium* genus, *Pneumocystis jirovecii*, Poliovirus, Rabies virus, Respiratory syncytial virus (RSV), Rhino virus, rhino viruses, *Rickettsia akari*, *Rickettsia* genus, *Rickettsia prowazekii*, *Rickettsia rickettsii*, *Rickettsia typhi*, Rift Valley fever virus, Rotavirus, Rubella virus, Sabia virus, *Salmonella* genus, *Sarcoptes scabiei*, SARS coronavirus, *Schistosoma* genus, *Shigella* genus, Sin Nombre virus, Hantavirus, *Sporothrix schenckii*, *Staphylococcus* genus, *Staphylococcus* genus, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Strongyloides stercoralis*, *Taenia* genus, *Taenia solium*, Tick-borne encephalitis virus (TBEV), *Toxocara canis* or *Toxocara cati*, *Toxoplasma gondii*, *Treponema pallidum*, *Trichinella spiralis*, *Trichomonas vaginalis*, *Trichophyton* spp, *Trichuris trichiura*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Ureaplasma urealyticum*, Varicella zoster virus (VZV), Varicella zoster virus (VZV), Variola major or Variola minor, vCJD prion, Venezuelan equine encephalitis virus, *Vibrio cholerae*, West Nile virus, Western equine encephalitis virus, *Wuchereria bancrofti*, Yellow fever virus, *Yersinia enterocolitica*, *Yersinia pestis*, and *Yersinia pseudotuberculosis*.

[0103] In certain embodiments, relevant antigens may be derived from the pathogens selected from: Severe Acute Respiratory Syndrome (SARS), Severe Acute Respiratory Syndrome Coronavirus and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-1 and SARS-CoV-2), Influenza virus, respiratory syncytial virus (RSV), Herpes simplex virus (HSV), human Papilloma virus (HPV), Human immunodeficiency virus (HIV), *Plasmodium*, *Staphylococcus aureus*, Dengue virus, *Chlamydia trachomatis*, Cytomegalovirus (CMV), Hepatitis B virus (HBV), *Mycobacterium tuberculosis*, Rabies virus, and Yellow Fever Virus.

[0104] In embodiments wherein the infectious disease is influenza, the nucleic acid may have a coding region encoding at least one antigenic peptide or protein derived from hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein 1 (M1), matrix protein 2 (M2), non-structural protein 1 (NS1), non-structural protein 2 (NS2), nuclear export protein (NEP),

polymerase acidic protein (PA), polymerase basic protein PB1, PB1-F2, or polymerase basic protein 2 (PB2) of an influenza virus or a fragment or variant thereof.

[0105] In certain embodiments, the coding region encodes at least one antigenic peptide or protein derived from hemagglutinin (HA) and/or neuraminidase (NA) of an influenza virus or a fragment or variant thereof. The HA and/or NA may, independently, be derived from an influenza A virus or an influenza B virus or a fragment of either.

[0106] In embodiments wherein the infectious disease is influenza, the nucleic acid may have a coding region encoding at least one antigenic peptide or protein derived from Spike (S) protein.

[0107] In embodiments, the nucleic acid can encode for an antigen associated with a cancer of a subject or identified from a cancer cell of a subject. The nucleic acid may encode for an antigen determined from a subject's own cancer cell to provide a personalized cancer vaccine.

[0108] In particular vaccine embodiments, nucleic acids can include mRNA.

[0109] In particular embodiments, a delivered nucleic acid encodes a genome editing agent, a cytosolic protein (e.g., a transcription factor or suicide gene, such as herpes simplex virus thymidine kinase, cytosine deaminase, diphtheria toxin, cytochrome P450, deoxycytidine kinase, or tumor necrosis factor), a transmembrane protein (e.g., a TCR, CAR, cytokine receptor, costimulatory ligand, death receptor such as TNF-related apoptosis-inducing ligand (TRAIL-R1/DR4, TRAIL-R2/DR5), or a “don’t eat me” signal), or a secreted protein (e.g., a chemoattractant, an antibody, a multispecific binding molecule wherein one binding domain activates an immune cell, or a growth factor, such as VEGF, insulin-like growth factors 1 and 2, platelet derived growth factor, epidermal growth factor, nerve growth factor, neurotrophic factor-3 and -4, brain-derived neurotrophic factor, glial derived growth factor, transforming growth factor α and β).

[0110] Multispecific binding molecules wherein one binding domain activates an immune cell include those which bind both an immune cell (e.g., T-cell or NK-cells) activating epitope and a targeted antigen, with the goal of bringing immune cells to the targeted antigen-expressing cells to destroy them. See, for example, US 2008/0145362. Examples of multi-domain immune cell engaging molecules include those which bind both an immune cell (e.g., T-cell or NK-cells) activating epitope and ROR1, with the goal of bringing immune cells to ROR1-expressing cells to destroy them. See, for example, US 2008/0145362. Such molecules can be referred to herein as immune-activating multi-specifics or I-AMS. BiTEs® (Amgen, Thousand Oaks, CA) or bispecific T cell engagers are a form of I-AMS. Immune cells that can be targeted for localized activation by I-AMS within the current disclosure include, for example, B-cells, T-cells, natural killer (NK) cells, and macrophages which are discussed in more detail herein.

[0111] I-AMS can target any T-cell activating epitope that upon binding induces T-cell activation. Examples of such T-cell activating epitopes are on T-cell markers including CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, 4-1BB (CD137), OX40, lymphocyte function-associated antigen-1 (LFA-1), LIGHT, NKG2C, and B7-H3.

[0112] In particular embodiments, the CD3 binding domain (e.g., scFv) is derived from the OKT3 antibody (the same as the one utilized in blinatumomab), oteixizumab, teplizumab, visilizumab, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, or TR66. The OKT3 antibody is described in detail in U.S. Patent No. 5,929,212.

[0113] In particular embodiments, the OKT3 binding domain includes a variable light chain of QIVLTQSPAIMASASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLGSGVPAHFR GSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO: 84) and a variable heavy chain of

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYN QKFKDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTTLTVSSAKTTA PSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSV TVTSS (SEQ ID NO: 85).

[0114] In particular embodiments, the binding domain includes a variable light chain including a CDRL1 sequence including SASSSVSYMN (SEQ ID NO: 86), a CDRL2 sequence including DTSKLG (SEQ ID NO: 87), a CDRL3 sequence including QQWSSNPFTF (SEQ ID NO: 88), a CDRH1 sequence including RYTMH (SEQ ID NO: 89), a CDRH2 sequence including YINPSRGYTNYNQKFKD (SEQ ID NO: 90), and a CDRH3 sequence including YYDDHYCL (SEQ ID NO: 91). In particular embodiments, the binding domain is human or humanized. For more information regarding binding domains that bind CD3, see U.S. Pat. No. 8785604, PCT/US 17/42264, and/or WO02051871.

[0115] In particular embodiments, a binding domain is “derived from” a reference antibody when the binding domain includes the CDRs of the reference antibody, according to a known numbering scheme (e.g., Kabat, Chothia, Martin, or others).

[0116] CD28 binds to B7-1 (CD80) and B7-2 (CD86) and is the most potent of the known co-stimulatory molecules (June et al., Immunol. Today 15:321, 1994; Linsley et al., Ann. Rev. Immunol. 11:191, 1993). In particular embodiments, the CD28 binding domain is derived from TGN1412, CD80, CD86 or the 9D7 antibody. Additional antibodies that bind CD28 include 9.3, KOLT-2, 15E8, 248.23.2, and EX5.3D10.

[0117] In particular embodiments, the binding domain that binds CD28 is derived from TGN-1412 and/or theralizumab. In particular embodiments, the binding domain includes a variable light chain

of

DIQMTQSPSSLSASVGDRVTITCKTNENIYSNLAWYQQKDGKSPQLLIYAATHLVEGVPSRFSGSGSGTQYSLTISSLQPEDFGNYYCQHFHWGTPXTFGGGTKLEI KR, wherein X=C, A, or N. (SEQ ID NO: 92) and a variable heavy chain of VQLQQSGAELKKPGASVKVSCKASGYTFTEYIIHWIKLRSGQGLEWIGWYFPGSNDIQYNAQFKGKATLTADKSSSTVYMELTGLTPEDSAVYFCARRDDFSGYDALPYWGQGTLVTVSA (SEQ ID NO: 93). In particular embodiments, the binding domain includes a variable light chain including a CDRL1 sequence including HASQNIYVWLN (SEQ ID NO: 94), a CDRL2 sequence including KASNLHT (SEQ ID NO: 95), a CDRL3 sequence including QQGQTYPYT (SEQ ID NO: 96), a CDRH1 sequence including SYYIH (SEQ ID NO: 97), a CDRH2 sequence including CIYPGNVNTNYNEKFKD (SEQ ID NO: 98), and a CDRH3 sequence including SHYGLDWNFDV (SEQ ID NO: 99). In particular embodiments, the binding domain is human or humanized. For more information regarding binding domains that bind CD28, see U.S. Pat. No. US8785604 and/or WO02051871.

[0118] Activated T-cells express 4-1BB (CD137). In particular embodiments, the 4-1BB binding domain includes a variable light chain including a CDRL1 sequence including RASQSVS (SEQ ID NO: 100), a CDRL2 sequence including ASNRAT (SEQ ID NO: 102), and a CDRL3 sequence including QRSNWPPALT (SEQ ID NO: 103) and a variable heavy chain including a CDRH1 sequence including YYWS (SEQ ID NO: 104), a CDRH2 sequence including INH, and a CDRH3 sequence including YGPGNYDWYFDL (SEQ ID NO: 105).

[0119] Particular embodiments disclosed herein including binding domains that bind epitopes on CD8. In particular embodiments, the CD8 binding domain (e.g., scFv) is derived from the OKT8 antibody.

[0120] In particular embodiments natural killer cells (also known as NK-cells, K-cells, and killer cells) are targeted for localized activation by I-AMS. NK cells can induce apoptosis or cell lysis by releasing granules that disrupt cellular membranes and can secrete cytokines to recruit other immune cells.

[0121] Examples of commercially available antibodies that bind to an NK cell receptor and induce and/or enhance activation of NK cells include: 5C6 and 1D11, which bind and activate NKG2D (available from BioLegend® San Diego, CA); mAb 33, which binds and activates KIR2DL4 (available from BioLegend®); P44-8, which binds and activates NKp44 (available from BioLegend®); SK1, which binds and activates CD8; and 3G8 which binds and activates CD16.

[0122] Binding domains of I-AMS and other engineered formats described herein may be joined through a linker. A linker is an amino acid sequence which can provide flexibility and room for

conformational movement between the binding domains of a I-AM. Any appropriate linker may be used.

[0123] In particular embodiments, a delivered nucleic acid results in expression of genes that make tumors more susceptible to chemotherapy or radiation therapy, chemoattractants, cytokines, or immune costimulatory genes.

[0124] Chemoattractants are chemicals or proteins released by various cells in the body that signal other cells to migrate to the location of the chemoattractants. Many cancer cells have commonly overexpressed receptors for certain chemoattractants, which help them migrate to new parts of a subject's body. In some embodiments, one or more chemoattractants are cancer cell chemoattractants. Common growth factors (i.e., chemoattractants) that have been shown to induce cancer migration are EGF, VEGF, ILGF-1, and HGF. Overexpressed cell surface receptors such as CXCR4 and EpCAM have similarly been involved in chemotactic migration and cell attachment. Because many cell adhesion factors are responsible for the expression of factors such as ILGF-1, HGF, and the ligand binding CXCR4, directing migration of fibroblasts away from the tumor mass is possible. For example, EGF, VEGF, ILGF-1, and/or HGF may be utilized as chemoattractants for breast cancer cells; VEGF, ILGF-1, and/or CXCL-12 may be utilized as chemoattractants for pancreatic cancer cells; VEGF may be utilized as a chemoattractant for brain cancer cells; EGF, VEGF, and/or HGF may be utilized as chemoattractants for lung cancer cells; and ILGF-1 may be utilized as a chemoattractant for melanoma cells. CCL-21, CCL-19, SDF-1, VEGF, and IL-4 are also examples of chemoattractants.

[0125] In other embodiments, one or more of the chemoattractants are fibroblast chemoattractants. Commonly known signaling molecules which enhance fibroblast movements and may be used for this purpose are platelet derived growth factor (PDGF) and collagen. Conversely, substances such as tissue factor pathway inhibitor-2 (TFPI-2) and fibroblast activation protein (FAP) pro-toxin limit fibroblast growth and migration. For example, IL-4 may be utilized as a chemoattractant for fibroblasts, and TFPI-2 and/or FAP may be utilized to hinder fibroblast migration.

[0126] In particular embodiments, a delivered nucleic acid results in expression of a bacterial toxin, such as *Pseudomonas* exotoxin A. Bacterial toxins derived from *Neisseria meningitidis*, *Escherichia coli* (E.coli), *Pseudomonas*, *Proteus*, *Enterobacter*, or *Klebsiella* can also be used.

[0127] In particular embodiments, a delivered nucleic acid results in overexpression of IRF5 in tumor-associated macrophages, P53 tumor suppression gene therapy, delivery of an oncolytic virus, genome editing (e.g. targeting mutant KRAS), promotion of wound healing (e.g., of the skin due to diabetic ulcers, burns or epidermolysis bullosa by overexpressing HOXA3), in situ gene

therapy of HSCs (e.g., correcting hereditary mutations using genome editing for sickle cell anemia, thalassemia, SCID), or expression of CXCR4 or CCR5 to treat HIV.

[0128] In particular embodiments, a delivered nucleic acid introduces or alters a gene to treat an immune-mediated condition, an inherited genetic defect, a blood disorder, a lysosomal storage disorder, a hyperproliferative disease, or an infectious disease. The introduced or altered gene can be selected from ABCD1, ABCA3, ABLI, ADA, AKT1, APC, APP, ARSA, ARSB, BCL11A, BLC1, BLC6, BRCA1, BRCA2, BRIP1, C9ORF72, C46 CAS9, C-CAM, CBFAI, CBL, CCR5, CD4, CD19, CD40, CDA, CFTR, CLN3, C-MYC, CRE, CSCR4, CSFIR, CTLA, CTS-I, CYB5R3, DCC, DHFR, DKC1, DLL1, DMD, EGFR, ERBA, ERBB, EBRB2, ETSI, ETS2, ETV6, F8, F9, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FasL, FCC, FGR, FOX, FUS, FUSI, FYN, GALNS, GATA1, GLB1, GNS, GUSB, HBB, HBD, HBE1, HBG1, HBG2, HCR, HGSNAT, HOXB4, HRAS, HYAL1, ICAM-1, iCaspase, IDUA, IDS, JUN, KLF4, KRAS, LCK, LRRK2, LYN, MCC, MDM2, MGMT, MLL, MMACI, MYB, MEN-I, MEN-II, MYC, NAGLU, NANOG, NF-1, NF-2, NKX2.1, NOTCH, OCT4, p16, p2I, p27, p53, p57, p73, PALB2, PARK2, PARK7, phox, PINK1, PK, PSEN1, PSEN2, PTPN22, RAD51C, ras, RPL3 through RPL40, RPLP0, RPLP1, RPLP2, RPS2 through RPS30, RPSA, SFTPB, SFTPC, SGSH, SLX4, SNCA, SOD1, SOX2, TERC, TERT, TDP43, TNF2, TK, ubiquilin 2, VHL, WAS or WT-I.

[0129] In particular embodiments, a delivered nucleic acid treats an inflammatory bowel disease such ulcerative colitis, with IL-10 gene therapy or by delivering siRNA targeting GATA4. In particular embodiments, a delivered nucleic acid encodes IL-10 to treat rheumatoid arthritis.

[0130] (iv) Lipid Nanoparticles. The term “lipid nanoparticle”, also referred to as LNP, refers to a particle having at least one dimension on the order of nanometers (e.g., 1-1,000 nm) and which includes one or more lipids.

[0131] Exemplary LNP include liposomes (microscopic vesicles including at least one concentric lipid bilayer surrounding an aqueous core), liposomal nanoparticles (a liposome structure used to encapsulate another smaller nanoparticle within its core); and lipid-like nanoparticles (liposome-like structures that lack the continuous lipid bilayer characteristic of liposomes).

[0132] An LNP may include any lipid capable of forming a particle to which the one or more nucleic acid molecules can be attached or encapsulated. The term “lipid” refers to a group of organic compounds that are derivatives of fatty acids (e.g., esters) and are generally characterized by being insoluble in water but soluble in many organic solvents. Lipids are usually divided in at least three classes: (1) “simple lipids” which include fats and oils as well as waxes; (2) “compound lipids” which include phospholipids and glycolipids; and (3) “derived lipids” such as steroids.

[0133] In particular embodiments, the LNP includes one or more cationic lipids. The cationic lipid can be cationisable, i.e., it becomes protonated as the pH is lowered below the pKa of the ionizable group of the lipid but is progressively more neutral at higher pH values. When positively charged, the lipid is then able to associate with negatively charged nucleic acids. In certain embodiments, the cationic lipid includes a zwitterionic lipid that assumes a positive charge on pH decrease. The LNP may include any lipid capable of forming a particle to which the one or more nucleic acid molecules are attached, or in which the one or more nucleic acid molecules are encapsulated.

[0134] In certain embodiments, the LNP may include any further cationic or cationisable lipid, i.e., any of a number of lipid species which carry a net positive charge at a selective pH, such as physiological pH. Such lipids include N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC); N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA); N,N-distearyl-N,N-dimethylammonium bromide (DDAB); N-(2,3dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP); 3-(N—(N',N'dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol), N-(1-(2,3-dioleoyloxy)propyl)N-2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate (DOSPA), dioctadecylamidoglycyl carboxyspermine (DOGS), 1,2-dioleoyl-3-dimethylammonium propane (DODAP), N,N-dimethyl-2,3-dioleoyloxy)propylamine (DODMA), and N-(1,2dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE).

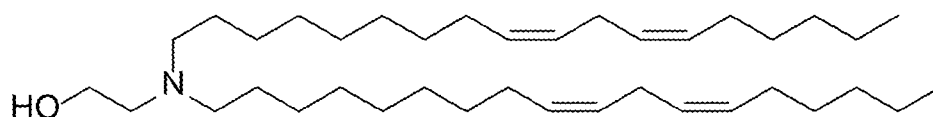
[0135] Additionally, a number of commercial preparations of cationic lipids are available which can be used in the present invention. These include, for example, LIPOFECTIN® (commercially available cationic liposomes including DOTMA and 1,2-dioleoyl-sn-3phosphoethanolamine (DOPE), from GIBCO/BRL, Grand Island, N.Y.); LIPOFECTAMINE® (commercially available cationic liposomes including N-(1-(2,3dioleoyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate (DOSPA) and (DOPE), from GIBCO/BRL); and TRANSFECTAM® (commercially available cationic lipids including dioctadecylamidoglycyl carboxyspermine (DOGS) in ethanol from Promega Corp., Madison, Wis.). The following lipids are cationic and have a positive charge at below physiological pH: DODAP, DODMA, DMDMA, 1,2-dilinoyleoxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinoleyenloxy-N,N-dimethylaminopropane (DLinDMA).

[0136] In particular embodiments, the further cationic lipid is an amino lipid. Suitable amino lipids include those described in WO2012/016184. Representative amino lipids include 1,2-dilinoyleoxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-dilinoyleoxy-3morpholinopropane (DLin-MA), 1,2-dilinoyleoyl-3-dimethylaminopropane (DLinDAP), 1,2-dilinoyleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-linoyleoyl-2-linoyleoxy-

3dimethylaminopropane (DLin-2-DMA), 1,2-dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), 3-(N,Ndilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-dioleylamino)-1,2-propanediol (DOAP), 1,2-dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 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-(dimethyl amino)butanoate (DLin-MC3-DMA), and 2,2-dilinoleyl-4-(2-dimethylaminoethyl)[1,3]-dioxolane (DLin-KC2-DMA).

[0137] Particular embodiments utilize an amino lipid selected from

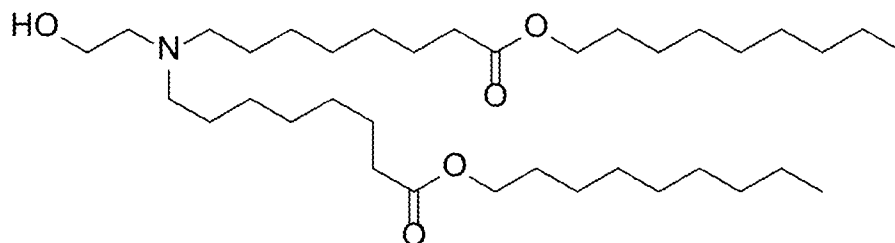
2-(Di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethan-1-ol



Chemical Formula: $C_{38}H_{71}NO$

Molecular Weight: 557.99

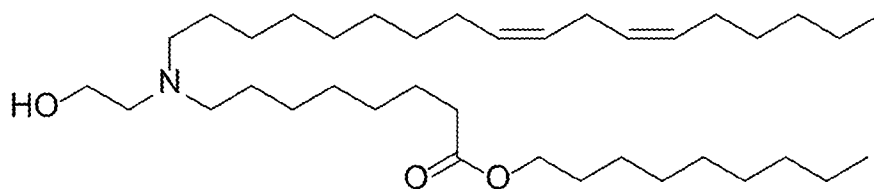
Dinonyl 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate



Chemical Formula: $C_{36}H_{71}NO_5$

Molecular Weight: 597.97

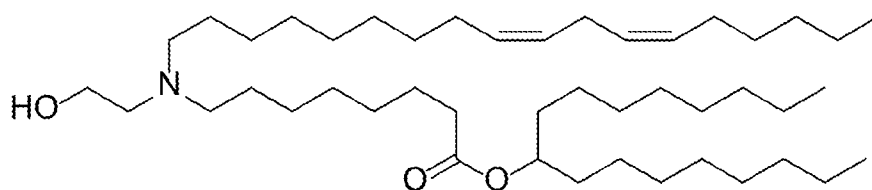
Nonyl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate



Chemical Formula: $C_{37}H_{71}NO_3$

Molecular Weight: 577.98

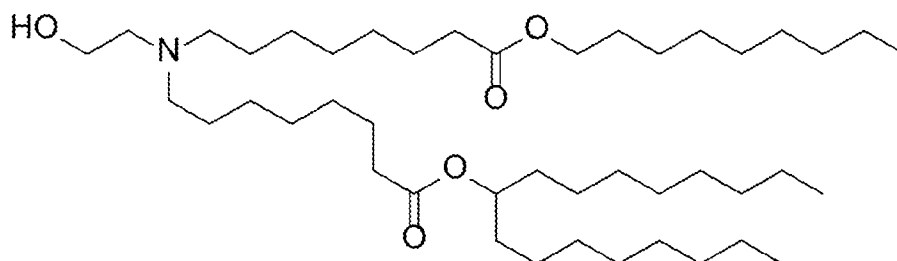
Heptadecan-9-yl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate



Chemical Formula: $C_{45}H_{87}NO_3$

Molecular Weight: 690.20

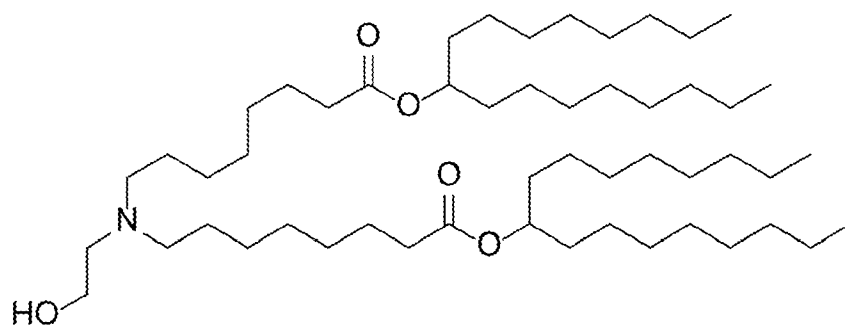
Heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate



Chemical Formula: $C_{44}H_{87}NO_5$

Molecular Weight: 710.18

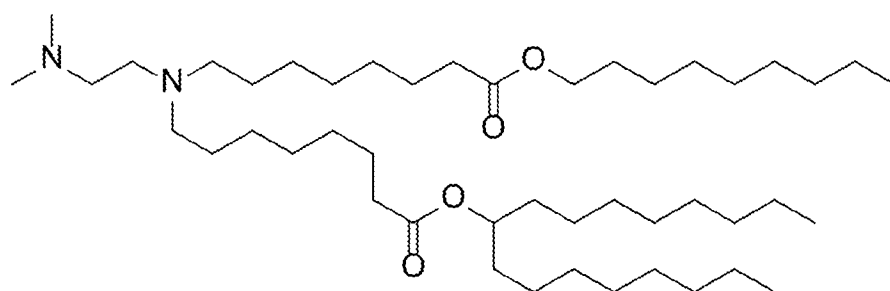
Di(heptadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate



Chemical Formula: $C_{52}H_{103}NO_5$

Molecular Weight: 822.40

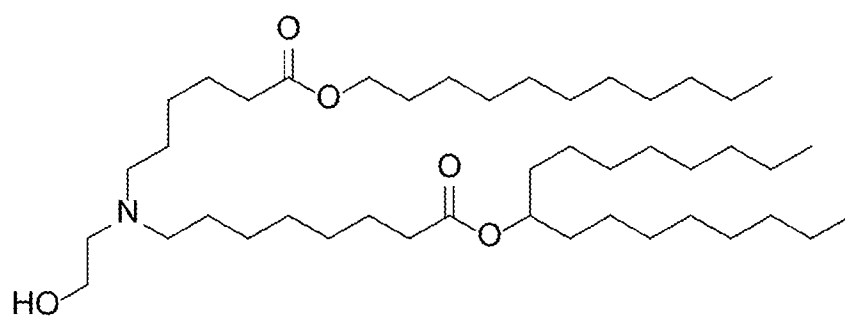
Heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate



Chemical Formula: $C_{46}H_{92}N_2O_4$

Molecular Weight: 737.252

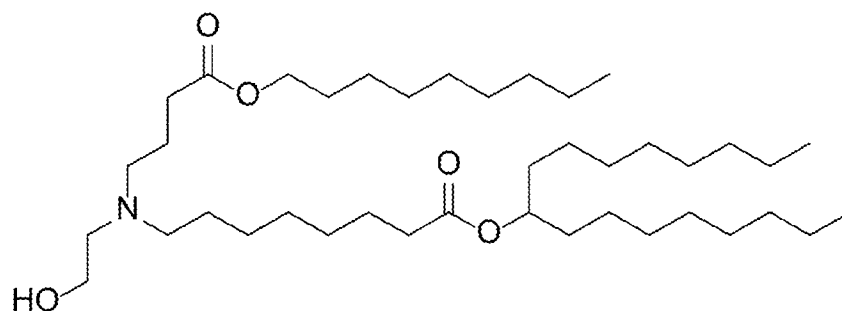
Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate



Chemical Formula: $C_{44}H_{87}NO_5$

Molecular Weight: 710.182

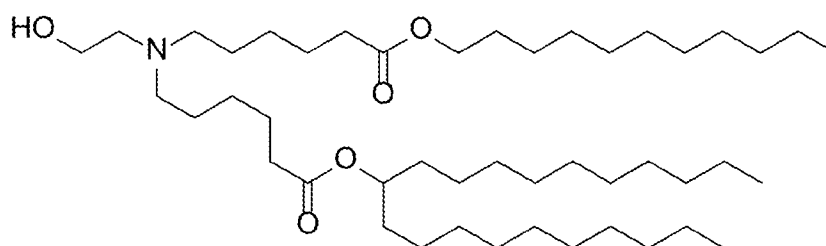
Heptadecan-9-yl 8-((2-hydroxyethyl)(4-(nonyloxy)-4-oxobutyl)amino)octanoate



Chemical Formula: $C_{40}H_{79}NO_5$

Molecular Weight: 654.074

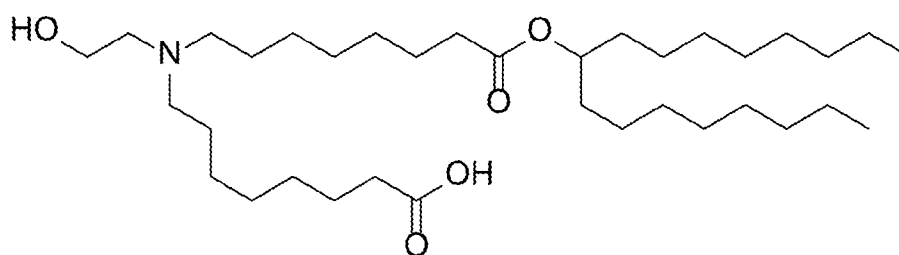
Henicosan-11-yl 6-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)hexanoate, or



Chemical Formula: $C_{46}H_{91}NO_5$

Molecular Weight: 738.24

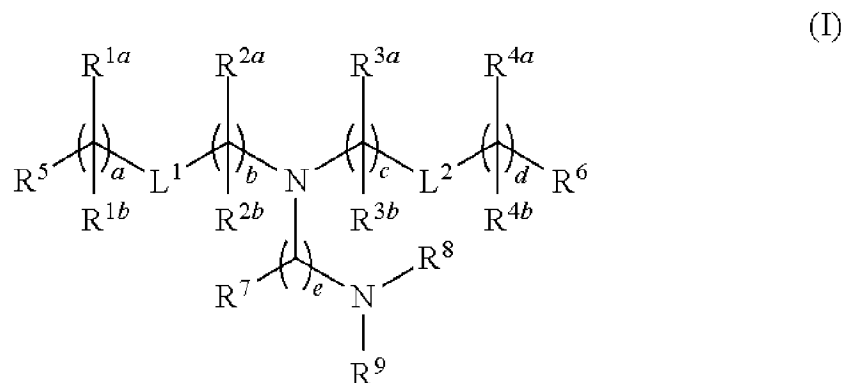
8-((8-(Heptadecan-9-yloxy)-8-oxooctyl)(2-hydroxyethyl)amino)octanoic acid



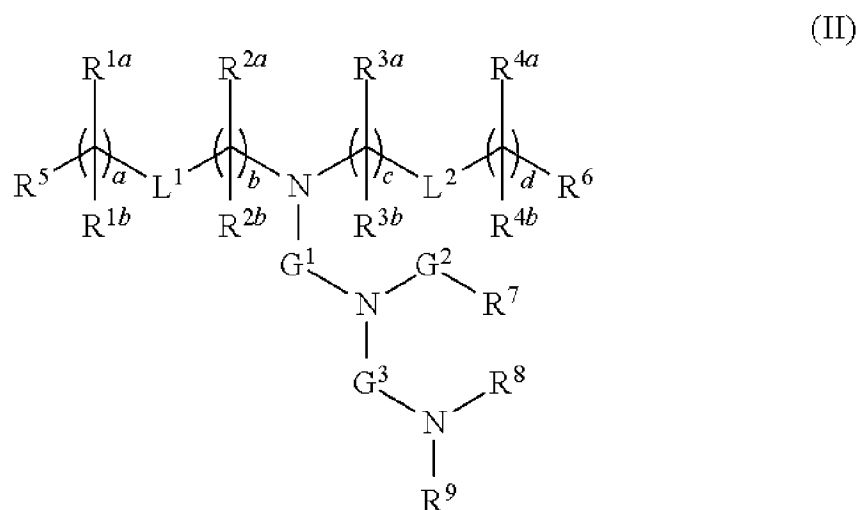
Chemical Formula: $C_{35}H_{69}NO_5$

Molecular Weight: 583.93

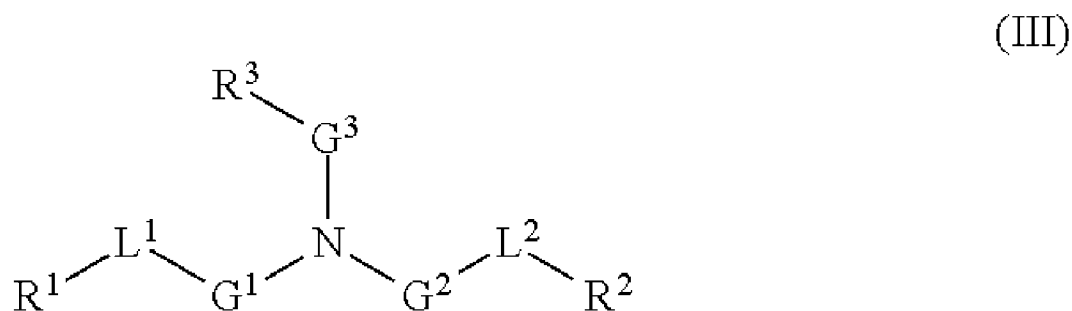
[0138] In particular embodiments, the cationic lipid has a Formula (I), (II) or (III):



or a pharmaceutically acceptable salt, tautomer or stereoisomer thereof, wherein R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^{4a}, R^{4b}, R⁵, R⁶, R⁷, R⁸, R⁹, L¹, L², a, b, c, d and e are as defined herein;



or a pharmaceutically acceptable salt, tautomer, prodrug or stereoisomer thereof, wherein R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b}, R^{4a}, R^{4b}, R⁵, R⁶, R⁷, R⁸, R⁹, L¹, L², G¹, G², G³, a, b, c and d are as defined herein;



or a pharmaceutically acceptable salt, tautomer, prodrug or stereoisomer thereof, wherein R¹, R², R³, L¹, L², G¹, G², and G³ are as defined herein.

[0139] In particular embodimentse, the LNP includes one or more cationic lipids and one or more stabilizing lipids. Stabilizing lipids include neutral lipids and pegylated lipids.

[0140] In some embodiments, LNP include a cationic lipid and one or more excipient selected from neutral lipids, charged lipids, steroids and polymer conjugated lipids.

[0141] In some embodiments LNP include a phospholipid. In some embodiments, the phospholipid includes 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-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 (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosaheptaenoyl-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-dilinenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, or mixtures thereof.

[0142] In some embodiments, the phospholipid includes 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-docosaheptaenoyl-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-docosaheptaenoyl-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 (OChemPC), and any combination thereof.

[0143] In some embodiments, the LNP include a structural lipid. In some embodiments, the structural lipid is cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, or mixtures thereof.

[0144] In some embodiments the LNP include a PEG lipid. In some embodiments, the PEG lipid is a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, a PEG-modified dialkylglycerol, or mixtures thereof.

[0145] In some embodiments the LNP include an ionizable lipid selected from 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10), N1-[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-dilinoley-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethyl amino)butanoate (DLin-MC3-DMA), 2,2-dilinoley-4-(2-dimethylaminoethyl)[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3 β)-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-[(3 β)-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-[(3 β)-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)).

[0146] In some embodiments the LNP include a quaternary amine compound. In some embodiments, the quaternary amine compound includes 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), 2,3-dioleyloxy-

N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), N-(1,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DOME), N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC), 1,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), 1,2-distearoyl-3-trimethylammonium-propane (DSTAP), 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), 1,2-dilinoleoyl-3-trimethylammonium-propane (DLTAP), 1,2-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.

[0147] In particular embodiments, LNP include liposomes and lipids are selected to achieve a specified degree of fluidity or rigidity of the final complex. As indicated, in particular embodiments, liposomes provide a lipid composition that is an outer layer surrounding a particle.

[0148] Liposomes can be neutral (cholesterol) or bipolar and include phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and sphingomyelin (SM) and other type of bipolar lipids including dioleoylphosphatidylethanolamine (DOPE), with a hydrocarbon chain length in the range of 14-22, and saturated or with one or more double C=C bonds. Examples of lipids capable of producing a stable liposome, alone, or in combination with other lipid components are phospholipids, such as hydrogenated soy phosphatidylcholine (HSPC), lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cephalin, cardiolipin, phosphatidic acid, cerebro sides, distearoylphosphatidylethanolamine (DSPE), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoylphosphatidylethanolamine (POPE) and dioleoylphosphatidylethanolamine 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (DOPE-mal). Additional non-phosphorous containing lipids that can become incorporated into liposomes include stearylamine, dodecylamine, hexadecylamine, isopropyl myristate, triethanolamine-lauryl sulfate, alkyl-aryl sulfate, acetyl palmitate, glycerol ricinoleate, hexadecyl stearate, amphoteric acrylic polymers, polyethoxylated fatty acid amides, and the cationic lipids mentioned above (DDAB, DODAC, DMRIE, DMTAP, DOGS, DOTAP (DOTMA), DOSPA, DPTAP, DSTAP, DC-Chol). Negatively charged lipids include phosphatidic acid (PA), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylglycerol and (DOPG),

dicetylphosphate that are able to form vesicles. In particular embodiments, lipids used to create liposomes disclosed herein include cholesterol, hydrogenated soy phosphatidylcholine (HSPC) and, the derivatized vesicle-forming lipid PEG-DSPE.

[0149] Methods of forming liposomes are described in, for example, US Patent Nos. 4,229,360; 4,224,179; 4,241,046; 4,737,323; 4,078,052; 4,235,871; 4,501,728; and 4,837,028, as well as in Szoka et al., *Ann. Rev. Biophys. Bioeng.* 9:467 (1980) and Hope et al., *Chem. Phys. Lip.* 40:89 (1986).

[0150] In the context of the present disclosure, LNP are not restricted to any particular morphology, and should be interpreted as to include any morphology generated when a cationic lipid and optionally one or more further lipids are combined, e.g., in an aqueous environment and/or in the presence of a nucleic acid compound. For example, a liposome, a lipid complex, a lipoplex and the like are within the scope of an LNP.

[0151] The size of LNP can vary over a wide range and can be measured in different ways. For example, LNP can have a minimum dimension of 100 nm. The LNP of the present disclosure can also have a minimum dimension of equal to or less than 500 nm, less than 150 nm, less than 100 nm, less than 90 nm, less than 80 nm, less than 70 nm, less than 60 nm, less than 50 nm, less than 40 nm, less than 30 nm, less than 20 nm, or less than 10 nm. In particular embodiments, the LNP can have a minimum dimension ranging between 5 nm and 500 nm, between 10 nm and 100 nm, between 20 nm and 90 nm, between 30 nm and 80 nm, between 40 nm and 70 nm, and between 40 nm and 60 nm. In particular embodiments, the dimension is the diameter of the LNP. In particular embodiments, a population of LNP can have a mean minimum dimension of equal to or less than 500 nm, less than 100 nm, less than 90 nm, less than 80 nm, less than 70 nm, less than 60 nm, less than 50 nm, less than 40 nm, less than 30 nm, less than 20 nm, or less than 10 nm. In particular embodiments, a population of LNP in a foam can have a mean diameter ranging between 5 nm and 500 nm, between 10 nm and 100 nm, between 20 nm and 90 nm, between 30 nm and 80 nm, between 40 nm and 70 nm, and between 40 nm and 60 nm.

[0152] In various embodiments, the LNP have a mean diameter of from 30 nm to 150 nm, from 40 nm to 150 nm, from 50 nm to 150 nm, from 60 nm to 130 nm, from 70 nm to 110 nm, from 70 nm to 100 nm, from 80 nm to 100 nm, from 90 nm to 100 nm, from 70 to 90 nm, from 80 nm to 90 nm, from 70 nm to 80 nm, or 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm, and are substantially non-toxic. In certain embodiments, the nucleic acid, when present in the LNP, is resistant in aqueous solution to degradation with a nuclease. Dimensions of the LNP can be determined using, e.g., conventional techniques, such

as dynamic light scattering and/or electron microscopy. In particular embodiments, the mean diameter may be represented by the z-average as determined by dynamic light scattering.

[0153] In some embodiments, the nucleic acid or a portion thereof, is encapsulated in the lipid portion of the LNP or an aqueous space enveloped by some or all of the lipid portion of the LNP, thereby protecting it from enzymatic degradation or other undesirable effects induced by the mechanisms of the host organism or cells e.g., an adverse immune response. In some embodiments, the nucleic acid or a portion thereof is associated with the LNP.

[0154] (v) Viral Vectors. A "vector" is a nucleic acid molecule that is capable of transporting another nucleic acid. Vectors may be, e.g., viruses, phage, a DNA vector, an RNA vector, a viral vector, a bacterial vector, a plasmid vector, a cosmid vector, and an artificial chromosome vector. An "expression vector" is any type of vector that is capable of directing the expression of a protein encoded by one or more genes carried by the vector when it is present in the appropriate environment.

[0155] Viral vectors are usually non-replicating or replication-impaired vectors, which means that the viral vector cannot replicate to any significant extent in normal cells (e.g., normal human cells), as measured by conventional means (e.g., via measuring DNA synthesis and/or viral titer). Non-replicating or replication-impaired vectors may have become so naturally (i.e., they have been isolated as such from nature) or artificially (e.g., by breeding in vitro or by genetic manipulation). There will generally be at least one cell-type in which the replication-impaired viral vector can be grown—for example, modified vaccinia Ankara (MVA) can be grown in CEF cells. Typically, viral vectors are incapable of causing a significant infection in a subject, typically in a mammalian subject.

[0156] Retroviral vectors (see Miller, et al., 1993, Meth. Enzymol. 217:581-599) can be used. In such embodiments, the gene to be expressed is cloned into the retroviral vector for its delivery into cells. In particular embodiments, a retroviral vector includes all of the cis-acting sequences necessary for the packaging and integration of the viral genome, i.e., (a) a long terminal repeat (LTR), or portions thereof, at each end of the vector; (b) primer binding sites for negative and positive strand DNA synthesis; and (c) a packaging signal, necessary for the incorporation of genomic RNA into virions. More detail about retroviral vectors can be found in Boesen, et al., 1994, Biotherapy 6:291-302; Clowes, et al., 1994, J. Clin. Invest. 93:644-651; Kiem, et al., 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114. Adenoviruses, adeno-associated viruses (AAV) and alphaviruses can also be used. See Kozarsky and Wilson, 1993, Current Opinion in Genetics and Development 3:499-503, Rosenfeld, et al., 1991, Science

252:431-434; Rosenfeld, et al., 1992, Cell 68:143-155; Mastrangeli, et al., 1993, J. Clin. Invest. 91:225-234; Walsh, et al., 1993, Proc. Soc. Exp. Bioi. Med. 204:289-300; and Lundstrom, 1999, J. Recept. Signal Transduct. Res. 19: 673-686. Other methods of gene delivery include use of mammalian artificial chromosomes (Vos, 1998, Curr. Op. Genet. Dev. 8:351-359); liposomes (Tarahovsky and Ivanitsky, 1998, Biochemistry (Mosc) 63:607-618); ribozymes (Branch and Klotman, 1998, Exp. Nephrol. 6:78-83); and triplex DNA (Chan and Glazer, 1997, J. Mol. Med. 75:267-282).

[0157] "Gammaretroviruses" refers to a genus of the retroviridae family. Exemplary gammaretroviruses include mouse stem cell virus, murine leukemia virus, feline leukemia virus, feline sarcoma virus, and avian reticuloendotheliosis viruses.

[0158] Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), simian immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations thereof (see, e.g., Buchscher et al., J. Virol. 66:2731-2739, 1992; Johann et al., J. Virol. 66:1635-1640, 1992; Sommerfelt et al., Virol. 176:58-59, 1990; Wilson et al., J. Virol. 63:2374-2378, 1989; Miller et al., J. Virol. 65:2220-2224, 1991; and PCT/US94/05700).

[0159] Particularly suitable are lentiviral vectors. "Lentivirus" refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells and typically produce high viral titers. Lentiviral vectors have been employed in gene therapy for a number of diseases. For example, hematopoietic gene therapies using lentiviral vectors or gamma retroviral vectors have been used for x-linked adrenoleukodystrophy and beta thalassaemia. See, e.g., Kohn et al., Clin. Immunol. 135:247-54, 2010; Cartier et al., Methods Enzymol. 507:187-198, 2012; and Cavazzana-Calvo et al., Nature 467:318-322, 2010. Several examples of lentiviruses include HIV (human immunodeficiency virus: including HIV type 1, and HIV type 2); equine infectious anemia virus; feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

[0160] In particular embodiments, other retroviral vectors can be used in the practice of the methods of the disclosure. These include, e.g., vectors based on human foamy virus (HFV) or other viruses in the Spumavirus genera.

[0161] Foamy viruses (FVs) are the largest retroviruses known today and are widespread among different mammals, including all non-human primate species, however are absent in humans. This complete apathogenicity qualifies FV vectors as ideal nucleic acid transfer vehicles for genetic therapies in humans and clearly distinguishes FV vectors as gene delivery system from HIV -derived and also gammaretrovirus-derived vectors.

[0162] FV vectors are suitable for gene therapy applications because they can (1) accommodate large transgenes (> 9kb), (2) transduce slowly dividing cells efficiently, and (3) integrate as a provirus into the genome of target cells, thus enabling stable long-term expression of the transgene(s). FV vectors do need cell division for the pre-integration complex to enter the nucleus, however the complex is stable for at least 30 days and still infective. The intracellular half-life of the FV pre-integration complex is comparable to the one of lentiviruses and significantly higher than for gammaretroviruses, therefore FV are also, similar to LV vectors, able to transduce rarely dividing cells. FV vectors are natural self-inactivating vectors and characterized by the fact that they seem to have hardly any potential to activate neighboring genes. In addition, FV vectors can enter any cells known (although the receptor is not identified yet) and infectious vector particles can be concentrated 100-fold without loss of infectivity due to a stable envelope protein. FV vectors achieve high transduction efficiency in pluripotent hematopoietic stem cells and have been used in animal models to correct monogenetic diseases such as leukocyte adhesion deficiency (LAD) in dogs and Fanconi anemia in mice. FV vectors are also used in preclinical studies of β -thalassemia.

[0163] Additional examples of viral vectors include those derived from adenoviruses (e.g., adenovirus 5 (Ad5), adenovirus 35 (Ad35), adenovirus 11 (Ad11), adenovirus 26 (Ad26), adenovirus 48 (Ad48) or adenovirus 50 (Ad50)), adeno-associated virus (AAV; see, e.g., U.S. Pat. No. 5,604,090; Kay et al., Nat. Genet. 24:257 (2000); Nakai et al., Blood 91:4600 (1998)), alphaviruses, cytomegaloviruses (CMV), flaviviruses, herpes viruses (e.g., herpes simplex), influenza viruses, papilloma viruses (e.g., human and bovine papilloma virus; see, e.g., U.S. Pat. No. 5,719,054), poxviruses, vaccinia viruses, etc. See Kozarsky and Wilson, 1993, Current Opinion in Genetics and Development 3:499-503, Rosenfeld, et al., 1991, Science 252:431-434; Rosenfeld, et al., 1992, Cell 68:143-155; Mastrangeli, et al., 1993, J. Clin. Invest. 91:225-234; Walsh, et al., 1993, Proc. Soc. Exp. Biol. Med. 204:289-300; and Lundstrom, 1999, J. Recept. Signal Transduct. Res. 19: 673-686. Examples include modified vaccinia Ankara (MVA) and NYVAC, or strains derived therefrom. Other examples include avipox vectors, such as a fowlpox vectors (e.g., FP9) or canarypox vectors (e.g., ALVAC and strains derived therefrom).

[0164] Particular embodiments utilize oncolytic viruses. Oncolytic viruses (OVs) are replication-competent viruses that infect and preferentially lyse tumor cells while leaving non-neoplastic cells intact. OVs can be selected from native viral species on the basis of their innate ability to induce immunogenic cell death (ICD) in cancer cells, although they can also be genetically engineered to enhance tumor selectivity, promote replication competence, limit pathogenicity and increase immunogenicity. Engineered OV can be manipulated by the deletion or modification of viral genes

or, in viruses with larger genomes, eukaryotic transgenes can be included as an additional 'payload' usually for the purpose of increasing the extent of tumor cell death or promoting antitumor immunity. Both DNA and RNA viruses capable of oncolytic activity in mammalian cells are available (Shalhout, S.Z., et al., Nat Rev Clin Oncol 20, 160–177, 2023). Particular OV can include an adenovirus, reovirus, measles, herpes simplex, Newcastle disease virus, senecavirus, or vaccinia virus.

[0165] OV can be derived from clinical isolates. For example, in some embodiments, the OV is a herpes simplex virus. Such strains can be isolated from infected individuals, such as those with recurrent cold sores. Clinical isolates may be screened for a desired ability or characteristic such as enhanced replication in tumor and/or other cells in vitro and/or in vivo in comparison to standard laboratory strains, as described in U.S. Pat. Nos. 7,063,835 and 7,223,593. In one embodiment the herpes simplex virus is a clinical isolate from a recurrent cold sore.

[0166] Particular embodiments utilize Herpes simplex virus 1 (HSV-1). Appropriate HSV-1 strains include strain JS1, strain 17+, strain F, strain KOS, and strain Patton.

[0167] The HSV-1 may be modified such that it lacks functional ICP34.5 genes and/or lacks a functional ICP47 gene. ICP34.5 acts as a virulence factor during HSV infection, limits replication in non-dividing cells and renders the virus non-pathogenic. ICP47 down-regulates major histocompatibility complex (MHC) class I expression on the surface of infected host cells and MHC Class I binding to transporter associated with antigen presentation (TAP). Such actions block antigenic peptide transport in the endoplasmic reticulum and loading of MHC class I molecules. Another HSV gene that can be modified is ICP6, the large subunit of ribonucleotide reductase, involved in nucleotide metabolism and viral DNA synthesis in non-dividing cells but not in dividing cells. Thymidine kinase, responsible for phosphorylating acyclovir to acyclovir-monophosphate, virion trans-activator protein vmw65, glycoprotein H, vhs, ICP43, and immediate early genes encoding ICP4, ICP27, ICP22 and/or ICP0, may also be modified.

[0168] Herpes virus strains and how to make such strains are also described in U.S. Pat. Nos. 5,824,318; 6,764,675; 6,770,274; 7,063,835; 7,223,593; 7,749,745; 7,744,899; 8,273,568; 8,420,071; and 8,470,577; WIPO Publication Numbers WO199600007; WO199639841; WO199907394; WO200054795; WO2006002394; and WO201306795; Chinese Patent Numbers CN128303, CN10230334 and CN 10230335; Varghese and Rabkin, (2002) Cancer Gene Therapy 9:967-97, and Cassady and Ness Parker, (2010) The Open Virology Journal 4:103-108.

[0169] In one embodiment, the OV is talimogene laherparepvec (IMLYGIC®), derived from a clinical strain (HSV-1 strain JS1) deposited at the European collection of cell cultures (ECAAC) under accession number 01010209. In talimogene laherparepvec, the HSV-1 viral genes

encoding ICP34.5 and ICP47 have been functionally deleted. Functional deletion of ICP47 leads to earlier expression of US11, a gene that promotes virus growth in tumor cells without decreasing tumor selectivity. The coding sequence for human GM-CSF, has been inserted into the viral genome at the former ICP34.5 sites (see Liu et al., *Gene Ther* 10: 292-303, 2003).

[0170] In some embodiments, the OV is a herpes simplex 2 virus (HSV-2). Additional embodiments can include inter-type recombinants containing DNA from HSV-1 and HSV-2 strains. Such inter-type recombinants are described in, for example Thompson et al., (1998) *Virus Genes* 1(3); 275286, and Meignier et al., (1998) *J. Infect. Dis.* 159; 602614.

[0171] Further examples of OV based on HSV-1 or HSV-2 include:

G207, an oncolytic HSV-1 derived from wild-type HSV-1 strain F having deletions in both copies of the major determinant of HSV neurovirulence, the ICP 34.5 gene, and an inactivating insertion of the *E. coli* lacZ gene in UL39, which encodes the infected-cell protein 6 (ICP6), see Mineta et al. (1995) *Nat Med.* 1:938-943;

OrienX010, a herpes simplex virus with deletion of both copies of γ 34.5 and the ICP47 genes as well as an interruption of the ICP6 gene and insertion of the human GM-CSF gene, see Liu et al., (2013) *World Journal of Gastroenterology* 19(31):5138-5143;

NV1020, a herpes simplex virus with the joint region of the long (L) and short (S) regions is deleted, including one copy of ICP34.5, UL24, and UL56.34,35. The deleted region was replaced with a fragment of HSV-2 US DNA (US2, US3 (PK), gJ, and gG), see Todo, et al. (2001) *Proc Natl Acad Sci USA.* 98:6396-6401;

M032, a herpes simplex virus with deletion of both copies of the ICP34.5 genes and insertion of interleukin 12, see Cassady and Ness Parker, (2010) *The Open Virology Journal* 4:103-108;

ImmunoVEX HSV2, is a herpes simplex virus (HSV-2) having functional deletions of the genes encoding vhs, ICP47, ICP34.5, UL43 and US5; and

OncoVEXGALV/CD is also derived from HSV-1 strain JS1 with the genes encoding ICP34.5 and ICP47 having been functionally deleted and the gene encoding cytosine deaminase and gibbon ape leukemia fusogenic glycoprotein inserted into the viral genome in place of the ICP34.5 genes.

[0172] In particular embodiments, the OV may be selected from Oncorine (H101) a genetically modified (E1B-deletion) adenovirus, Riggvir (Riga virus) an oncolytic Enteric Cytopathogenic Human Orphan type 7 echovirus (ECHO-7), Teserpaturev a third generation (triple-mutated) recombinant oncolytic herpes simplex virus type 1, and talimogene laherparepvec (T-VEC, IMLYGIC®).

[0173] Other examples of OV include RP1 (HSV-1/ICP34.5-/ICP47-/GM-CSF/GALV-GP R(-); RP2 (HSV-1/ICP34.5-/ICP47-/GM-CSF/GALV-GP R(-)/anti-CTLA-4 binder; and RP3 (HSV-1/ICP34.5-/ICP47-/GM-CSF/GALV-GP R(-)/anti-CTLA-4 binder/co-stimulatory ligands (e.g., CD40L, 4-1BBL, GITRL, OX40L, ICOSL)). In such oncolytic viruses, GALV (gibbon ape leukemia virus) has been modified with a specific deletion of the R-peptide, resulting in GALV-GP R(-). Such oncolytic viruses are described in WO2017118864, WO2017118865, WO2017118866, WO2017118867, and WO2018127713A1.

[0174] Additional examples of OV include NSC-733972, HF-10, BV-2711, JX-594, Myb34.5, AE-618, Brainwel™, and Heapwel™, Cavatak® (coxsackievirus, CVA21), HF-10, Seprehvir®, Reolysin®, enadenotucirev, ONCR-177, and those described in U.S. Pat. No. 10,105,404, WO2018006005, WO2018026872A1, and WO2017181420.

[0175] Vectors, including OV, may be modified to include one or more heterologous genes in addition to a nucleic acid as otherwise described herein. Heterologous genes refer to a gene to be introduced to the genome of a virus, wherein that gene is not normally found in the virus' genome or is a homolog of a gene expressed in the virus from a different species which has a different nucleic acid sequence and acts via a different biochemical mechanism. The heterologous genes may encode one or more proteins, for example, a cytotoxin, an immunomodulatory protein (i.e., a protein that either enhances or suppresses a host immune response to an antigen), a tumor antigen, prodrug activator, a tumor suppressor, a prodrug converting enzyme, proteins capable of causing cell to cell fusion, a TAP inhibitor antisense RNA molecule, or a ribozyme. Examples of immunomodulatory proteins include, for example, cytokines. Cytokines include an interleukins, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-20; α , β or γ -interferons, tumor necrosis factor alpha (TNF α), CD40L, granulocyte macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), and granulocyte colony stimulating factor (G-CSF), chemokines (such as neutrophil activating protein (NAP), macrophage chemoattractant and activating factor (MCAF), RANTES, and macrophage inflammatory peptides MIP-1a and MIP-1b), complement components and their receptors, immune system accessory molecules (e.g., B7.1 and B7.2), adhesion molecules (e.g., ICAM-1, 2, and 3), and adhesion receptor molecules. Tumor antigens include the E6 and E7 antigens of human papillomavirus, EBV-derived proteins, mucins, such as MUC1, melanoma tyrosinase, and MZ2-E. Pro-drug activators include nitroreductase and cytochrome p450, tumour suppressors include p53. a prodrug converting enzymes include cytosine deaminase. Proteins capable of causing cell to cell fusion include gibbon ape leukaemia fusogenic glycoprotein. TAP inhibitors include the bovine herpesvirus (BHV) UL49.5 polypeptide.

Antisense RNA molecules that can be used to block expression of a cellular or pathogen mRNA. RNA molecules that can be a ribozyme (e.g., a hammerhead or a hairpin-based ribozyme) designed either to repair a defective cellular RNA, or to destroy an undesired cellular or pathogen-encoded RNA.

[0176] Other methods of gene delivery include use of artificial chromosome vectors such as mammalian artificial chromosomes (Vos, 1998, *Curr. Op. Genet. Dev.* 8:351-359) and yeast artificial chromosomes (YAC). YAC are typically used when the inserted nucleic acids are too large for more conventional vectors (e.g., greater than 12 kb).

[0177] There are a large number of available viral vectors suitable within the current disclosure, including those identified for human gene therapy applications (see Pfeifer and Verma, 2001, *Ann. Rev. Genomics Hum. Genet.* 2:177). Methods of using retroviral and lentiviral viral vectors and packaging cells for transducing mammalian host cells with viral particles including CAR transgenes are described in, e.g., US 8,119,772; Walchli, et al., 2011, *PLoS One* 6:327930; Zhao, et al., 2005, *J. Immunol.* 174:4415; Engels, et al., 2003, *Hum. Gene Ther.* 14:1155; Frecha, et al., 2010, *Mol. Ther.* 18:1748; and Verhoeyen, et al., 2009, *Methods Mol. Biol.* 506:97. Retroviral and lentiviral vector constructs and expression systems are also commercially available.

[0178] Particular embodiments utilize DNA constructs (e.g., chimeric genes, expression cassettes, expression vectors, recombination vectors, etc.) including a nucleic acid sequence encoding the protein or proteins of interest operatively linked to appropriate regulatory sequences. Such DNA constructs are not naturally occurring DNA molecules and are useful for introducing DNA into host-cells to express selected proteins of interest.

[0179] The term “gene” refers to a nucleic acid sequence that encodes and results in expression of a molecule (e.g., a therapeutic molecule). This definition includes various sequence polymorphisms, mutations, and/or sequence variants wherein such alterations do not substantially affect the function of the encoded genetic construct. The term “gene” may include not only coding sequences but also regulatory regions such as promoters, enhancers, and termination regions. Gene sequences encoding the molecule can be DNA or RNA. These nucleic acid sequences may be a DNA strand sequence that is transcribed into RNA or an RNA sequence that is translated into protein. The nucleic acid sequences include both the full-length nucleic acid sequences as well as non-full-length sequences derived from the full-length protein. The sequences can also include degenerate codons of the native sequence or sequences that may be introduced to provide codon preference in a specific cell type. Portions of complete gene sequences are referenced throughout the disclosure as is understood by one of ordinary skill in the art.

[0180] "Encoding" refers to the property of specific sequences of nucleotides in a gene, such as a cDNA, or an mRNA, to serve as templates for synthesis of other macromolecules such as a defined sequence of amino acids. Thus, a gene codes for a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. A "gene sequence encoding a protein" includes all nucleotide sequences that are degenerate versions of each other and that code for the same amino acid sequence or amino acid sequences of substantially similar form and function.

[0181] Gene sequences encoding therapeutic molecules are provided herein and can also be readily prepared by synthetic or recombinant methods from the relevant amino acid sequences and other description provided herein. In embodiments, the gene sequence encoding any of these sequences can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the gene sequence encoding the sequence with another gene sequence encoding a different sequence. In embodiments, the gene sequence encoding the sequences can be codon optimized for expression in mammalian cells.

[0182] Nucleic acid sequences encoding a molecule can be operably linked to relevant regulatory sequences. For example, there can be a functional linkage between a regulatory sequence and an exogenous nucleic acid sequence resulting in expression of the latter. For another example, a first nucleic acid sequence can be operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary or helpful, can join multiple coding regions, into the same reading frame.

[0183] Operatively linked refers to the linking of DNA sequences (including the order of the sequences, the orientation of the sequences, and the relative spacing of the various sequences) in such a manner that the encoded protein is expressed. Methods of operatively linking expression control sequences to coding sequences are well known in the art. See, e.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, N. Y., 1982; and Green and Sambrook, *Molecular Cloning: A Laboratory Manual*, 4th Edition, 2012.

[0184] Expression control or regulatory sequences are DNA sequences involved in any way in the control of transcription or translation. Suitable expression control sequences and methods of making and using them are well known in the art. Expression control sequences generally include a promoter. The promoter may be inducible or constitutive. It may be naturally occurring, may be

composed of portions of various naturally occurring promoters, or may be partially or totally synthetic. Guidance for the design of promoters is provided by studies of promoter structure, such as that of Harley and Reynolds, *Nucleic Acids Res.*, 15, 2343-2361, 1987. Also, the location of the promoter relative to the transcription start may be optimized. See, e.g., Roberts et al., *Proc. Natl. Acad. Sci. USA*, 76:760-764, 1979.

[0185] The promoter may include, or be modified to include, one or more enhancer elements. In particular embodiments, the promoter will include a plurality of enhancer elements. Promoters including enhancer elements can provide for higher levels of transcription as compared to promoters that do not include them.

[0186] For efficient expression, the coding sequences can be operatively linked to a 3' untranslated sequence. In particular embodiments, the 3' untranslated sequence can include a transcription termination sequence and a polyadenylation sequence. The 3' untranslated region can be obtained, for example, from the flanking regions of genes.

[0187] In particular embodiments, a 5' untranslated leader sequence can also be employed. The 5' untranslated leader sequence is the portion of an mRNA that extends from the 5' CAP site to the translation initiation codon. In particular embodiments, the nucleic acid is stably integrated into the genome of a cell. In particular embodiments, the nucleic acid is stably maintained in a cell as a separate, episomal segment.

[0188] (vi) Targeting Ligands. Targeting ligands include binding domains that result in binding of the targeting ligand to a targeted antigen. Targeted antigens can include antigens expressed by any cell type. Examples include cells that line body cavities (oral, thoracic or abdominal), cells that line the intestinal tract, cells that line the female genital lumen, cells within injectable tissues (especially muscle for vaccine applications, bone marrow or lymph nodes), cells within tumors, and skin cells.

[0189] A targeted antigen should be preferentially expressed by the cell type to which a nucleic acid is intended for delivery. "Preferentially expressed" means that a targeted antigen is found at higher levels on the cell type to which a nucleic acid is intended for delivery as compared to other cell types. In some instances, the antigen is only expressed by the cell type to which a nucleic acid is intended for delivery. In other instances, the targeted antigen is expressed on the cell type to which a nucleic acid is intended for delivery at least 25%, 35%, 45%, 55%, 65%, 75%, 85%, 95%, 96%, 97%, 98%, 99%, or 100% more than on cells not targeted for nucleic acid delivery.

[0190] Taking cancer cells as just one example, cancer cell antigens are preferentially expressed by cancer cells. Exemplary antigens preferentially expressed by cancer cells include A33; BAGE; Bcl-2; β -catenin; CA125; CA19-9; CD5; CD19; CD20; CD21; CD22; CD33; CD37; CD45; CD123;

CEA; c-Met; CS-1; cyclin B1; DAGE; EBNA; EGFR; ephrinB2; estrogen receptor; FAP; ferritin; folate-binding protein; GAGE; G250; GD-2; GM2; gp75, gp100 (Pmel 17); HER-2/neu; HPV E6; HPV E7; Ki-67; LRP; mesothelin, p53, PRAME; progesterone receptor; PSA; PSMA; MAGE; MART; mesothelin; MUC; MUM-1-B; myc; NYESO-1; ras; ROR1; survivin; tenascin; TSTA tyrosinase; VEGF; and WT1. The following table provides examples of particular cancers and cancer antigens that can be bound by targeting ligands.

Targeted Cancer	Cancer Antigens
Prostate Cancer	PSMA, WT1, Prostate Stem Cell antigen (PSCA), SV40 T
Breast Cancer	HER2, ERBB2, ROR1
Ovarian Cancer	L1-CAM, extracellular domain of MUC16 (MUC-CD), folate binding protein (folate receptor), Lewis Y, ROR1, mesothelin, WT-1
Mesothelioma	mesothelin
Renal Cell Carcinoma	carboxy-anhydrase-IX (CAIX);
Melanoma	GD2
Pancreatic Cancer	mesothelin, CEA, CD24, ROR1
Lung Cancer	ROR1
Leukemia/Lymphoma	CD19, CD20, CD22, ROR1, CD33, WT-1, CD123
Multiple Myeloma	B-cell maturation antigen (BCMA)

[0191] Foams of the disclosure can also be used to target and kill infected cells that are clustered at an anatomical site.

[0192] Exemplary viruses include adenoviruses, arenaviruses, bunyaviruses, coronaviruses, flaviruses, hantaviruses, hepadnaviruses, herpesviruses, papillomaviruses, paramyxoviruses, parvoviruses, picornaviruses, poxviruses, orthomyxoviruses, retroviruses, reoviruses, rhabdoviruses, rotaviruses, spongiform viruses or togaviruses. In additional embodiments, viral targeted antigens include peptides expressed by CMV, cold viruses, Epstein-Barr, flu viruses, hepatitis A, B, and C viruses, herpes simplex, HIV, influenza, Japanese encephalitis, measles, polio, rabies, respiratory syncytial, rubella, smallpox, varicella zoster or West Nile virus.

[0193] As further particular examples, coronavirus antigen includes the spike protein, cytomegaloviral antigens include envelope glycoprotein B and CMV pp65; Epstein-Barr antigens include EBV EBNA1, EBV P18, and EBV P23; hepatitis antigens include the S, M, and L proteins

of hepatitis B virus, the pre-S antigen of hepatitis B virus, HBCAG DELTA, HBV HBE, hepatitis C viral RNA, HCV NS3 and HCV NS4; herpes simplex viral antigens include immediate early proteins and glycoprotein D; HIV antigens include gene products of the gag, pol, and env genes such as HIV gp32, HIV gp41, HIV gp120, HIV gp160, HIV P17/24, HIV P24, HIV P55 GAG, HIV P66 POL, HIV TAT, HIV GP36, the Nef protein and reverse transcriptase; influenza antigens include hemagglutinin and neuraminidase; Japanese encephalitis viral antigens include proteins E, M-E, M-E-NS1, NS1, NS1-NS2A and 80% E; measles antigens include the measles virus fusion protein; rabies antigens include rabies glycoprotein and rabies nucleoprotein; respiratory syncytial viral antigens include the RSV fusion protein and the M2 protein; rotaviral antigens include VP7sc; rubella antigens include proteins E1 and E2; and varicella zoster viral antigens include gpl and gpII.

[0194] Additional particular exemplary viral antigen sequences include:

Source	Sequence	SEQ ID NO.
Nef (66-97):	VGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGL	1
Nef (116-145)	HTQGYFPDWQNYTPGPGVRYPLTFGWLYKL	2
Gag p17 (17-35)	EKIRLRPGGKKKYKLKHIV	3
Gag p17-p24 (253-284)	NPPIPVGEIYKRWILGLNKIVRMYSPTSILD	4
Pol 325-355 (RT 158-188)	AIFQSSMTKILEPFRKQNPDIVIYQYMDDLY	5

See Fundamental Virology, Second Edition, eds. Fields, B. N. and Knipe, D. M. (Raven Press, New York, 1991) for additional examples of viral antigens.

[0195] In particular embodiments, the targeting agent targets HIV gag protein, gp120 or the Hepatitis B envelope protein (S domain).

[0196] In particular embodiments, targeted antigens are expressed by cells associated with bacterial infections. Exemplary bacteria include anthrax; gram-negative bacilli, chlamydia, diptheria, haemophilus influenza, Helicobacter pylori, malaria, Mycobacterium tuberculosis, pertussis toxin, pneumococcus, rickettsiae, staphylococcus, streptococcus and tetanus.

[0197] As particular examples of bacterial targeted antigens, anthrax antigens include anthrax protective antigen; gram-negative bacilli antigens include lipopolysaccharides; haemophilus influenza antigens include capsular polysaccharides; diptheria antigens include diptheria toxin; Mycobacterium tuberculosis antigens include mycolic acid, heat shock protein 65 (HSP65), the 30 kDa major secreted protein and antigen 85A; pertussis toxin antigens include hemagglutinin,

pertactin, FIM2, FIM3 and adenylate cyclase; pneumococcal antigens include pneumolysin and pneumococcal capsular polysaccharides; rickettsiae antigens include rompA; streptococcal antigens include M proteins; and tetanus antigens include tetanus toxin.

[0198] In particular embodiments, targeted antigens are expressed by cells associated with fungal infections. Exemplary fungi include candida, coccidioides, cryptococcus, histoplasma, leishmania, plasmodium, protozoa, parasites, schistosomae, tinea, toxoplasma, and trypanosoma cruzi.

[0199] As further particular examples of fungal antigens, coccidioides antigens include spherule antigens; cryptococcal antigens include capsular polysaccharides; histoplasma antigens include heat shock protein 60 (HSP60); leishmania antigens include gp63 and lipophosphoglycan; plasmodium falciparum antigens include merozoite surface antigens, sporozoite surface antigens, circumsporozoite antigens, gametocyte/gamete surface antigens, protozoal and other parasitic antigens including the blood-stage antigen pf 155/RESA; schistosomae antigens include glutathione-S-transferase and paramyosin; tinea fungal antigens include trichophytin; toxoplasma antigens include SAG-1 and p30; and trypanosoma cruzi antigens include the 75-77 kDa antigen and the 56 kDa antigen.

[0200] In particular embodiments, targeted antigens are expressed by cells associated with autoimmune or allergic conditions. Exemplary autoimmune conditions include acute necrotizing hemorrhagic encephalopathy, allergic asthma, alopecia areata, anemia, aphthous ulcer, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), asthma, autoimmune thyroiditis, conjunctivitis, Crohn's disease, cutaneous lupus erythematosus, dermatitis (including atopic dermatitis and eczematous dermatitis), diabetes, diabetes mellitus, erythema nodosum leprosum, keratoconjunctivitis, multiple sclerosis, myasthenia gravis, psoriasis, scleroderma, Sjogren's syndrome, including keratoconjunctivitis sicca secondary to Sjogren's syndrome, Stevens-Johnson syndrome, systemic lupus erythematosus, ulcerative colitis, vaginitis and Wegener's granulomatosis.

[0201] Examples of autoimmune antigens include glutamic acid decarboxylase 65 (GAD 65), native DNA, myelin basic protein, myelin proteolipid protein, acetylcholine receptor components, thyroglobulin, and the thyroid stimulating hormone (TSH) receptor. Examples of allergic antigens include pollen antigens such as Japanese cedar pollen antigens, ragweed pollen antigens, rye grass pollen antigens, animal derived antigens (such as dust mite antigens and feline antigens), histocompatibility antigens, and penicillin and other therapeutic drugs.

[0202] Alternatively, foams can be targeted to cells to increase the function or health of the targeted cells. Numerous examples are described elsewhere herein. Particular embodiments

include HSC genome editing applications where genetic defects are corrected (e.g., sickle cell anemia, thalassemia, SCID) and promotion of wound healing through foam application to the skin.

[0203] Antibodies are one example of binding domains and include whole antibodies or binding fragments of an antibody, e.g., Fv, Fab, Fab', F(ab')₂, and single chain (sc) forms and fragments thereof that bind specifically a cellular marker. Antibodies or antigen binding fragments can include all or a portion of polyclonal antibodies, monoclonal antibodies, human antibodies, humanized antibodies, synthetic antibodies, non-human antibodies, recombinant antibodies, chimeric antibodies, bispecific antibodies, mini bodies, and linear antibodies.

[0204] Antibodies are produced from two genes, a heavy chain gene and a light chain gene. Generally, an antibody includes two identical copies of a heavy chain, and two identical copies of a light chain. Within a variable heavy chain and variable light chain, segments referred to as complementary determining regions (CDRs) dictate epitope binding. Each heavy chain has three CDRs (i.e., CDRH1, CDRH2, and CDRH3) and each light chain has three CDRs (i.e., CDRL1, CDRL2, and CDRL3). CDR regions are flanked by framework residues (FR).

[0205] The assignment of amino acids to each domain can be in accordance with Kabat numbering (Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. ("Kabat" numbering scheme)); Chothia (Al-Lazikani et al., (1997) JMB 273, 927-948 ("Chothia" numbering scheme)), Martin (Abinandan et al., Mol Immunol. 45:3832-3839 (2008), "Analysis and improvements to Kabat and structurally correct numbering of antibody variable domains"), Gelfand, Contact (MacCallum et al., J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745." (Contact numbering scheme)), IMGT (Lefranc M P et al., "IMGT unique numbering for immunoglobulin and T-cell receptor variable domains and Ig superfamily V-like domains," Dev Comp Immunol, 2003 January; 27(1):55-77 ("IMGT" numbering scheme)), AHo (Honegger A and Plückthun A, "Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool," J Mol Biol, 2001 Jun. 8; 309(3):657-70, (AHo numbering scheme)), North (North et al., J Mol Biol. 406(2):228-256 (2011), "A new clustering of antibody CDR loop conformations"), or other numbering schemes. CDR residues can be identified using software programs such as ABodyBuilder. The boundaries of a given CDR or FR may vary depending on the scheme used for identification.

[0206] In some instances, scFvs can be prepared according to methods known in the art (see, for example, Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). ScFv molecules can be produced by linking VH and VL regions of an antibody together using flexible polypeptide linkers. If a short polypeptide linker is employed (e.g.,

between 5-10 amino acids) intrachain folding is prevented. Interchain folding is also required to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientations and sizes see, e.g., Hollinger et al. 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448, US 2005/0100543, US 2005/0175606, US 2007/0014794, and WO2006/020258 and WO2007/024715. More particularly, linker sequences that are used to connect the VL and VH of an scFv are generally five to 35 amino acids in length. In particular embodiments, a VL-VH linker includes from five to 35, ten to 30 amino acids or from 15 to 25 amino acids. Variation in the linker length may retain or enhance activity.

[0207] In particular embodiments, the binding domain includes a humanized antibody or an engineered fragment thereof. In particular embodiments, a non-human antibody is humanized, where one or more amino acid residues of the antibody are modified to increase similarity to an antibody naturally produced in a human or fragment thereof. These nonhuman amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. As provided herein, humanized antibodies or antibody fragments include one or more CDRs from nonhuman immunoglobulin molecules and framework regions wherein the amino acid residues including the framework are derived completely or mostly from human germline. A humanized antibody can be produced using a variety of techniques known in the art, including CDR-grafting (see, e.g., European Patent No. EP 239,400; WO 91/09967; and US 5,225,539, US 5,530,101, and US 5,585,089), veneering or resurfacing (see, e.g., EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering, 7(6):805-814; and Roguska et al., 1994, PNAS, 91:969-973), chain shuffling (see, e.g., US 5,565,332), and techniques disclosed in, e.g., US 2005/0042664, US 2005/0048617, US 6,407,213, US 5,766,886, WO 93/17105, Tan et al., J. Immunol., 169:1119-25 (2002), Caldas et al., Protein Eng., 13(5):353-60 (2000), Morea et al., Methods, 20(3):267-79 (2000), Baca et al., J. Biol. Chem., 272(16): 10678-84 (1997), Roguska et al., Protein Eng., 9(10):895-904 (1996), Couto et al., Cancer Res., 55 (23 Supp):5973s-5977s (1995), Couto et al., Cancer Res., 55(8):1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen et al., J. Mol. Biol., 235(3):959-73 (1994). Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, for example improve, binding. These framework substitutions are identified by methods well-known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., US 5,585,089; and Riechmann et al., 1988, Nature, 332:323).

[0208] Although chimeric and humanized antibodies often incorporate all six CDRs from a non-

human antibody, they can also be made with less than all CDRs (e.g., at least 3, 4, or 5) CDRs from a non-human antibody (e.g., Pascalis et al., J. Immunol. 169:3076, 2002; Vajdos et al., Journal of Molecular Biology, 320: 415-428, 2002; Iwahashi et al., Mol. Immunol.).

[0209] Additional examples of antibody-based binding domain formats include scFv-based grababodies and soluble VH domain antibodies. These antibodies form binding regions using only heavy chain variable regions. See, for example, Jespers et al., Nat. Biotechnol. 22:1161, 2004; Cortez-Retamozo et al., Cancer Res. 64:2853, 2004; Baral et al., Nature Med. 12:580, 2006; and Barthelemy et al., J. Biol. Chem. 283:3639, 2008.

[0210] In particular embodiments, a VL region in a binding domain of the present disclosure is derived from or based on a known VL and contains one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (e.g., conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the known VL. An insertion, deletion or substitution may be anywhere in the VL region, including at the amino- or carboxy-terminus or both ends of this region, provided that each CDR includes zero changes or at most one, two, or three changes and provided a binding domain containing the modified VL region can still specifically bind its target with an affinity similar to the wild type binding domain.

[0211] In particular embodiments, a binding domain VH region of the present disclosure can be derived from or based on a known VH of an antibody and can contain one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (e.g., conservative amino acid substitutions or non-conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the known VH of the antibody. An insertion, deletion or substitution may be anywhere in the VH region, including at the amino- or carboxy-terminus or both ends of this region, provided that each CDR includes zero changes or at most one, two, or three changes and provided a binding domain containing the modified VH region can still specifically bind its target with an affinity similar to the wild type binding domain.

[0212] In particular embodiments, a binding domain includes or is a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to a known amino acid sequence of a light chain variable region (VL) or to a known heavy chain variable region (VH), or both, wherein each CDR includes zero changes or at most one, two, or three changes, from an antibody disclosed herein or fragment or derivative thereof that specifically binds a targeted antigen.

[0213] "Specifically binds" refers to an association of a binding domain (of, for example, a targeting ligand) to its cognate binding molecule (target antigen) with an affinity or K_a (i.e., an equilibrium association constant of a particular binding interaction with units of $1/M$) equal to or greater than $10^5 M^{-1}$, while not significantly associating with any other molecules or components in a relevant environment sample. Binding domains may be classified as "high affinity" or "low affinity". In particular embodiments, "high affinity" binding domains refer to those binding domains with a K_a of at least $10^7 M^{-1}$, at least $10^8 M^{-1}$, at least $10^9 M^{-1}$, at least $10^{10} M^{-1}$, at least $10^{11} M^{-1}$, at least $10^{12} M^{-1}$, or at least $10^{13} M^{-1}$. In particular embodiments, "low affinity" binding domains refer to those binding domains with a K_a of up to $10^7 M^{-1}$, up to $10^6 M^{-1}$, up to $10^5 M^{-1}$. Alternatively, affinity may be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (e.g., $10^{-5} M$ to $10^{-13} M$). In certain embodiments, a binding domain may have "enhanced affinity," which refers to a selected or engineered binding domains with stronger binding to a cognate binding molecule than a wild type (or parent) binding domain. For example, enhanced affinity may be due to a K_a (equilibrium association constant) for the cognate binding molecule that is higher than the reference binding domain or due to a K_d (dissociation constant) for the cognate binding molecule that is less than that of the reference binding domain, or due to an off-rate (K_{off}) for the cognate binding molecule that is less than that of the reference binding domain. A variety of assays are known for detecting binding domains that specifically bind a particular cognate binding molecule as well as determining binding affinities, such as Western blot, ELISA, and BIACORE® analysis (see also, e.g., Scatchard, et al., 1949, Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0214] In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including ASGFDFSAYYM (SEQ ID NO: 6), a CDRL2 sequence including TIYPSSG (SEQ ID NO: 7), and a CDRL3 sequence including ADRATYFCA (SEQ ID NO: 8). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including DTIDWY (SEQ ID NO: 9), a CDRH2 sequence including VQSDGSYTKRPGVPDR (SEQ ID NO: 10), and a CDRH3 sequence including YIGGYVFG (SEQ ID NO: 22).

[0215] In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including QASQSIDSNLA (SEQ ID NO: 12), a CDRL2 sequence including RASNLAS (SEQ ID NO: 13), and a CDRL3 sequence including LGGVGNVSYRTS (SEQ ID NO: 14). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized

binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including DYPIS (SEQ ID NO: 15), a CDRH2 sequence including FINSGGSTWYASWWKG (SEQ ID NO: 16), and a CDRH3 sequence including GYSTYYCDFNI (SEQ ID NO: 17). These reflect CDR sequences of the R11 antibody.

[0216] In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including TLSSAHKTDITD (SEQ ID NO: 18), a CDRL2 sequence including GSYTKRP (SEQ ID NO: 19), and a CDRL3 sequence including GADYIGGYV (SEQ ID NO: 20). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including AYYMS (SEQ ID NO: 21), a CDRH2 sequence including TIYPSSGKTTYATWNG (SEQ ID NO: 22), and a CDRH3 sequence including DSYADDGALFNI (SEQ ID NO: 23). These reflect CDR sequences of the R12 antibody.

[0217] In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including KASQNVDAAVA (SEQ ID NO: 24), a CDRL2 sequence including SASNRYT (SEQ ID NO: 25), and a CDRL3 sequence including QQYDIYPYT (SEQ ID NO: 26). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including DYEMH (SEQ ID NO: 27), a CDRH2 sequence including AIDPETGGTAYNQKFKG (SEQ ID NO: 28), and a CDRH3 sequence including YYDYDSFTY (SEQ ID NO: 29). These reflect CDR sequences of the 2A2 antibody.

[0218] In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including QASQSIGSYLA (SEQ ID NO: 30), a CDRL2 sequence including YASNLAS (SEQ ID NO: 31), and a CDRL3 sequence including LGSLSNSDNV (SEQ ID NO: 32). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including SHWMS (SEQ ID NO: 33), a CDRH2 sequence including IIAASGSTYYANWAKG (SEQ ID NO: 34), and a CDRH3 sequence including DYGDYRLVTFNI (SEQ ID NO: 35). These reflect CDR sequences of the Y31 antibody.

[0219] A number of additional antibodies specific for RORI are known to those of skill in the art and can be readily characterized for sequence, epitope binding, and affinity. See, for example, WO2008076868, WO/2008103849, WO201008069, WO2010124188, WO2011079902,

WO2011054007, WO2011159847, WO2012076066, WO2012076727, WO 2012045085, and WO2012097313.

[0220] In particular embodiments, the targeting ligand binds CD19 epitope. In particular embodiments, cancer antigen epitope binding domain includes a binding domain (e.g., scFv) that include VH and VL regions specific for CD19. In particular embodiments, the VH and VL regions are human. Exemplary VH and VL regions include the segments of anti-CD19 specific monoclonal antibody FMC63. In particular embodiments, the binding domain (e.g., scFv) is human or humanized and including a variable light chain including a CDRL1 sequence including RASQDISKYLN (SEQ ID NO: 36), a CDRL2 sequence including SRLHSGV (SEQ ID NO: 37), and a CDRL3 sequence including GNTLPYTFG (SEQ ID NO: 38). In particular embodiments, the binding domain (e.g., scFv) is human or humanized and includes a variable heavy chain including a CDRH1 sequence including DYGVS (SEQ ID NO: 39), a CDRH2 sequence including VTWGSETTYNSALKS (SEQ ID NO: 40), and a CDRH3 sequence including YAMDYWG (SEQ ID NO: 41). Other CD19-targeting antibodies such as SJ25C1 and HD37 are known. (SJ25C1: Bejcek et al. Cancer Res 2005, PMID 7538901; HD37: Pezutto et al. JI 1987, PMID 2437199).

[0221] In particular embodiments, the targeting ligand binds PSMA epitope. A number of antibodies specific for PSMA are known to those of skill in the art and can be readily characterized for sequence, epitope binding, and affinity. Binding domains can also include anti-Mesothelin ligands (associated with treating ovarian cancer, pancreatic cancer, and mesothelioma). As will be understood by one of ordinary skill in the art, the different cancer antigen epitope binding domains can bind any number of different epitopes on the cancer antigens disclosed herein (among others). As previously indicated, in particular embodiments, the different epitopes are on the same cancer antigen. In particular embodiments, the different epitopes are on different cancer antigens.

[0222] In particular embodiments, the targeting ligand binds CD20 epitope. Rituxan (Rituximab, Genentech) targets CD20 for CD20-positive non-Hodgkin's lymphoma and Arzerra (Ofatumumab, Novartis), targets a different epitope of CD20.

[0223] In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including RASSSVSYIH (SEQ ID NO: 42), a CDRL2 sequence including ATSNLAS (SEQ ID NO: 43), and a CDRL3 sequence including QQWTSNPPT (SEQ ID NO: 44). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including SYNMH (SEQ ID NO: 45), a CDRH2 sequence including AIYPGNGDTSYNQKFKG (SEQ ID NO:

46), and a CDRH3 sequence including STYYGGDWYFNV (SEQ ID NO: 47). These reflect CDR sequences of the 2B8 antibody.

[0224] In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including RASQDVNTAVAW (SEQ ID NO: 48), a CDRL2 sequence including YSASFLES (SEQ ID NO: 49), and a CDRL3 sequence including QQHYTTPT (SEQ ID NO: 50). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including SGFNTKDTYIHW (SEQ ID NO: 51), a CDRH2 sequence including RIYPTNGYTRYADSVKGR (SEQ ID NO: 52), and a CDRH3 sequence including WGGDGFYAMDV (SEQ ID NO: 53). These reflect CDR sequences of the 4D5 antibody.

[0225] In particular embodiments, the targeting ligand binds PD-L1 epitope. In particular embodiments, the PD-L1 binding domain includes a variable light chain including a CDRL1 sequence including RASQDVSTAVA (SEQ ID NO: 54), a CDRL2 sequence including SASFLYS (SEQ ID NO: 55), and a CDRL3 sequence including QQYLYHPAT (SEQ ID NO: 56). In particular embodiments, the PD-L1 binding domain includes a variable heavy chain including a CDRH1 sequence including SGFTFSDSWIH (SEQ ID NO: 57), a CDRH2 sequence including WISPYGGSTYYADSVKG (SEQ ID NO: 58), and a CDRH3 sequence including RHWPGGFDY (SEQ ID NO: 59).

[0226] In particular embodiments, the PD-L1 binding domain includes a variable light chain including a CDRL1 sequence including TGTSSDVGGYNYVS (SEQ ID NO: 60), a CDRL2 sequence including DVSNRPS (SEQ ID NO: 61), and a CDRL3 sequence including SSYTSSSTRV (SEQ ID NO: 62). In particular embodiments, the PD-L1 binding domain includes a variable heavy chain including a CDRH1 sequence including SGFTFSSYIMM (SEQ ID NO: 63), a CDRH2 sequence including SIYPSGGITFYADTVKG (SEQ ID NO: 64), and a CDRH3 sequence including IKLGTVTTVDY (SEQ ID NO: 65).

[0227] In particular embodiments, the PD-L1 binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including RASQSVSSYL (SEQ ID NO: 66), a CDRL2 sequence including DASNRAT (SEQ ID NO: 67), and a CDRL3 sequence including QQRSNWPRT (SEQ ID NO: 68). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including DYGFSS (SEQ ID NO: 69), a CDRH2 sequence including WITAYNGNTNYAQKLQG (SEQ ID NO: 70), and a CDRH3 sequence including DYFYGMDY (SEQ ID NO: 71). These reflect CDR sequences of the 3G10

antibody. Numerous additional sequences that bind PD-L1 are described in, for example, US 2016/0222117.

[0228] In particular embodiments, the targeting ligand binds CD123 epitope. In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including the CDRs of the anti-CD123 7G3 antibody. In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including RASESVDNYGNTFMH (SEQ ID NO: 72), a CDRL2 sequence including RASNLES (SEQ ID NO: 73), and a CDRL3 sequence including QQSNEPPT (SEQ ID NO: 74). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including NYGMN (SEQ ID NO: 75), a CDRH2 sequence including WINTYTGESTYSADFKG (SEQ ID NO: 76), and a CDRH3 sequence including SGGYDPMDY (SEQ ID NO: 77). These reflect CDR sequences of antibody 32716 described in US Patent Number 8,163,279.

[0229] In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable light chain including a CDRL1 sequence including RSNKSLHNSNGNTYLY (SEQ ID NO: 78), a CDRL2 sequence including RMSNLAS (SEQ ID NO: 79), and a CDRL3 sequence including MQHLEYPYT (SEQ ID NO: 80). In particular embodiments, the cancer antigen epitope binding domain is a human or humanized binding domain (e.g., scFv) including a variable heavy chain including a CDRH1 sequence including NYWMN (SEQ ID NO: 81), a CDRH2 sequence including RIDPSDSESHYNQKFKD (SEQ ID NO: 82), and a CDRH3 sequence including YDYDDTMDY (SEQ ID NO: 83). These reflect CDR sequences of antibody 32703 described in US Patent Number 8,163,279.

[0230]

[0231] (vii) Foam Expansion Agents. In particular embodiments, foams are self-expanding. Foams can be made to be self-expanding by including an oxygen releasing biomaterial. See, for example, Camci-Unal et al., Polym Int. 2013 June 1; 62(6): 843-848. Examples of oxygen releasing biomaterials include perfluorocarbons. When perfluorocarbons are exposed to high concentrations of oxygen, large amounts of oxygen dissolve into the perfluorocarbons. If the perfluorocarbon/oxygen solution is then exposed to a low oxygen environment, then oxygen diffuses out of the solution. This oxygen release then expands the foam.

[0232] Other oxygen releasing materials include sodium percarbonate, calcium peroxide, magnesium peroxide, hydrogen peroxide, and fluorinated compounds. Solid peroxides decompose upon exposure to water to release oxygen. For additional information, see, for

example, Jagers et al., *European Journal of Physiology* (2021) 473: 139-150.

[0233] As disclosed herein, any reaction that releases a gas can be engineered into a foam to make it expandable, so long as the byproducts are biocompatible and desirable (e.g., releasing CO₂ in tumors is undesirable). Foams can also be created under pressure, wherein when the pressure is released, they expand.

[0234] (viii) Methods of Forming Foams. In some instances, simply adding cells within a liquid to a foam precursor will generate a foam. Foams also can be formed by turbulently mixing a foam precursor, a nucleic acid, a liquid, and a gas before adding cells. Turbulently mixing refers to applying a sufficient amount of turbulent stress (also referred to as Reynolds Stress) to the foam precursor, nucleic acid, liquid, and gas such that a foam with characteristics described herein results. This approach can be preferable to the direct addition of cells within a liquid to a foam precursor because it facilitates cell dispersion throughout the foam, reduces shear stress for the cells, and improves the overall texture of the foam.

[0235] FIG. 11A depicts an exemplary clinical workflow for generating a nucleic acid delivering foam. A syringe can be filled with a foam precursor and a liquid (which can be referred to as a foam precursor solution) and nucleic acid vector (e.g., LNP or viral vector) and can be connected to a syringe containing a gas, such as air or other gasses described herein (FIG. 11A). The foaming liquid and gas can then be moved back and forth through a constriction that connects the two syringes to generate a substantially homogenous foam which contains substantially evenly distributed vector. While this method of making a foam described herein is provided, one can envision additional approaches to admix a foam precursor solution with nucleic acid vector to generate a foam with the characteristics described herein. In certain examples, these additional approaches can include automation and the use of software and processors to control various aspects of foam formation.

[0236] In particular embodiments, and particularly in relation to the ex vivo cell manufacturing, foam would most likely not be made in syringes but rather in a continuous flow microfluidics system. Such a system could include (1) one large syringe with a gas, (2) one syringe with a foam precursor solution, and (3) one syringe with cells and/or a nucleic acid vector. Using automated syringe pumps, these components would be mixed in a continuous flow static mixer to minimize stress on cells. Such systems are commercially available from, for example, Koflo Corporation, Cary, IL.

[0237] In making foams, 1 mL of foam precursor solution with 9 mL of gas can generate 4 mL of foam. A starting amount of foam precursor solution can be selected based on the desired amount of foam to be generated. For example, a starting amount of foam could be 0.25 mL, 0.50 mL, 0.75

mL, 1 mL, 1.5 mL, 2 mL, 3 mL, 4 mL, 8 mL, 10 mL, 20 mL, 50 mL or other larger or smaller amounts.

[0238] The amount of gas mixed with a selected amount foam precursor solution can be adjusted depending on the desired volume and thickness of a resulting foam. Certain examples can include 1 mL of foam precursor solution and 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mL of gas. Certain examples can include 1 mL of foam precursor solution and 9 mL of gas. The amount of gas can be adjusted down for smaller amounts of starting foam precursor solutions or up for larger amounts of foam precursor solutions.

[0239] Appropriate amounts of foam for in situ programming of immune cells (e.g., CAR T cells) or stem cells can be in the range of 0.5 – 4 mL 1-4 mL, 1-3 mL, or 2-3 mL, particularly when administering foam subcutaneously or into the bone marrow. Repeated administrations of these amounts can be appropriate.

[0240] For edible or drinkable foams (e.g., for treating esophageal cancers), or rectally applied foams (e.g., for the treatment of rectal cancer) the foam volume should be bigger as targeted body cavities should be substantially filled. Here, foams with volumes of 5 – 50 mL, 5-40mL, 10-30 mL, or 15-25 mL are envisioned.

[0241] For intraperitoneal administrations, for example to treat disseminated disease, a foam volume of 100-500 mL, 150-400 mL, 200-350 mL, or 200-300 mL could be used to substantially fill the peritoneal cavity (similar to intraperitoneal chemotherapy). In this scenario, foam could be applied through a catheter.

[0242] Exemplary appropriate amounts of cells and vectors for a starting 1 mL foam precursor solution include $1-10 \times 10^6$ cells, 5-20 μ g nucleic acid (e.g., mRNA) within LNP, and $1.25-5 \times 10^6$ transfection units lentiviral vector.

[0243] For in situ cell engineering, an appropriate amount of vector depends on the number of cells mixed into a foam. For lentivirus, the MOI can be, for example, 1-2 and for LNPs an appropriate range can be 5-20 μ g nucleic acid for 1-10 million cells. When using foam to transfect tissue (e.g., a drinkable foam for esophageal cancer or rectally applied foam for rectal cancer), the amount of vector/mL can be similar (e.g., 10-50 μ g nucleic acid/mL) but also depends on the particular gene being provided and any potential or expected toxicities associated with the same.

[0244] In particular embodiments, liquids used to make the foams disclosed herein are biocompatible liquids. Examples of biocompatible liquids include cell culture media, phosphate buffered saline (PBS), human serum or serum substitutes, or other solutions routinely used in the clinic to administer drugs, such as Lactated Ringer's solution, saline (0.9% sodium chloride), sterile water, or 5% dextrose solution. Cell culture media provide an artificial environment for cells

that is conducive to their survival and/or proliferation in an artificial environment. Cell culture media generally include a complement of amino acids, vitamins, inorganic salts, glucose, growth factors, and/or hormones. Exemplary cell culture media include Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640, Minimum Essential Medium (MEM), Iscove's Modified Dulbecco's Medium (IMDM), McCoy's 5A, and Eagle's Basal Medium (EBSS), optionally supplemented with fetal bovine serum (FBS).

[0245] Exemplary cell culture media for T cells include (i) RPMI supplemented with non-essential amino acids, sodium pyruvate, and penicillin/streptomycin; (ii) RPMI with HEPES, 5-15% human serum, 1-3% L-Glutamine, 0.5-1.5% penicillin/streptomycin, and 0.25×10^{-4} - 0.75×10^{-4} M β -MercaptoEthanol; (iii) RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 10mM HEPES, 100 U/ml penicillin and 100 m/mL streptomycin; (iv) DMEM medium supplemented with 10% FBS, 2mM L-glutamine, 10mM HEPES, 100 U/ml penicillin and 100 m/mL streptomycin; and (v) X-Vivo 15 medium (Lonza, Walkersville, MD) supplemented with 5% human AB serum (Gemcell, West Sacramento, CA), 1% HEPES (Gibco, Grand Island, NY), 1% Pen-Strep (Gibco), 1% GlutaMax (Gibco), and 2% N-acetyl cysteine (Sigma-Aldrich, St. Louis, MO). T cell culture media are also commercially available from Hyclone (Logan, UT) and Stemcell Technologies (e.g., ImmunoCult™ (ImmC). CTL-Test™ Medium (ImmunoSpot®, Cellular Technology, Ltd; Cleveland, OH) may also be used. T cell media can be functionalized with growth factors and cytokines, such as IL-2, IL7, and IL15.

[0246] Exemplary cell culture media for stem cells may include (i) α -MEM supplemented with 10% fetal bovine serum; α -MEM supplemented with 1-10% (v/v) of Ultrosor G, 1- 20% (v/v) of KOSR, 1- 5 mM of stabilized dipeptide of L-alanyl-L-glutamine, one or more growth factors (e.g., at least one of VEGF, bFGF, EGF, TGF β , PDGF) at a concentration of 1-100 ng/ml, and at least one glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline, and L-serine at a concentration of 0.1-0.5mM; (ii) RPMI-1640 DMEM medium supplemented with FGF7, retinoic acid, noggin, and cyclopamine-KAAD; (iii) DMEM F12 medium supplemented with 5 μ g/mL insulin, 2 nM (3,3', 5-triiodo-L-thyronine), 400 ng/mL hydrocortisone, 24.3 μ g/mL adenine, 10 ng/mL EGF, 10% FBS (no heat inactivation), 1 μ M Jagid-1; 100 ng/mL Noggin, 125 ng/mL R-spondin 1, 2.5 μ M Y-27632, and 1.35 mM L-glutamine; (iv) DMEM F12 medium optionally supplemented with 0.1 nM cholera enterotoxin, 2 μ M SB431542, 10 mM nicotinamide, or 2 μ M SB431542 and 10 mM nicotinamide. Stem cell culture media are also commercially available from Thermo Fisher Scientific (e.g., StemPro™ MSC SFM) and Stemcell Technologies (e.g., StemSpan™-AOF).

[0247] In particular embodiments, stem cell culture media can be supplemented with fms-like

tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO). In particular embodiments, a stem cell culture media can include 50-300 ng/ml stem cell factor, 50-300 ng/ml Flt-3 receptor ligand, 50-100 ng/ml thrombopoietin, 50-100 ng/ml interleukin-6 and 10 ng/ml interleukin-3. In more specific embodiments, 300 ng/ml stem cell factor, 300 ng/ml of Flt-3 receptor ligand, 100 ng/ml thrombopoietin, 100 ng/ml interleukin-6 and 10 ng/ml interleukin-3, or 50 ng/ml stem cell factor, 50 ng/ml of Flt-3 receptor ligand, 50 ng/ml thrombopoietin, 50 ng/ml interleukin-6 and 10 ng/ml interleukin-3 can be used.

[0248] (ix) Methods of Use Using Foam Application to an Anatomical Site. Methods disclosed herein include treating subjects (humans, veterinary animals (dogs, cats, reptiles, birds, etc.) livestock (horses, cattle, goats, pigs, chickens, etc.) and research animals (monkeys, rats, mice, fish, etc.) with compositions disclosed herein. Treating subjects includes delivering therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments and/or therapeutic treatments.

[0249] An "effective amount" is the amount of a composition necessary to result in a desired physiological change in the subject. Effective amounts are often administered for research purposes. Effective amounts disclosed herein can cause a statistically significant effect in an animal model or in vitro assay relevant to the assessment of a condition's development or progression.

[0250] A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of a condition or displays only early signs or symptoms of a condition such that treatment is administered for the purpose of diminishing or decreasing the risk of developing the condition further. Thus, a prophylactic treatment functions as a preventative treatment against a condition. In particular embodiments, prophylactic treatments reduce, delay, or prevent metastasis from a primary cancer tumor site from occurring.

[0251] A "therapeutic treatment" includes a treatment administered to a subject who displays symptoms or signs of a condition and is administered to the subject for the purpose of diminishing or eliminating those signs or symptoms of the condition. The therapeutic treatment can reduce, control, or eliminate the presence or activity of the condition and/or reduce control or eliminate side effects of the condition.

[0252] Function as an effective amount, prophylactic treatment or therapeutic treatment are not mutually exclusive, and in particular embodiments, administered dosages may accomplish more than one treatment type.

[0253] As will be understood by one of ordinary skill in the art, foams can be implanted in close proximity to a treatment site. The foams can be administered in a number of different sizes and

shapes and can be shape-conformable to fit the particular needs of individual subjects. In particular embodiments, the foams can be injected using ultrasound guidance in close proximity to (or in physical contact with) a treatment site. Depending on the stage, size or severity of a treatment site, foams may be provided with different therapeutic strengths. Therapeutic strength can be manipulated by altering the size of the foams, volume of the foams, the number of nucleic acid vectors within the foams, the presence of additional therapeutic agents within the foam, etc. Each of these parameters can be assessed and determined by a treating physician.

[0254] For the purposes of the present disclosure, the term “proximity” refers to a distance within 10 cm, within 9 cm, within 8 cm, within 7 cm, within 6 cm, within 5 cm, within 4 cm, within 3 cm, within 2 cm, within 1 cm, within 0.9 cm, within 0.8 cm, within 0.7 cm, within 0.6 cm, within 0.5 cm, within 0.4 cm, within 0.3 cm, within 0.2 cm, or within 0.1 cm of a treatment site.

[0255] It is also understood by one of ordinary skill in the art that the foams can be administered only once or can be administered a plurality of times to provide ongoing therapy over months or years. Such treatment regimens can be determined by a treating physician.

[0256] In particular embodiments, therapeutically effective amounts provide anti-cancer effects. Anti-cancer effects include a decrease in the number of cancer cells, decrease in the number of metastases, a decrease in tumor volume, an increase in life expectancy, induced chemo- or radiosensitivity in cancer cells, inhibited angiogenesis near cancer cells, inhibited cancer cell proliferation, inhibited tumor growth, prevented or reduced metastases, prolonged subject life, reduced cancer-associated pain, and/or reduced relapse or re-occurrence of cancer following treatment.

[0257] A “tumor” is a swelling or lesion formed by an abnormal growth of cells (called neoplastic cells or tumor cells). A “tumor cell” is an abnormal cell that divides by a rapid, uncontrolled cellular proliferation and continues to divide after the stimuli that initiated the new division cease. Tumors show partial or complete lack of structural organization and functional coordination with the normal tissue, and usually form a distinct mass of tissue, which may be either benign, pre-malignant or malignant.

[0258] As used herein, an anti-tumor effect refers to a biological effect, which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or a decrease of various physiological symptoms associated with the cancerous condition. An anti-tumor effect can also be manifested by a decrease in recurrence or an increase in the time before recurrence. Accordingly, the foams disclosed herein can be used to treat a variety of cancers, can prevent or significantly delay metastasis, and/or can prevent or significantly delay relapse. Foams can continue to provide anti-

tumor immunity after eradication/reduction of an initial tumor and can prevent and/or reduce metastases or development of secondary tumors.

[0259] Cancer (medical term: malignant neoplasm) refers to a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis. "Metastasis" refers to the spread of cancer cells from their original site of proliferation to another part of the body. The formation of metastasis is a very complex process and depends on detachment of malignant cells from the primary tumor, invasion of the extracellular matrix, penetration of the endothelial basement membranes to enter the body cavity and vessels, and then, after being transported by the blood, infiltration of target organs. Finally, the growth of a new tumor, i.e., a secondary tumor or metastatic tumor, at the target site depends on angiogenesis. Tumor metastasis often occurs even after the removal of the primary tumor because tumor cells or components may remain and develop metastatic potential.

[0260] Cancers that can be treated with the anti-tumor effects of the foams and methods disclosed herein include, for example, adrenal cancer, brain cancer, breast cancer, cervical cancer, colon cancer, colorectal cancer, ear, nose and throat (ENT) cancer, endometrial cancer, esophageal cancer, gastrointestinal cancer, gliomas, head and neck cancer, intestinal cancer, kidney cancer, liver cancer, lung cancer, lymph node cancer, melanomas, neuroblastomas, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, seminomas, skin cancer, stomach cancer, teratomas, thyroid cancer, uterine cancer, and metastases thereof.

[0261] As will be understood by one of ordinary skill in the art, foams can be implanted in close proximity to a tumor site. In particular embodiments, the tumor site includes a solid tumor, un-resectable tumor cells and/or a tumor resection bed following resection. In particular embodiments, the foam is applied within 10 cm, within 9 cm, within 8 cm, within 7 cm, within 6 cm, within 5 cm, within 4 cm, within 3 cm, within 2 cm, within 1 cm, within 0.9 cm, within 0.8 cm, within 0.7 cm, within 0.6 cm, within 0.5 cm, within 0.4 cm, within 0.3 cm, within 0.2 cm, or within 0.1 cm of a solid tumor, an un-resectable tumor, un-resectable tumor cells, and/or a tumor resection bed.

[0262] It is also understood by one of ordinary skill in the art that the foams can be administered only once, at the time of resection or at a first treatment time in a subject with a solid tumor, an un-resectable tumor, un-resectable tumor cells, and/or a tumor resection bed. Additionally, the foams can be administered a plurality of times to provide ongoing therapy over months or years.

[0263] As used herein, the term "surgical treatment failure" refers to relapse of cancer in a subject who had previously undergone tumor resection. Surgical treatment failure may include metastatic

relapse.

[0264] Without limiting the scope of the disclosure, the following cancer types are noted:

[0265] Brain tumor (Glioblastoma): An estimated 10,000 new cases/year in the U.S. are seen. Currently no curative therapy is available. Glioblastoma shows very infiltrative growth and cannot be resected completely. 90% of tumors relapse within a 2 cm margin from the originally resected tumor. Biomaterial wafers loaded with chemotherapy are United States Food and Drug Administration (FDA)-approved (GLIADEL®, MGI Pharma, Inc., Woodcliff Lake, NJ) for glioblastoma. However, due to insufficient tissue penetration, biomaterial implant delivered chemotherapy is mostly ineffective. In contrast, foams disclosed herein can better permeate tissue.

[0266] Pancreatic adenocarcinoma: An estimated 43,920 new cases of pancreatic cancer were expected to occur in the U.S. in 2012. Only 20% will have resectable disease at the time of diagnosis (80% of patients do not undergo surgery as their tumor is too advanced at the time of diagnosis). Even surgery is considered a palliative venture with a 5-year survival rate of only 20%. Local recurrence is usually attributed to the difficulty of achieving microscopically negative surgical margins. Beyond the current foam's ability to eradicate residual disease following surgical tumor resection, the foams can also provide pancreatic tumor patients with inoperable disease (80% of patients) with a highly effective treatment option. In particular embodiments, foams are implanted directly onto un-resectable established pancreatic adenocarcinomas.

[0267] Ovarian cancer: An estimated 22,000 new cases in 2012 in the U.S. were seen. Despite multimodality therapy with surgery and chemotherapy, most ovarian cancer patients have a poor prognosis (15,500 estimated deaths/year in U.S.). Ovarian cancer primarily disseminates within the peritoneal cavity. Adoptive T-cell therapy in ovarian cancer patients is currently being investigated at several centers. However, to date clinical results have been disappointing due to a poor survival of infused T-cells and a failure to combat immunosuppressive factors released by tumor cells to render T-cells dysfunctional. Multiple foams could be administered laparoscopically into the peritoneal cavity of ovarian cancer patients.

[0268] Referring to FIG. 13, foams can be used to deliver nucleic acids to treat ovarian cancers. Exemplary therapeutic nucleic acids for delivery include, for example, (1) CRISPR-CAS9 systems that silence critical drivers of the disease (e.g., CASC4 (Bapat et al., Cancer Gene Ther. 2024;31(2):300-10)), (2) transgenes that improve the efficiency of chemotherapy (e.g., SMAC (Hernandez et al., Cell Death Discov. 2021;7(1):134) or TRAIL (Nguyen et al., Chem Biol Drug Des. 2024;103(1):e14357)), (3) tumor suppressor genes (e.g., PTEN (Lee et al., Nat Rev Mol Cell Biol. 2018;19(9):547-62) or P53 (Wallis et al., Biomolecules. 2023;13(1)), (4) genes that suppress

migration and invasion of ovarian cancer cells (e.g., PLAA (Shen et al., *Oncogene*. 2022;41(35):4145-58)), (5) immunomodulatory genes (Hao et al., *J Control Release*. 2022;342:14-25; Yang et al., *Mol Ther Nucleic Acids*. 2022;30:184-97), (6) suicide genes (Nguyen et al., *Cancer Gene Ther*. 2023;30(6):812-21), (7) antiangiogenic genes (Li et al., *Oncol Lett*. 2018;16(1):687-702), or (8) oncolytic virotherapy (Mistarz et al. *Mol Ther Oncolytics*. 2021;23:38-50).

[0269] In certain embodiments, foams deliver nucleic acids that encode a bacterial toxin to treat cancer, for example *Pseudomonas* exotoxin A.

[0270] Referring to FIGs. 23 and 27, foams can be used to deliver nucleic acids to treat gastrointestinal cancers. In particular embodiments, the gastrointestinal cancer is esophageal cancer and the foam is applied orally, in certain instances by consuming an edible or drinkable foam. When foams and nucleic acids delivered in this manner encode a bacterial toxin, such as *Pseudomonas* exotoxin A, the toxin would only be active in successfully transfected tissue (e.g., in an around tumor). Once the foam reaches the stomach (pH-1), and in embodiments utilizing LNPs the LNP and nucleic acid (e.g., mRNA) would be rapidly destroyed by stomach acid, reducing or eliminating off target transfection and/or toxin-mediated side effects.

[0271] In particular embodiments, the gastrointestinal cancer is rectal cancer and the foam is applied rectally.

[0272] In particular embodiments, a method of treating bladder (e.g., urothelial) cancer within a subject includes intravesical administration. Intravesical administration includes the administration of a foam directly into the bladder (e.g., via catheterization). Prior to intravesical administration, an assessment of the subject's ability to retain solution for a select dwell time can be performed. In particular embodiments, the subject's bladder should be emptied before instillation of the foam. In particular embodiments, a patient experiencing bladder spasms can be administered an anticholinergic.

[0273] Intravesical instillation of a foam disclosed herein will establish a depot within the bladder, providing sustained release of nucleic acid vectors within the bladder over time. Intravesical instillation procedures are known in the art. For intravesical instillation, the foam should be at suitable volume to supply a sufficient dose volume for intravesical instillation, i.e., where the volume of the dose is sufficient to expose the bladder tissues to the foam. Generally, the volume of the foam is less than 100 mL. In some embodiments, the volume of the foam is 10 mL to 100 mL, or 20 mL to 80 mL, or 25 mL to 75 mL, or 10 mL to 50 mL, or 15 mL to 45 mL, or 20 mL to 40 mL, or 25 mL to 35 mL, or 20 mL to 30 mL, or 25 mL.

[0274] In particular embodiments, dwell time of the foam includes 15 minutes, 30 minutes, 1.0

hour, 1.1 hours, 1.2 hours, 1.3 hours, 1.4 hours, 1.5 hours, 1.6 hours, 1.7 hours, 1.8 hours, 1.9 hours, 2.0 hours, 2.1 hours, 2.2 hours, 2.3 hours, 2.4 hours, 2.5 hours, 2.6 hours, 2.7 hours, 2.8 hours, 2.9 hours, 3.0 hours, 3.1 hours, 3.2 hours, 3.3 hours, 3.4 hours, 3.5 hours, 3.6 hours, 3.7 hours, 3.8 hours, 3.9 hours, or 4.0 hours. In particular embodiments, dwell time includes 30 minutes to 2 hours.

[0275] One or more subsequent instillations of the foam can be administered following the initial instillation. The subsequent instillations can be separated by periodic intervals. In particular embodiments, periodic intervals include a day, two days, three days, four days, five days, six days, seven days, two weeks, three weeks, a month, two months, or three months.

[0276] In particular embodiments, 5 instillations can be administered on a weekly basis two weeks following the initial instillation, and additionally, 3 more instillations can be administered weekly 3 months after the 5th instillation, followed by 3 more instillations administered weekly 3 months after the 8th instillation, followed by 3 more instillations administered weekly 3 months after the 11th instillation, for a total of 14 instillations administered after the initial instillation. Devices such as catheters and needles can be used to administer the formulation to the bladder.

[0277] In particular embodiments, intravesical administration includes injecting foam into one or more tumor surgical resection sites, wherein the injecting is done following surgical resection of one or more tumors of the subject, thereby treating or inhibiting the recurrence of the cancer.

[0278] In certain examples, foams are administered as a combination therapy with radiotherapy.

[0279] In particular embodiments, the foams can be used to deliver nucleic acids to anatomical sites with an infection, for example, a bacterial, viral, or fungal infection. In these instances, the foams can provide an anti-infection effect.

[0280] Anti-infection effects include a reducing or preventing infection of a cell by a virus, bacteria, or fungus, decreasing the number of infected cells, decreasing the volume of infected tissue, increasing lifespan, increasing life expectancy, reducing or eliminating infection-associated symptoms.

[0281] In particular embodiments, therapeutically effective amounts provide anti-allergic effects. Anti-allergic effects can decrease the occurrence or severity of allergic rhinitis, allergic asthma, atopic dermatitis, allergic gastroenteropathy, anaphylaxis, urticaria, food allergies, allergic bronchopulmonary aspergillosis, parasitic diseases, interstitial cystitis, hyper-IgE syndrome, ataxia-telangiectasia, Wiskott-Aldrich syndrome, athymic lymphoplasia, and allergic purpura. symptoms of an IgE-mediated disorder or disease is considered to be a treatment thereof.

[0282] In particular embodiments, a treated condition is a wound. A “wound” refers to open wounds, such as incisions, lacerations, abrasions, avulsions, puncture wounds, penetration

wounds, gunshot wounds, burn wounds, thermal burns, chemical burns, electrical burns, and radiation burns. Wounds also include pressure wounds. As used herein, "wound" also includes internal injuries, such as retinal or macular tears. "Chronic wounds" include wounds that take longer to heal than would be expected by a physician. In diabetics, "chronic wounds" include wounds that take longer to heal as compared to a wound of a healthy control subject. For example, a corneal incision wound is expected to heal within 42 hours following its occurrence in a healthy control subject.

[0283] The present disclosure describes foams delivering a nucleic acid to promote wound healing. In particular embodiments, the foams can be used to reduce the occurrence of chronic wounds. In particular embodiments, the foams can be used to promote wound healing in healthy or in diabetic subjects. In particular embodiments, the foams can be used to reduce the occurrence of chronic wounds in diabetic subjects.

[0284] Wound healing generally can be divided into three steps: re-epithelialization, granulation, and neovascularization. Delayed re-epithelialization and inadequate formation of granulation tissue can lead to the development of chronic wounds. Endothelial progenitor cells (EPCs), which derive from bone marrow, normally travel to sites of injury and are essential for the formation of blood vessels and wound healing.

[0285] Objective measures for the promotion of wound healing include the time required for the closure of an open wound or establishment of a biological barrier.

[0286] Particular embodiments utilize topical administration of foams to the surface of the skin, injection of foams into dermal layers of the skin, injection of foams into tumor or diseased tissues, subcutaneous or intramuscular injection of foams (e.g., for vaccine applications), direct application of foams into body cavities (gastrointestinal, genitourinary, pleural, peritoneal, tumor resection, or bone marrow), intra-articular injections of foams (e.g., for arthritis treatments), and intra-lymph node injections of foams. Importantly, foams described herein can be edible, for example for delivery of nucleic acids to the mouth, throat, and upper gastrointestinal tract (e.g., esophagus).

[0287] (x) Methods of Use Using Ex Vivo Cell Manufacturing. The present disclosure describes use of the foams disclosed herein in ex vivo cell manufacturing, for example manufacturing cell populations to express chimeric antigen receptors or other types of therapeutic molecules. Genetically modified cells can include, for example, T-cells, B cells, natural killer (NK) cells, NK-T cells, monocytes/macrophages, lymphocytes, hematopoietic stem cells (HSCs), hematopoietic progenitor cells (HPC), a mixture of HSC and HPC (i.e., HSPC), induced pluripotent stem cells (iPSC), and cells differentiated from iPSC. In particular embodiments, genetically modified cells

include T-cells.

[0288] Cells can be incorporated into the foams of the current disclosure through a variety of methods. For example, cells may be added to either the foam precursor starting material or the nucleic acid vector material. Cells may be added before mixing of the foam precursor and nucleic acid vector begins or may be added once mixing has begun. In particular embodiments, cells can be added when mixing the components to form the foam is almost complete. For example, if a syringe system were to be plunged 30 times, cells could be added before the 25th plunge, before the 26th plunge, before the 27th plunge, before the 28th plunge, before the 29th plunge, or before the 30th plunge. Alternatively, cells can be directly vortexed into a foam or gently inserted using, for example, a dual-syringe spiral mixing tip.

[0289] Several different subsets of T-cells have been discovered, each with a distinct function. For example, a majority of T-cells have a T-cell receptor (TCR) existing as a complex of several proteins. The actual T-cell receptor is composed of two separate peptide chains, which are produced from the independent T-cell receptor alpha and beta (TCR α and TCR β) genes and are called α - and β -TCR chains.

[0290] $\gamma\delta$ T-cells represent a small subset of T-cells that possess a distinct T-cell receptor (TCR) on their surface. In $\gamma\delta$ T-cells, the TCR is made up of one γ -chain and one δ -chain. This group of T-cells is much less common (2% of total T-cells) than the $\alpha\beta$ T-cells.

[0291] CD3 is expressed on all mature T cells. Activated T-cells express 4-1BB (CD137), CD69, and CD25. CD5 and transferrin receptor are also expressed on T-cells.

[0292] T-cells can further be classified into helper cells (CD4 $^{+}$ T-cells) and cytotoxic T-cells (CTLs, CD8 $^{+}$ T-cells), which include cytolytic T-cells. T helper cells assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and activation of cytotoxic T-cells and macrophages, among other functions. These cells are also known as CD4 $^{+}$ T-cells because they express the CD4 protein on their surface. Helper T-cells become activated when they are presented with peptide antigens by MHC class II molecules that are expressed on the surface of antigen presenting cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response.

[0293] Cytotoxic T-cells destroy virally infected cells and tumor cells and are also implicated in transplant rejection. These cells are also known as CD8 $^{+}$ T-cells because they express the CD8 glycoprotein on their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body.

[0294] "Central memory" T-cells (or "TCM") as used herein refers to an antigen experienced CTL that expresses CD62L or CCR7 and CD45RO on the surface thereof and does not express or

has decreased expression of CD45RA as compared to naive cells. In particular embodiments, central memory cells are positive for expression of CD62L, CCR7, CD25, CD127, CD45RO, and CD95, and have decreased expression of CD45RA as compared to naive cells.

[0295] "Effector memory" T-cell (or "TEM") as used herein refers to an antigen experienced T-cell that does not express or has decreased expression of CD62L on the surface thereof as compared to central memory cells and does not express or has decreased expression of CD45RA as compared to a naive cell. In particular embodiments, effector memory cells are negative for expression of CD62L and CCR7, compared to naive cells or central memory cells, and have variable expression of CD28 and CD45RA. Effector T-cells are positive for granzyme B and perforin as compared to memory or naive T-cells.

[0296] "Naive" T-cells as used herein refers to a non-antigen experienced T cell that expresses CD62L and CD45RA and does not express CD45RO as compared to central or effector memory cells. In particular embodiments, naive CD8⁺ T lymphocytes are characterized by the expression of phenotypic markers of naive T-cells including CD62L, CCR7, CD28, CD127, and CD45RA.

[0297] Natural killer cells (also known as NK cells, K cells, and killer cells) are activated in response to interferons or macrophage-derived cytokines. They serve to contain viral infections while the adaptive immune response is generating antigen-specific cytotoxic T cells that can clear the infection. NK cells express CD8, CD16 and CD56 but do not express CD3.

[0298] NK cells include NK-T cells. NK-T cells are a specialized population of T cells that express a semi invariant T cell receptor (TCR $\alpha\beta$) and surface antigens typically associated with natural killer cells. NK-T cells contribute to antibacterial and antiviral immune responses and promote tumor-related immunosurveillance or immunosuppression. Like natural killer cells, NK-T cells can also induce perforin-, Fas-, and TNF-related cytotoxicity. Activated NK-T cells are capable of producing IFN- γ and IL-4. In particular embodiments, NK-T cells are CD3⁺/CD56⁺.

[0299] Macrophages (and their precursors, monocytes) reside in every tissue of the body (in certain instances as microglia, Kupffer cells and osteoclasts) where they engulf apoptotic cells, pathogens and other non-self-components. Monocytes/macrophages express CD11b, F4/80; CD68; CD11c; IL-4R α ; and/or CD163.

[0300] Immature dendritic cells (i.e., pre-activation) engulf antigens and other non-self-components in the periphery and subsequently, in activated form, migrate to T-cell areas of lymphoid tissues where they provide antigen presentation to T cells. Dendritic cells express CD1a, CD1b, CD1c, CD1d, CD21, CD35, CD39, CD40, CD86, CD101, CD148, CD209, and DEC-205.

[0301] Hematopoietic Stem/Progenitor Cells or HSPC refer to a combination of hematopoietic stem cells and hematopoietic progenitor cells.

[0302] Hematopoietic stem cells refer to undifferentiated hematopoietic cells that are capable of self-renewal either in vivo, essentially unlimited propagation in vitro, and capable of differentiation to all other hematopoietic cell types.

[0303] A hematopoietic progenitor cell is a cell derived from hematopoietic stem cells or fetal tissue that is capable of further differentiation into mature cell types. In certain embodiments, hematopoietic progenitor cells are CD24^{lo} Lin⁻ CD117⁺ hematopoietic progenitor cells. HPC can differentiate into (i) myeloid progenitor cells which ultimately give rise to monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, or dendritic cells; or (ii) lymphoid progenitor cells which ultimately give rise to T-cells, B-cells, and NK-cells.

[0304] HSPC can be positive for a specific marker expressed in increased levels on HSPC relative to other types of hematopoietic cells. For example, such markers include CD34, CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD133, CD166, HLA DR, or a combination thereof. Also, the HSPC can be negative for an expressed marker relative to other types of hematopoietic cells. For example, such markers include Lin, CD38, or a combination thereof. Preferably, the HSPC are CD34⁺ cells.

[0305] A statement that a cell or population of cells is "positive" for or expressing a particular marker refers to the detectable presence on or in the cell of the particular marker. When referring to a surface marker, the term can refer to the presence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is detectable by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions and/or at a level substantially similar to that for cell known to be positive for the marker, and/or at a level substantially higher than that for a cell known to be negative for the marker.

[0306] A statement that a cell or population of cells is "negative" for a particular marker or lacks expression of a marker refers to the absence of substantial detectable presence on or in the cell of a particular marker. When referring to a surface marker, the term can refer to the absence of surface expression as detected by flow cytometry, for example, by staining with an antibody that specifically binds to the marker and detecting said antibody, wherein the staining is not detected by flow cytometry at a level substantially above the staining detected carrying out the same procedure with an isotype-matched control under otherwise identical conditions, and/or at a level substantially lower than that for cell known to be positive for the marker, and/or at a level substantially similar as compared to that for a cell known to be negative for the marker.

[0307] Stems cells can be an important precursor to generate or regenerate organs, repair tissues, prepare or deliver certain biological factors, or treat diseases or disorders. Presently, there are two sources of pluripotent stem cells: embryonic stem cells and induced pluripotent stem cells. Induced pluripotent stem cells (iPSC) are a type of stem cell that is derived from somatic cells and have been dedifferentiated back to pluripotency (also referred to as “reprogrammed”). Human pluripotent stem cells express at least some, and in some embodiments, all of the markers from the following list: SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog. Cell morphologies associated with pluripotent stem cells are also pluripotent stem cell characteristics. In particular embodiments, pluripotency can be verified by reviewing cell morphology, TRA1-60 live staining.

[0308] In particular embodiments, somatic cells to be reprogrammed are derived from bone marrow. In particular embodiments, somatic cells to be reprogrammed are hematopoietic stem cells or mesenchymal stem cells. In particular embodiments, the type of cell to be reprogrammed includes fibroblasts, hepatocytes, myoblasts, neurons, osteoblasts, osteoclasts, kidney cells, immune cells, or stem cells.

[0309] The somatic cells to be reprogrammed can be isolated from a sample obtained from a mammalian subject. The subject can be any mammal (e.g., bovine, ovine, porcine, canine, feline, equine, primate), including a human. The sample of cells may be obtained from any of a number of different sources including, for example, bone marrow, skin, foreskin, fetal tissue (e.g., fetal liver tissue), peripheral blood, umbilical cord blood, pancreas and the like.

[0310] In particular embodiments, iPSC are differentiated, for example, for a research or therapeutic purpose (e.g., before administration to a subject). Where differentiation of iPSC is desired, stem cells (e.g., iPSC) can be exposed to one or more activation factors (e.g., growth factors, differentiation factors, and/or survival factors) that promote differentiation into a more committed cell type.

[0311] Many activation factors and cell culture conditions that promote differentiation are known in the art (see, e.g., U.S. Patent No. 7,399,633 at Section 5.2 and Section 5.5). For example, stem cell factor (SCF) can be used in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) or interleukin (IL)-7 to promote differentiation into myeloid stem/progenitor cells or lymphoid stem/progenitor cells, respectively. In particular embodiments, iPSC can be differentiated into a lymphoid stem/progenitor cell by exposing iPSC to 100 ng/ml of each of SCF and GM-CSF or IL-7. In particular embodiments, a retinoic acid receptor (RAR) agonist, or preferably all trans retinoic acid (ATRA) is used to promote the differentiation of iPSC. Differentiation into natural killer cells, e.g., can be achieved by exposing cultured iPSC to RPMI

media supplemented with human serum, IL-2 at 50 U/mL and IL-15 at 500ng/mL. In additional embodiments, RPMI media can also be supplemented L-glutamine.

[0312] Cardiomyocytes have been generated in vitro from a wide range of stem cells, including iPSC (see, e.g., Gai, et al., 2009, *Cell. Biol. Int.* 33:1184-93; Kuzmenkin, et al., 2009, *FASEB J.* 23:4168-80; Pfannkuche, et al., 2009, *Cell Physiol. Biochem.* 24:73-86), ESCs (see, e.g., Beqqali, et al., 2009, *Cell. Mol. Life Sci.* 66:800-13; Steel, et al., 2009, *Curr. Opin. Drug Discov. Dev* 12:133-40), HSPC (see, e.g., Choi, et al., 2008, *Biotechnol. Lett* 30:835-43; Antonitsis, et al., 2008, *Thorac. Cardiovasc. Surg* 56:77-82; Ge, et al., 2009, *Biochem. Biophys. Res. Commun.* 381:317-21; Gwak, et al., 2009, *Cell. Biochem. Funct.* 27:148-54), and cardiomyocyte progenitor cells (see, e.g., Smits, et al., 2009, *Nat. Protoc.* 4:232-43). Mummery, et al., 2012 July 20, *Circ. Res.* 111(3): 344-358 provides a summary of methods to differentiate iPSC and hESCs into cardiomyocytes. Methods to differentiate stem cells (e.g., iPSC) into cardiac cells are also described in, e.g., U.S. Publication No. 2015/0017718.

[0313] In particular embodiments, cardiomyocyte progenitors can be generated from embryoid bodies (EBs) treated with Activin A, BMP4 or with 2+Wnt3 and bFGF. These progenitors express Nkx2.5, Tbx5/20, Gata-4, Mef2c and Hand1/2. Their further differentiation to functional cardiomyocytes can be promoted with VEGF and Dkk1 (Vidarsson, et al., 2010, *Stem Cell Rev.* 6:108-20).

[0314] A protocol for generating insulin producing beta-cells involves stepwise lineage restriction generating in sequence: definitive endodermal cells (Activin+Wnt3), primitive foregut endoderm (FGF10+KAAD-cyclopamine), posterior foregut endoderm (RA+FGF10+KAAD-cyclopamine), pancreatic endoderm and endocrine precursors (Extendin-4), and hormone producing cells (IGF1+HGF). Transcription factor profiles include: Sox17, CER, FoxA2, and the cytokine receptor CXCR4 (definitive endodermal cells), Hnf1B, Hnf4A (primitive foregut endoderm), Pdx1, Hnf6, H1xB9 (posterior foregut endoderm), and Nkx6.1, Nkx2.2, Ngn3, Pax4 (pancreatic endoderm and endocrine precursors). See, e.g., D'Amour, et al., 2006, *Nat. Biotechnol.* 24:1392-401; Kroon, et al., 2008, *Nat. Biotechnol.* 26:443-52). Another method to induce stem cells (e.g., iPSC) to commit to definitive endoderm, then to pancreatic endoderm, to pancreatic endocrine/exocrine cells and finally to more mature islet cells is described in Jiang, et al., 2007, *Stem Cells* 25(8): p. 1940-53.

[0315] Various types of retinal cells can be generated from stem cells (e.g., iPSC) (see, e.g., Lamba, et al., 2006, *Proc. Natl. Acad. Sci. USA* 103:12769-74; Reh, et al., 2010, *Methods Mol. Biol.* 636:139-53). EBs can be produced and thereafter treated with IGF1, Noggin (BMP inhibitor) and Dkk1 (Wnt inhibitor). This treatment with IGF1, Noggin (BMP inhibitor), and Dkk1 (Wnt inhibitor) can direct stem cells (e.g., iPSC) to adopt a retinal progenitor phenotype, expressing

Pax6 and Chx10. Exposing these progenitors to N-(N-(3,5-difluorophenacetyl)-1-alanyl)-S-phenylglycine t-butyl ester (DAPT), a blocker of Notch signaling, promotes neuronal differentiation (Lamba, et al., 2010, PLoS One 5:e8763). The decision to undergo photoreceptor differentiation is under the control of the transcription factor, Blimp1 (Brzezinski, et al., 2010, Development 137:619-29).

[0316] In particular embodiments, neuronal differentiation can be achieved by replacing a stem cell culture media with a media including basic fibroblast growth factor (bFGF) heparin, and an N2 supplement (e.g., transferrin, insulin, progesterone, putrescine, and selenite). Two days later, differentiating cells can be attached by plating them onto dishes coated with laminin or polyornithine. After an additional 10–11 days in culture, primitive neuroepithelial cells will have formed. The identity of the cells can be confirmed by staining for PAX6 (paired box protein 6, a transcription factor), SOX2 (sex-determining region Y-box 2, another transcription factor), and N-cadherin (a calcium-dependent cell adhesion molecule specific to neural tissue). Neuroepithelial cells can be further differentiated into, e.g., motor neurons (see, e.g., Li, et al. 2005, Nat. Biotechnol. 23, 215–221), dopaminergic neurons (see, e.g., Yan, et al. 2005, Stem Cells 23, 781–790), and oligodendrocytes (Nistor, et al. 2005, Glia 49, 385–396).

[0317] Additional information regarding differentiation to motor neurons includes treatment with RA (Pax6 expressing primitive neuroepithelial cells), RA+Shh (Pax6/Sox1 expressing neuroepithelial cells), which gradually start to express the motor neuron progenitor marker Olig2. Reducing RA+Shh concentration promotes the emergence of motor neurons expressing HB9 and Islet1. The addition of brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), insulin-like growth factor-1 (IGF1), and cAMP promotes process outgrowth (see, e.g., Hu, et al., 2009, Nat. Protoc. 4:1614-22; Hu, et al., 2010, Proc. Natl. Acad. Sci. USA; 107:4335-40).

[0318] Additional information regarding differentiation to dopaminergic neurons includes overexpression of the transcription factor Nurr1 followed by exposure to Shh, FGF-8 and ascorbic acid (see, e.g., Lee, et al., 2000 June, Nat. Biotechnol. 18(6):675-9; Kriks and Studer, 2009, Adv. Exp. Med. Biol. 651:101-11; Lindvall and Kokaia, 2009 May, Trends Pharmacol. Sci. 30(5):260-7.). The combination of stromal cell-derived factor 1 (SDF-1/CXCL12), pleiotrophin (PTN), insulin-like growth factor 2 (IGF2), and ephrin B1 (EFNB1) can induce stem cells (e.g., iPSC) to differentiate to TH-positive neurons in vitro, expressing midbrain specific markers, including Engrailed 1, Nurr1, Pitx3, and dopamine transporter (DAT). These neurons are capable of generating action potentials and forming functional synaptic connections (Vazin, et al., 2009, PLoS One 4:e6606).

[0319] A protocol to produce mature myelinating oligodendrocytes includes directing stem cells (e.g., iPSC) toward neuroectoderm differentiation in the absence of growth factors for 2 weeks. These cells express neuroectoderm transcription factors, including Pax6 and Sox1. Next stem cells (e.g., iPSC) are exposed to the caudalizing factor retinoic acid (RA) and the ventralizing morphogen Shh for 10 days to begin expression of Olig2. To prevent the differentiation to motor neurons and promote the generation of oligodendrocyte precursor cells (OPC)s, cells are cultured with FGF2 for 10 days. By day 35, the Olig2 progenitors co-express Nkx2.2 and no longer give rise to motor neurons. The co-expression of Olig2 and Nkx2.2 reflects a stage prior to human OPCs (pre-OPCs). These pre-OPCs are finally cultured in a glia medium including triiodothyronine (T3), neurotrophin 3 (NT3), PDGF, cAMP, IGF-1 and biotin, which individually or synergistically can promote the survival and proliferation of the OPCs, for another 8 weeks to generate OPCs. These OPCs are bipolar or multipolar, express Olig2, Nkx2.2, Sox10 and PDGFR α , become motile and are able to differentiate to competent oligodendrocytes. WO2007/066338 also describes differentiation protocols for the generation of oligodendrocyte-like cells.

[0320] A protocol to produce glutamatergic neurons includes use of stem cells (e.g., iPSC) to produce cell aggregates which are then treated for 8 days with RA. This results in Pax6 expressing radial glial cells, which after additional culturing in N2 followed by "complete" medium results in 95% glutamate neurons (Bibel, et al., 2007, Nat. Protoc. 2:1034-43).

[0321] A protocol to produce GABAergic neurons includes exposing EBs for 3 days to all-trans-RA. After subsequent culture in serum-free neuronal induction medium including Neurobasal medium supplemented with B27, bFGF and EGF, 95% GABA neurons develop (see, e.g., Chatzi, et al., 2009, Exp. Neurol. 217:407-16).

[0322] U.S. Publication No. 2013/0330306 describes compositions and methods to induce differentiation and proliferation of neural precursor cells or neural stem cells into neural cells using umbilical cord blood-derived mesenchymal stem cells; U.S. Publication No. 2007/0179092 describes use of pituitary adenylate cyclase activating polypeptide (PACAP) to enhance neural stem cell proliferation, differentiation and survival; U.S. Publication No. 2012/0329714 describes use of prolactin to increase neural stem cell numbers; while U.S. Publication No. 2012/0308530 describes a culture surface with amino groups that promotes neuronal differentiation into neurons, astrocytes and oligodendrocytes. U.S. Publication No. 2006/211109 describes improved methods for efficiently producing neuroprogenitor cells and differentiated neural cells such as dopaminergic neurons and serotonergic neurons from pluripotent stem cells, e.g., iPSCs.

[0323] Thus, the fate of neural stem cells can be controlled by a variety of extracellular factors.

Commonly used factors include amphiregulin; BMP-2 (U.S. Pat. Nos. 5,948,428 and 6,001,654); brain derived growth factor (BDNF; Shetty and Turner, 1998, J. Neurobiol. 35:395-425); neurotrophins (e.g., Neurotrophin-3 (NT-3) and Neurotrophin-4 (NT-4); Caldwell, et al., 2001, Nat. Biotechnol. 1;19:475-9); ciliary neurotrophic factor (CNTF); cyclic adenosine monophosphate; epidermal growth factor (EGF); dexamethasone (glucocorticoid hormone); fibroblast growth factor (bFGF; U.S. Pat. NO.5,766,948; FGF-1, FGF-2); forskolin; GDNF family receptor ligands; growth hormone; interleukins; insulin-like growth factors; isobutyl 3-methylxanthine; leukemia inhibitory growth factor (LIF; U.S. Patent No. 6,103,530); Notch antagonists (U.S. Patent No. 6,149,902); platelet derived growth factor (PDGF; U.S. Patent No. 5,753,506); potassium; retinoic acid (U.S. Patent No. 6,395,546); somatostatin; tetanus toxin; and transforming growth factor- α and TGF- β (U.S. Pat. Nos. 5,851,832 and 5,753,506).

[0324] In particular embodiments, preferred proliferation-inducing neural growth factors include BDNF, EGF and FGF-1 or FGF-2. Growth factors can be usually added to the culture medium at concentrations ranging between 1 fg/ml of a pharmaceutically acceptable composition (including, e.g., CNS compatible carriers, excipients and/or buffers) to 1 mg/ml.

[0325] Growth factor expanded stem cells (e.g., iPSC) can also differentiate into neurons and glia after mitogen withdrawal from a culture medium.

[0326] Additionally, WO 2004/046348 describes differentiation protocols for the generation of neural-like cells from bone marrow-derived stem cells. WO 2006/134602 describes differentiation protocols for the generation of neurotrophic factor secreting cells. Commercial kits are also available from Life Technologies and include PSC Neural Induction Medium, Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix, and a Human Neural Stem Cell Immunocytochemistry kit. Stem cells (e.g., iPSC) differentiated into neural cells using the Life Technology kits can be further terminally differentiated into neurons, astrocytes and oligodendrocytes using Life Technologies' B-27® supplements, with N-2 supplement and NEUROBASAL® Medium.

[0327] Additional methods to assist with stem cell (e.g., iPSC) differentiation protocols include, e.g., culture vessels with a portion including an oxygen permeable substrate at least partially coated with a synthetic matrix having an average thickness of less than 100 nm. See, e.g., U.S. Publication No. 2014/0370598.

[0328] U.S. Publication No. 2013/0251690 describes methods to support stem cell (e.g., iPSC) differentiation in elderly populations.

[0329] A number of different differentiation methods have been described. Additional methods that can be used within the teaching of the current disclosure can be found in the art by those with

ordinary skill. Furthermore, and as indicated, differentiation of stem cells (e.g., iPSC) can be confirmed by measuring cellular markers expressed by the desired differentiated cell.

[0330] The foregoing discussion describes in vitro or ex vivo differentiation methods. Modified stem cells (e.g., iPSC) disclosed herein can also differentiate in vivo following administration, as described elsewhere herein.

[0331] Cells to be genetically modified according to the teachings of the current disclosure can be patient-derived cells (autologous) or allogeneic when appropriate and can also be in vivo or ex vivo.

[0332] Methods of sample collection and enrichment are known by those skilled in the art. In some embodiments, cells are derived from cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, or pig. In particular embodiments, cells are derived from humans, for example a patient to be treated.

[0333] In some embodiments, T cells are derived or isolated from samples such as whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. In particular embodiments, cells from the circulating blood of a subject are obtained, e.g., by apheresis or leukapheresis. The samples, in particular embodiments, contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, HSC, HPC, HSPC, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets and further processing is necessary. In particular embodiments, T cells are derived from PBMCs.

[0334] In some embodiments, blood cells collected from a subject are washed, e.g., to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In particular embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. Washing can be accomplished using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, Baxter) according to the manufacturer's instructions. Tangential flow filtration (TFF) can also be performed. In particular embodiments, cells can be re-suspended in a variety of biocompatible buffers after washing, such as, Ca⁺⁺/Mg⁺⁺ free PBS.

[0335] The isolation can include one or more of various cell preparation and separation steps, including separation based on one or more properties, such as size, density, sensitivity or

resistance to particular reagents, and/or affinity, e.g., immunoaffinity, to antibodies or other binding partners. In particular embodiments, the isolation is carried out using the same apparatus or equipment sequentially in a single process stream and/or simultaneously. In particular embodiments, the isolation, culture, and/or engineering of the different populations is carried out from the same starting composition or material, such as from the same sample.

[0336] In particular embodiments, a sample can be enriched for T cells by using density-based cell separation methods and related methods. For example, white blood cells can be separated from other cell types in the peripheral blood by lysing red blood cells and centrifuging the sample through a Percoll or Ficoll gradient.

[0337] In particular embodiments, a bulk T cell population can be used that has not been enriched for a particular T cell type. In particular embodiments, a selected T cell type can be enriched for and/or isolated based on cell-marker based positive and/or negative selection. In positive selection, cells having bound cellular markers are retained for further use. In negative selection, cells not bound by a capture agent, such as an antibody to a cellular marker are retained for further use. In some examples, both fractions can be retained for a further use. In particular embodiments, CD4+ and/or CD8+ T cells are enriched from PBMCs.

[0338] The separation need not result in 100% enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type refers to increasing the number or percentage of such cells but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular type refers to decreasing the number or percentage of such cells but need not result in a complete removal of all such cells.

[0339] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection.

[0340] In some embodiments, an antibody or binding domain for a cellular marker is bound to a solid support or matrix, such as a magnetic bead or paramagnetic bead, to allow for separation of cells for positive and/or negative selection. For example, in some embodiments, the cells and cell populations are separated or isolated using immunomagnetic (or affinity magnetic) separation techniques (reviewed in *Methods in Molecular Medicine*, vol. 58: *Metastasis Research Protocols*, Vol. 2: *Cell Behavior In Vitro and In Vivo*, p 17-25 Edited by: S. A. Brooks and U. Schumacher © Humana Press Inc., Totowa, NJ); see also US 4,452,773; US 4,795,698; US 5,200,084; and EP 452342.

[0341] In some embodiments, affinity-based selection is via magnetic-activated cell sorting

(MACS) (Miltenyi Biotec, Auburn, CA). MACS systems are capable of high-purity selection of cells having magnetized particles attached thereto. In certain embodiments, MACS operates in a mode wherein the non-target and target species are sequentially eluted after the application of the external magnetic field. That is, the cells attached to magnetized particles are held in place while the unattached species are eluted. Then, after this first elution step is completed, the species that were trapped in the magnetic field and were prevented from being eluted are freed in some manner such that they can be eluted and recovered. In certain embodiments, the non-target cells are labelled and depleted from the heterogeneous population of cells.

[0342] In some embodiments, a cell population described herein is collected and enriched (or depleted) via flow cytometry, in which cells stained for multiple cell surface markers are carried in a fluidic stream. In some embodiments, a cell population described herein is collected and enriched (or depleted) via preparative scale (FACS)-sorting. In certain embodiments, a cell population described herein is collected and enriched (or depleted) by use of microelectromechanical systems (MEMS) chips in combination with a FACS-based detection system (see, e.g., WO 2010/033140, Cho et al. (2010) Lab Chip 10, 1567-1573; and Godin et al. (2008) J Biophoton. 1(5):355— 376). In both cases, cells can be labeled with multiple markers, allowing for the isolation of well-defined cell subsets at high purity.

[0343] Cell-markers for different T cell subpopulations are described above. In particular embodiments, specific subpopulations of T cells, such as cells positive or expressing high levels of one or more surface markers, e.g., CCR7, CD45RO, CD8, CD27, CD28, CD62L, CD127, CD4, and/or CD45RA T cells, are isolated by positive or negative selection techniques.

[0344] CD3+, CD28+ T cells can be positively selected for and expanded using anti-CD3/anti-CD28 conjugated magnetic beads (e.g., DYNABEADS® M-450 CD3/CD28 T Cell Expander).

[0345] In particular embodiments, a CD8+ or CD4+ selection step is used to separate CD4+ helper and CD8+ cytotoxic T cells. Such CD8+ and CD4+ populations can be further sorted into sub-populations by positive or negative selection for markers expressed or expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations.

[0346] In some embodiments, enrichment for central memory T (TCM) cells is carried out. In particular embodiments, memory T cells are present in both CD62L subsets of CD8+ peripheral blood lymphocytes. PBMC can be enriched for or depleted of CD62L, CD8 and/or CD62L+CD8+ fractions, such as by using anti-CD8 and anti-CD62L antibodies.

[0347] In some embodiments, the enrichment for central memory T (TCM) cells is based on positive or high surface expression of CCR7, CD45RO, CD27, CD62L, CD28, CD3, and/or CD127; in some aspects, it is based on negative selection for cells expressing or highly

expressing CD45RA and/or granzyme B. In some aspects, isolation of a CD8⁺ population enriched for TCM cells is carried out by depletion of cells expressing CD4, CD14, CD45RA, and positive selection or enrichment for cells expressing CCR7, CD45RO, and/or CD62L. In one aspect, enrichment for central memory T (TCM) cells is carried out starting with a negative fraction of cells selected based on CD4 expression, which is subjected to a negative selection based on expression of CD14 and CD45RA, and a positive selection based on CD62L. Such selections in some aspects are carried out simultaneously and in other aspects are carried out sequentially, in either order. In some aspects, the same CD4 expression-based selection step used in preparing the CD8⁺ cell population or subpopulation, also is used to generate the CD4⁺ cell population or sub-population, such that both the positive and negative fractions from the CD4-based separation are retained, optionally following one or more further positive or negative selection steps.

[0348] Other cell types can be enriched based on known marker profiles and techniques. For example, CD34⁺ HSC, HSP, and HSPC can be enriched using anti-CD34 antibodies directly or indirectly conjugated to magnetic particles in connection with a magnetic cell separator, for example, the CliniMACS® Cell Separation System (Miltenyi Biotec, Bergisch Gladbach, Germany).

[0349] Cell populations can be genetically modified to express a therapeutic molecule, such as a pro-inflammatory cytokine, a CAR, an engineered T cell receptor (TCR), an interfering RNA, or any other therapeutic molecule.

[0350] Desired genes or genetic constructs can be introduced into cells by any method known in the art, including transfection, electroporation, microinjection, lipofection, calcium phosphate mediated transfection, infection with a viral or bacteriophage vector including the gene sequences, cell fusion, chromosome-mediated nucleic acid transfer, microcell-mediated nucleic acid transfer, spheroplast fusion, in vivo nanoparticle-mediated delivery, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see e.g., Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen, et al., 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used, provided that the necessary developmental and physiological functions of the recipient cells are not unduly disrupted. The technique can provide for the stable transfer of the gene to the cell, so that the gene is expressible by the cell and, in certain instances, preferably heritable and expressible by its cell progeny.

[0351] Viral vectors are described above and can be used in ex vivo cell manufacturing. Targeted genetic engineering approaches may also be utilized. The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated protein) nuclease system is an engineered nuclease system used for genetic engineering that is based on a bacterial system.

Information regarding CRISPR-Cas systems and components thereof are described in, for example, US8697359, US8771945, US8795965, US8865406, US8871445, US8889356, US8889418, US8895308, US8906616, US8932814, US8945839, US8993233 and US8999641 and applications related thereto; and WO2014/018423, WO2014/093595, WO2014/093622, WO2014/093635, WO2014/093655, WO2014/093661, WO2014/093694, WO2014/093701, WO2014/093709, WO2014/093712, WO2014/093718, WO2014/145599, WO2014/204723, WO2014/204724, WO2014/204725, WO2014/204726, WO2014/204727, WO2014/204728, WO2014/204729, WO2015/065964, WO2015/089351, WO2015/089354, WO2015/089364, WO2015/089419, WO2015/089427, WO2015/089462, WO2015/089465, WO2015/089473 and WO2015/089486, WO2016205711, WO2017/106657, WO2017/127807 and applications related thereto.

[0352] Particular embodiments utilize zinc finger nucleases (ZFNs) as gene editing agents. ZFNs are a class of site-specific nucleases engineered to bind and cleave DNA at specific positions. ZFNs are used to introduce double stranded breaks (DSBs) at a specific site in a DNA sequence which enables the ZFNs to target unique sequences within a genome in a variety of different cells. A zinc finger is a domain of 30 amino acids within the zinc finger binding domain whose structure is stabilized through coordination of a zinc ion. Examples of zinc fingers include C2H2 zinc fingers, C3H zinc fingers, and C4 zinc fingers. A designed zinc finger domain is a domain not occurring in nature whose design/composition results principally from rational criteria, e.g., application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP designs and binding data. A well-known example of a ZFN is a fusion of the FokI nuclease with a zinc finger DNA binding domain. For additional information regarding ZFNs and ZFNs useful within the teachings of the current disclosure, see, e.g., US 6,534,261; US 6,607,882; US 6,746,838; US 6,794,136; US 6,824,978; 6,866,997; US 6,933,113; 6,979,539; US 7,013,219; US 7,030,215; US 7,220,719; US 7,241,573; US 7,241,574; US 7,585,849; US 7,595,376; US 6,903,185; US 6,479,626; US 2003/0232410 and US 2009/0203140 as well as Gaj et al., *Nat Methods*, 2012, 9(8):805-7; Ramirez et al., *Nucl Acids Res*, 2012, 40(12):5560-8; Kim et al., *Genome Res*, 2012, 22(7): 1327-33; Urnov et al., *Nature Reviews Genetics*, 2010, 11:636-646; Miller, et al. *Nature biotechnology* 25, 778-785 (2007); Bibikova, et al. *Science* 300, 764 (2003); Bibikova, et al. *Genetics* 161, 1169-1175 (2002); Wolfe, et al. *Annual review of biophysics and biomolecular structure* 29, 183-212 (2000); Kim, et al. *Proceedings of the National Academy of Sciences of the United States of America* 93, 1156-1160 (1996); and Miller, et al. *The EMBO journal* 4, 1609-1614 (1985).

[0353] Particular embodiments can use transcription activator like effector nucleases (TALENs)

as gene editing agents. TALENs refer to fusion proteins including a transcription activator-like effector (TALE) DNA binding protein and a DNA cleavage domain. TALENs are used to edit genes and genomes by inducing double DSBs in the DNA, which induce repair mechanisms in cells. Generally, two TALENs must bind and flank each side of the target DNA site for the DNA cleavage domain to dimerize and induce a DSB. For additional information regarding TALENs, see US 8,440,431; US 8,440,432; US 8,450,471; US 8,586,363; and US 8,697,853; as well as Joung and Sander, *Nat Rev Mol Cell Biol*, 2013, 14(l):49-55; Beurdeley et al., *Nat Commun*, 2013, 4: 1762; Scharenberg et al., *Curr Gene Ther*, 2013, 13(4):291-303; Gaj et al., *Nat Methods*, 2012, 9(8):805-7; Miller, et al. *Nature biotechnology* 29, 143-148 (2011); Christian, et al. *Genetics* 186, 757-761 (2010); Boch, et al. *Science* 326, 1509-1512 (2009); and Moscou, & Bogdanove, *Science* 326, 1501 (2009).

[0354] Particular embodiments can utilize MegaTALs as gene editing agents. MegaTALs have a rare-cleaving nuclease structure in which a TALE is fused with the DNA cleavage domain of a meganuclease. Meganucleases, also known as homing endonucleases, are single peptide chains that have both DNA recognition and nuclease function in the same domain. In contrast to the TALEN, the megaTAL only requires the delivery of a single peptide chain for functional activity.

[0355] Particular embodiments can use transposon-based systems as gene editing agents to mediate the integration of a genetic construct into cells. Generally, such methods will involve introducing into cells (i) a first vector encoding a transposase (or a transposase polypeptide) and (ii) a second vector encoding a desired genetic element that is flanked by transposon repeats. Transposons or transposable elements include a (short) nucleic acid sequence with terminal repeat sequences upstream and downstream thereof and encode enzymes that facilitate the excision and insertion of the nucleic acid into target DNA sequences.

[0356] Several transposon/transposase systems have been adapted for genetic insertions of heterologous DNA sequences. Examples of such transposases include sleeping beauty ("SB", e.g., derived from the genome of salmonid fish); piggyback (e.g., derived from lepidopteran cells and/or the *Myotis lucifugus*); mariner (e.g., derived from *Drosophila*); frog prince (e.g., derived from *Rana pipiens*); Tol1; Tol2 (e.g., derived from medaka fish); TcBuster (e.g., derived from the red flour beetle *Tribolium castaneum*), Helraiser, Himar1, Passport, Minos, Ac/Ds, PIF, Harbinger, Harbinger3-DR, HSmar1, and spinON. Transposases and transposon systems are further described in U.S. Pat. Nos. 6,489,458; 7,148,203; 8,227,432; and 9,228,180.

[0357] In particular embodiments, genetically modified cells can be harvested from a culture medium and washed and concentrated into a carrier in a therapeutically effective amount. Herein, cell formulations refer to the formulations including cells genetically modified to express a

molecule and prepared for administration. Exemplary carriers include saline, buffered saline, physiological saline, water, Hanks' solution, Ringer's solution, Normosol-R (Abbott Labs), PLASMA-LYTE A® (Baxter Laboratories, Inc., Morton Grove, IL), and combinations thereof.

[0358] In particular embodiments, carriers can be supplemented with human serum albumin (HSA) or other human serum components or fetal bovine serum. In particular embodiments, a carrier for infusion includes buffered saline with 5% HSA or dextrose. Additional isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, or mannitol.

[0359] Carriers can include buffering agents, such as citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers, and/or trimethylamine salts.

[0360] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which helps to prevent cell adherence to container walls. Typical stabilizers can include polyhydric sugar alcohols; amino acids, such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, and threonine; organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol, and cyclitols, such as inositol; PEG; amino acid polymers; sulfur-containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, alpha-monothioglycerol, and sodium thiosulfate; low molecular weight polypeptides (i.e., <10 residues); proteins such as HSA, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides such as xylose, mannose, fructose and glucose; disaccharides such as lactose, maltose and sucrose; trisaccharides such as raffinose, and polysaccharides such as dextran.

[0361] Where necessary or beneficial, cell formulations can include a local anesthetic such as lidocaine to ease pain at a site of injection.

[0362] Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[0363] Therapeutically effective amounts of cells within cell formulations can be greater than 10^2 cells, greater than 10^3 cells, greater than 10^4 cells, greater than 10^5 cells, greater than 10^6 cells, greater than 10^7 cells, greater than 10^8 cells, greater than 10^9 cells, greater than 10^{10} cells, or greater than 10^{11} .

[0364] In cell formulations disclosed herein, cells are generally in a volume of a liter or less, 500

ml or less, 250 ml or less or 100 ml or less. Hence the density of administered cells is typically greater than 10^4 cells/ml, 10^7 cells/ml or 10^8 cells/ml.

[0365] In particular embodiments, cell formulations can include one or more genetically modified cell types (e.g., modified T-cells, NK cells, or stem cells). Cell formulations can include different types of genetically modified cells (e.g., T-cells, NK cells, and/or stem cells in combination).

[0366] Different types of genetically-modified cells or cell subsets (e.g., modified T-cells, NK cells, and/or stem cells) can be provided in different ratios e.g., a 1:1:1 ratio, 2:1:1 ratio, 1:2:1 ratio, 1:1:2 ratio, 5:1:1 ratio, 1:5:1 ratio, 1:1:5 ratio, 10:1:1 ratio, 1:10:1 ratio, 1:1:10 ratio, 2:2:1 ratio, 1:2:2 ratio, 2:1:2 ratio, 5:5:1 ratio, 1:5:5 ratio, 5:1:5 ratio, 10:10:1 ratio, 1:10:10 ratio, 10:1:10 ratio, etc. These ratios can also apply to numbers of cells expressing the same or different CAR components. Particular embodiments include a 1:1 ratio of CD4 T-cells and CD8 T-cells.

[0367] The cell formulations disclosed herein can be prepared for administration by, e.g., injection, infusion, perfusion, or lavage. The cell formulations can further be formulated for bone marrow, intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, intrathecal, intratumoral, intramuscular, intravesical, and/or subcutaneous injection.

[0368] Particular embodiments achieve all steps required to collect, modify, and readminister cells at a patient's bedside, greatly reducing the cost and complexity of more conventional ex vivo cell manufacturing (see, e.g., FIGs. 20 and 21).

[0369] For example, in relation to FIG. 20, PMBCs may be modified to express a chimeric antigen receptor or other therapeutic molecule, such as a TCR, cytokine receptor, costimulatory ligand, death receptor, chemoattractant, antibody, or multispecific binding molecule

[0370] In relation to FIG. 21, HSC may be modified to introduce or alter a gene selected from ABCD1, ABCA3, ABLI, ADA, AKT1, APC, APP, ARSA, ARSB, BCL11A, BLC1, BLC6, BRCA1, BRCA2, BRIP1, C9ORF72, C46 CAS9, C-CAM, CBFAI, CBL, CCR5, CD4, CD19, CD40, CDA, CFTR, CLN3, C-MYC, CRE, CSCR4, CSFIR, CTLA, CTS-I, CYB5R3, DCC, DHFR, DKC1, DLL1, DMD, EGFR, ERBA, ERBB, EBRB2, ETSI, ETS2, ETV6, F8, F9, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FasL, FCC, FGR, FOX, FUS, FUSI, FYN, GALNS, GATA1, GLB1, GNS, GUSB, HBB, HBD, HBE1, HBG1, HBG2, HCR, HGSNAT, HOXB4, HRAS, HYAL1, ICAM-1, iCaspase, IDUA, IDS, JUN, KLF4, KRAS, LCK, LRRK2, LYN, MCC, MDM2, MGMT, MLL, MMACI, MYB, MEN-I, MEN-II, MYC, NAGLU, NANOG, NF-1, NF-2, NKX2.1, NOTCH, OCT4, p16, p21, p27, p53, p57, p73, PALB2, PARK2, PARK7, phox, PINK1, PK, PSEN1, PSEN2, PTPN22, RAD51C, ras, RPL3 through RPL40, RPLP0, RPLP1, RPLP2, RPS2 through RPS30, RPSA, SFTPB, SFTPC, SGSH, SLX4, SNCA, SOD1,

SOX2, TERC, TERT, TDP43, TNF2, TK, ubiquitin 2, VHL, WAS or WT-1.

[0371] (xi) Kits include groupings of components to practice aspects of the current disclosure. For example, a kit could include the components of a system described herein. A kit could include components to form a foam as described herein. Kits can also include components to practice methods disclosed herein. In particular embodiments, kits can include one or more of a nucleic acid (e.g., plasmid DNA, minicircle DNA, self-replicating RNA, in vitro transcribed RNA, circular RNA, dogpybone DNA (dbDNA), siRNA, or guide RNA), a vector (e.g., viral vector, oncolytic vector, for example, derived from HSV-1 or HSV-2 or a lentiviral vector), a lipid, a lipid nanoparticle, a foam precursor (e.g., methylcellulose, sodium caseinate, or albumin), xanthum gum, guar gum, a flavoring agent, a sugar (e.g., sucrose, maltose, maltodextrin, or trehalose), a targeting ligand that binds an antigen expressed by a cell, hydrogen peroxide, potassium iodide, a syringe, two syringes, three syringes, and/or a continuous flow microfluidics system.

[0372] (xii) Exemplary Embodiments.

1. A gas-bubble based degrading liquid foam including a nucleic acid.
2. The gas-bubble based degrading liquid foam of embodiment 1, wherein the nucleic acid is within a vector.
3. The gas-bubble based degrading liquid foam of embodiment 2, wherein the vector includes a lipid nanoparticle or a viral vector.
4. The gas-bubble based degrading liquid foam of embodiment 3, wherein the lipid nanoparticle includes 2-(Di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethan-1-ol, Dinonyl 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Nonyl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Di(heptadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(4-(nonyloxy)-4-oxobutyl)amino)octanoate, Henicosan-11-yl 6-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)hexanoate, or 8-((8-(Heptadecan-9-yloxy)-8-oxooctyl)(2-hydroxyethyl)amino)octanoic acid.
5. The gas-bubble based degrading liquid foam of embodiment 3, wherein the lipid nanoparticle includes Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate.
6. The gas-bubble based degrading liquid foam of embodiment 2, wherein the vector includes an oncolytic vector.
7. The gas-bubble based degrading liquid foam of embodiment 6, wherein the oncolytic vector

is derived from HSV-1 or HSV-2.

8. The gas-bubble based degrading liquid foam of embodiment 3, wherein the viral vector includes a lentiviral vector.

9. The gas-bubble based degrading liquid foam of any of embodiments 1-8, wherein the foam includes a material generally recognized as safe (GRAS).

10. The gas-bubble based degrading liquid foam of embodiment 9, wherein the GRAS material includes cellulose, sodium caseinate, or albumin.

11. The gas-bubble based degrading liquid foam of embodiment 10, wherein the cellulose includes methylcellulose or a derivative thereof.

12. The gas-bubble based degrading liquid foam of embodiment 11, wherein the derivative thereof includes sodium carboxymethyl cellulose (SCMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), methyl hydroxyethyl cellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), carboxymethylcellulose, hydroxyethyl methyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose succinate (HPMCS), hydroxypropyl methylcellulose trimellitate (HPMCT), hydroxypropyl methylcellulose acetate phthalate (HPMCAP), or hydroxypropyl methylcellulose acetate maleate (HPMCAM)

13. The gas-bubble based degrading liquid foam of embodiment 11, wherein the derivative thereof includes hydroxypropyl methylcellulose or carboxymethyl cellulose.

14. The gas-bubble based degrading liquid foam of any of embodiments 1-13, further including xanthum gum or guar gum.

15. The gas-bubble based degrading liquid foam of any of embodiments 1-14, wherein gas within the gas-bubble based degrading liquid foam includes air, oxygen (O₂), nitrogen (N₂), and inert gasses such as Argon (Ar), Neon (Ne), Radon (Ra), Helium (He), Krypton (Kr), Nitric oxide (NO), or mixtures thereof.

16. The gas-bubble based degrading liquid foam of any of embodiments 1-15, wherein gas within the gas-bubble based degrading liquid foam has a higher than average oxygen content.

17. The gas-bubble based degrading liquid foam of any of embodiments 1-15, wherein gas within the gas-bubble based degrading liquid foam has a lower than average oxygen content.

18. The gas-bubble based degrading liquid foam of embodiment 17, wherein the lower than average oxygen content is 1-5% oxygen.

19. The gas-bubble based degrading liquid foam of any of embodiments 1-18, wherein the liquid within the gas-bubble based degrading liquid foam includes a cell culture media.

20. The gas-bubble based degrading liquid foam of any of embodiments 1-18, wherein the liquid

within the gas-bubble based degrading liquid foam includes phosphate buffered saline (PBS).

21. The gas-bubble based degrading liquid foam of any of embodiments 1-18, wherein the liquid within the gas-bubble based degrading liquid foam includes serum, Lactated Ringer's solution, 0.9% sodium chloride, sterile water, or 5% dextrose solution.

22. The gas-bubble based degrading liquid foam of any of embodiments 1-21, wherein the liquid within the gas-bubble based degrading liquid foam includes a cytokine.

23. The gas-bubble based degrading liquid foam of any of embodiments 1-22, wherein the liquid within the gas-bubble based degrading liquid foam includes fms-like tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO).

24. The gas-bubble based degrading liquid foam of any of embodiments 1-23, wherein the liquid within the gas-bubble based degrading liquid foam includes IL-2, IL-7, and IL-15.

25. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein the foam has an initial mean bubble count of 8,000 μm^2 to 13,000 μm^2 .

26. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein the foam has an initial mean bubble count of 9,000 μm^2 to 12,000 μm^2 .

27. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein the foam has an initial mean bubble count of 10,000 μm^2 to 11,000 μm^2 .

28. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein the foam has an initial mean bubble count of 10,528 μm^2 .

29. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein after 10 hours at room temperature the foam has a bubble count of 15/mm² to 35/mm².

30. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein after 10 hours at room temperature the foam has a bubble count of 20/mm² to 32/mm².

31. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein after 10 hours at room temperature the foam has a bubble count of 26/mm² $\pm$ 6.7 /mm².

32. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein after 10 hours at room temperature the foam has a mean bubble area of 30,000 μm^2 to 55,000 μm^2 .

33. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein after 10 hours at room temperature the foam has a mean bubble area of 32,000 μm^2 to 52,000 μm^2 .

34. The gas-bubble based degrading liquid foam of any of embodiments 1-24, wherein after 10 hours at room temperature the foam has a mean bubble area of 41,597 μm^2 .

35. The gas-bubble based degrading liquid foam of any of embodiments 1-34, wherein the nucleic acid encodes a therapeutic molecule.

36. The gas-bubble based degrading liquid foam of embodiment 35, wherein the therapeutic

molecule is cytotoxic.

37. The gas-bubble based degrading liquid foam of embodiment 35, wherein the therapeutic molecule restores a physiological function of a cell.

38. The gas-bubble based degrading liquid foam of embodiment 35, wherein the therapeutic molecule includes a recombinant protein.

39. The gas-bubble based degrading liquid foam of any of embodiments 1-34, wherein the nucleic acid encodes a vaccine antigen, a genome editing agent, a cytosolic protein, a transmembrane protein, or a secreted protein.

40. The gas-bubble based degrading liquid foam of embodiment 39, wherein the cytosolic protein includes a transcription factor or a suicide gene.

41. The gas-bubble based degrading liquid foam of embodiment 39, wherein the transmembrane protein includes a T cell receptor (TCR), a chimeric antigen receptor (CAR), a cytokine receptor, a costimulatory ligand, or a death receptor.

42. The gas-bubble based degrading liquid foam of embodiment 39, wherein the secreted protein includes a chemoattractant, an antibody, a multispecific binding molecule or a growth factor.

43. The gas-bubble based degrading liquid foam of embodiment 42, wherein the multispecific binding molecule activates an immune cell.

44. The gas-bubble based degrading liquid foam of any of embodiments 1-34, wherein the nucleic acid encodes IRF5, HOXA3, beta-globin, CXCR4, CCR5, IL-10, or siRNA targeting GATA4.

45. The gas-bubble based degrading liquid foam of any of embodiments 1-34, wherein the nucleic acid encodes PTEN, TRAIL, SMAC, TNF- α , P53, or PLAA.

46. The gas-bubble based degrading liquid foam of any of embodiments 1-34, wherein the nucleic acid encodes a bacterial toxin.

47. The gas-bubble based degrading liquid foam of embodiment 46, wherein the bacterial toxin is produced by *Neisseria meningitidis*, *Escherichia coli* (E.coli), *Pseudomonas*, *Proteus*, *Enterobacter*, or *Klebsiella*.

48. The gas-bubble based degrading liquid foam of embodiment 46, wherein the bacterial toxin includes *Pseudomonas* exotoxin A.

49. The gas-bubble based degrading liquid foam of any of embodiments 1-48, wherein the foam further includes a sugar.

50. The gas-bubble based degrading liquid foam of embodiment 49, wherein the sugar includes sucrose, maltose, maltodextrin, or trehalose.

51. The gas-bubble based degrading liquid foam of any of embodiments 1-50, wherein the foam further includes a binding domain that binds an antigen expressed by a cell.

52. The gas-bubble based degrading liquid foam of embodiment 51, wherein the cell is a lymphocyte.
53. The gas-bubble based degrading liquid foam of embodiment 52, wherein the lymphocyte is a T cell or an NK cell.
54. The gas-bubble based degrading liquid foam of embodiment 53, wherein the antigen expressed by the T cell includes CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, 4-1BB (CD137), OX40, lymphocyte function-associated antigen-1 (LFA-1), LIGHT, NKG2C, or B7-H3
55. The gas-bubble based degrading liquid foam of any of embodiments 1-54, wherein the binding domain is a binding fragment of OKT3, oteixizumab, teplizumab, visilizumab, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, or TR66.
56. The gas-bubble based degrading liquid foam of embodiment 51, wherein the cell is a stem cell.
57. The gas-bubble based degrading liquid foam of embodiment 56, wherein the antigen expressed by the stem cell is CD34, CD90, or CD117.
58. The gas-bubble based degrading liquid foam of embodiment 56 or 57, wherein the stem cell is a hematopoietic stem cell or an induced pluripotent stem cell (iPSC).
59. The gas-bubble based degrading liquid foam of embodiment 51, wherein the cell has been differentiated from a hematopoietic stem cell or an iPSC.
60. The gas-bubble based degrading liquid foam of embodiment 51, wherein the cell has been differentiated from a hematopoietic stem cell or an iPSC ex vivo.
61. The gas-bubble based degrading liquid foam of embodiment 51, wherein the cell is a cancer cell or an infected cell.
62. The gas-bubble based degrading liquid foam of embodiment 61, wherein the antigen expressed by the cancer cell includes A33, BAGE, Bcl-2, β -catenin, CA125, CA19-9, CD5, CD19, CD20, CD21, CD22, CD33, CD37, CD45, CD123, CEA, c-Met, CS-1, cyclin B1, DAGE, EBNA, EGFR, ephrinB2, estrogen receptor, FAP, ferritin, folate-binding protein, GAGE, G250, GD-2, GM2, gp75, gp100 (Pmel 17), HER-2/neu, HPV E6, HPV E7, Ki-67, LRP, mesothelin, p53, PRAME, progesterone receptor, PSA, PSMA, MAGE, MART, mesothelin, MUC, MUM-1-B, myc, NYESO-1, ras, RORI, survivin, tenascin, TSTA tyrosinase, VEGF, or WT1.
63. The gas-bubble based degrading liquid foam of any of embodiments 51-62, wherein the binding domain is a binding fragment of R11, R12, 2A2, Y31, FMC63, SJ25C1, HD37, Rituximab, Ofatumumab, 2B8, 4D5, 3G10, 32716, or 32703.
64. The gas-bubble based degrading liquid foam of any of embodiments 51-63, wherein the binding domain is linked to a sugar and/or a methylcellulose or derivative thereof within the foam.

65. The gas-bubble based degrading liquid foam of any of embodiments 51-64, wherein the binding domain is linked to xanthum gum within the foam.
66. The gas-bubble based degrading liquid foam of any of embodiments 51-65, wherein the binding domain is linked to a vector within the foam.
67. The gas-bubble based degrading liquid foam of embodiment 66, wherein the vector is a viral vector or a nanoparticle.
68. The gas-bubble based degrading liquid foam of any of embodiments 1-67, wherein the foam is self-expanding.
69. The gas-bubble based degrading liquid foam of embodiment 68, wherein the self-expanding foam includes an oxygen releasing biomaterial.
70. The gas-bubble based degrading liquid foam of embodiment 68 or 69, wherein the self-expanding foam includes a perfluorocarbon.
71. The gas-bubble based degrading liquid foam of any of embodiments 68-70, wherein the self-expanding foam includes sodium percarbonate, calcium peroxide, magnesium peroxide, hydrogen peroxide, or a fluorinated compound.
72. The gas-bubble based degrading liquid foam of any of embodiments 68-71, wherein the self-expanding foam includes 1-15% hydrogen peroxide.
73. The gas-bubble based degrading liquid foam of any of embodiments 68-71, wherein the self-expanding foam includes 1-6% hydrogen peroxide.
74. The gas-bubble based degrading liquid foam of any of embodiments 68-71, wherein the self-expanding foam includes 2-5% hydrogen peroxide.
75. The gas-bubble based degrading liquid foam of any of embodiments 68-71, wherein the self-expanding foam includes 3.75% hydrogen peroxide.
76. The gas-bubble based degrading liquid foam of any of embodiments 68-75, wherein the self-expanding foam includes 0.0025 – 0.03% potassium iodide.
77. The gas-bubble based degrading liquid foam of any of embodiments 68-75, wherein the self-expanding foam includes 0.01 – 0.02% potassium iodide.
78. The gas-bubble based degrading liquid foam of any of embodiments 68-75, wherein the self-expanding foam includes 0.015% potassium iodide.
79. The gas-bubble based degrading liquid foam of any of embodiments 1-78, wherein the foam is edible or drinkable.
80. The gas-bubble based degrading liquid foam of embodiment 79, wherein the edible or drinkable foam includes a flavoring agent.
81. The gas-bubble based degrading liquid foam of any of embodiments 1-80, wherein the nucleic

acid includes DNA or RNA.

82. The gas-bubble based degrading liquid foam of any of embodiments 1-81, wherein the nucleic acid includes plasmid DNA, minicircle DNA, self-replicating RNA, in vitro transcribed RNA, circular RNA, dogpybone DNA (dbDNA), siRNA, or guide RNA.

83. The gas-bubble based degrading liquid foam of any of embodiments 1-82, further including a cell.

84. The gas-bubble based degrading liquid foam of embodiment 83, wherein the cell is an immune cell.

85. The gas-bubble based degrading liquid foam of embodiment 84, wherein the immune cell is a lymphocyte or a stem cell.

86. The gas-bubble based degrading liquid foam of embodiment 85, wherein the lymphocyte is a T cell or a natural killer (NK) cell.

87. The gas-bubble based degrading liquid foam of embodiment 86, further including an anti-CD3 binding domain and/or an anti-CD28 binding domain.

88. The gas-bubble based degrading liquid foam of any of embodiments 1-87, further including a transduction enhancer.

89. The gas-bubble based degrading liquid foam of embodiment 88, wherein the transduction enhancer includes dimethyl sulfoxide (DMSO), polybrene, or protamine sulfate.

90. Use of a foam of any of embodiments 1-89, to treat a condition in a subject in need thereof.

91. The use of embodiment 90, wherein the condition is cancer.

92. The use of embodiment 91, wherein the cancer is esophageal cancer, rectal cancer, colon cancer, or ovarian cancer.

93. The use of embodiment 90, wherein the condition is a genetic disorder.

94. The use of embodiment 93, wherein the genetic disorder is a genetic blood disorder.

95. The use of embodiment 90, wherein the condition includes an immunodeficiency, a hemoglobinopathy, or an inherited metabolic disorder.

96. A method of forming a gas-bubble based degrading liquid foam of any of embodiments 1-89, the method including adding a population of cells within a liquid to a foam precursor.

97. A method of forming a gas-bubble based degrading liquid foam of any of embodiments 1-89, the method including turbulently mixing a foam precursor, a nucleic acid, a liquid, and a gas, thereby forming the gas-bubble based degrading liquid foam.

98. The method of embodiment 97, wherein the turbulently mixing is automatic.

99. The method of embodiment 97 or 98, wherein the turbulently mixing is within a continuous flow static mixer.

100. The method of any of embodiments 97-99, wherein the foam precursor, the liquid, and the nucleic acid are within a first syringe and the gas is within a second syringe, and the turbulently mixing includes drawing a plunger of the first syringe and a plunger of the second syringe back and forth.

101. The method of embodiment 100, wherein drawing the syringe plunger of the first syringe and the second syringe back and forth includes drawing the syringe plunger of the first syringe and the second syringe back and forth 10 – 50 times.

102. The method of embodiment 100, wherein drawing the syringe plunger of the first syringe and the second syringe back and forth includes drawing the syringe plunger of the first syringe and the second syringe back and forth 20 – 40 times.

103. The method of embodiment 100, wherein drawing the syringe plunger of the first syringe and the second syringe back and forth includes drawing the syringe plunger of the first syringe and the second syringe back and forth 30 times.

104. The method any of embodiments 100-103, wherein the drawing is automatic.

105. A method of delivering a nucleic to a cell, the method including applying a foam of any of embodiments 1-89 to an area in proximity to the cell.

106. The method of embodiment 105, wherein the area in proximity is within 10 centimeters of the cell.

107. The method of embodiment 105 or 106, wherein the cell is in vivo.

108. The method of embodiment 105 or 106, wherein the cell is ex vivo.

109. The method of any of embodiments 105-108, wherein the cell is a lymphocyte.

110. The method of embodiment 109, wherein the lymphocyte is a T cell or NK cell.

111. The method of any of embodiments 105-108, wherein the cell is a stem cell.

112. The method of embodiment 111, wherein the stem cell is a hematopoietic stem cell or an induced pluripotent stem cell (iPSC).

113. The method of any of embodiments 105-108, wherein the cell is a cell that has been differentiated from a hematopoietic stem cell or an iPSC.

114. The method of any of embodiments 105-108, wherein the cell is a cell that has been differentiated from a hematopoietic stem cell or an iPSC ex vivo.

115. The method of any of embodiments 105-114, wherein the cell is a cancer cell or an infected cell.

116. The method of any of embodiments 105-114, wherein the cell is a cancer cell within a solid tumor.

117. The method of any of embodiments 105-107 or 109-114, wherein the cell is within a tumor

resection bed.

118. The method of any of embodiments 105-107 or 109-114, wherein the cancer cell is an ovarian cancer cell within an abdominal cavity.

119. The method of any of embodiments 105-107 or 109-114, wherein the cell lines a body cavity.

120. The method of embodiment 119, wherein the body cavity is oral, thoracic, abdominal, or bladder.

121. The method of any of embodiments 105-107 or 109-114, wherein the cell is within an intestinal tract, a female genital lumen, a muscle, bone marrow, or a lymph node.

122. The method of any of embodiments 105-107 or 109-114, wherein the cell is within skin.

123. The method of any of embodiments 105-107 or 109-114, wherein the cell is within a mouth, throat, or upper gastrointestinal tract.

124. A method of modifying cells, the method including obtaining a population of cells and applying a foam of any of embodiments 1-89 to the population of cells, thereby modifying the population of cells.

125. The method of embodiment 124, wherein the obtaining is from a cell culture device.

126. The method of embodiment 124, wherein the obtaining is from a subject.

127. The method of embodiment 124, wherein the obtaining is from blood of a subject.

128. The method of embodiment 124, wherein the obtaining is from peripheral blood of a subject.

129. The method of embodiment 128, wherein the obtaining is from non-mobilized peripheral blood of a subject.

130. The method of embodiment 128, wherein the obtaining is from mobilized peripheral blood of a subject.

131. The method of embodiment 124, wherein the obtaining is from bone marrow of a subject.

132. The method of any of embodiments 124-131, wherein the obtaining further includes separating the population of cells from an initial environment.

133. The method of embodiment 132, wherein the separating includes density gradient separation or magnetic separation.

134. The method of embodiment 132, wherein the population of cells includes lymphocytes and the separating includes density gradient separation.

135. The method of embodiment 134, wherein the lymphocytes include T cells or NK cells.

136. The method of embodiment 135, wherein the lymphocytes include T cells and the method further includes incubating the T cells with at least one of stimulatory anti-CD3 antibodies, stimulatory anti-CD28 antibodies, interleukin (IL)-2, IL-7, and IL-15.

137. The method of embodiment 132, wherein the population of cells includes stem cells and the

separating includes magnetic separation.

138. The method of any of embodiments 124-137, further including administering the modified population of cells to a subject.

139. The method of embodiment 138, wherein the administering is at least one of subcutaneous, intraperitoneal, or intratumoral.

140. The method of embodiment 139, wherein the modified population of cells includes lymphocytes.

141. The method of embodiment 138, wherein the administering is into bone marrow.

142. The method of embodiment 141, wherein the modified population of cells includes stem cells.

143. A method of treating a gastrointestinal cancer, the method including administering a foam of any of embodiments 1-89 to a gastrointestinal area of a subject who has the gastrointestinal cancer.

144. The method of embodiment 143, wherein the administering is by providing an edible or drinkable foam of embodiment 79 to the subject and directing that the subject ingest the foam.

145. The method of embodiment 143, wherein the administering is rectal administering.

146. The method of any of embodiments 143-145, wherein the nucleic acid within the foam encodes phosphatase and tensin homolog (PTEN), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), second mitochondria-derived activator of caspase (SMAC), or tumor necrosis factor alpha (TNF- α).

147. The method of any of embodiments 143-146, further including administering a radiotherapy to the subject.

148. A system to form a gas-bubble based degrading liquid foam including a nucleic acid, the system including a foam precursor, a gas, a nucleic acid, and a liquid.

149. The system of embodiment 148, wherein the nucleic acid is within a vector.

150. The system of embodiment 149, wherein the vector includes a lipid nanoparticle or a viral vector.

151. The system of embodiment 150, wherein the lipid nanoparticle includes 2-(Di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethan-1-ol, Dinonyl 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Nonyl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Di(heptadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-

(undecyloxy)hexyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(4-(nonyloxy)-4-oxobutyl)amino)octanoate, Henicosan-11-yl 6-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)hexanoate, or 8-((8-(Heptadecan-9-yloxy)-8-oxooctyl)(2-hydroxyethyl)amino)octanoic acid.

152. The system of embodiment 150, wherein the lipid nanoparticle includes Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate.

153. The system of embodiment 150, wherein the viral vector includes an oncolytic vector.

154. The system of embodiment 153, wherein the oncolytic vector is derived from HSV-1 or HSV-2.

155. The system of embodiment 150, wherein the viral vector includes a lentiviral vector.

156. The system of any of embodiments 148-155, wherein the foam precursor includes a material generally recognized as safe (GRAS).

157. The system of embodiment 156, wherein the GRAS material includes cellulose, sodium caseinate, or albumin.

158. The system of embodiment 157, wherein the cellulose includes methylcellulose or a derivative thereof.

159. The system of embodiment 158, wherein the derivative thereof includes sodium carboxymethyl cellulose (SCMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), methyl hydroxyethyl cellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), carboxymethylcellulose, hydroxyethyl methyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose succinate (HPMCS), hydroxypropyl methylcellulose trimellitate (HPMCT), hydroxypropyl methylcellulose acetate phthalate (HPMCAP), or hydroxypropyl methylcellulose acetate maleate (HPMCAM)

160. The system of embodiment 158, wherein the derivative thereof includes hydroxypropyl methylcellulose or carboxymethyl cellulose.

161. The system of any of embodiments 148-160, further including xanthum gum or guar gum.

162. The system of any of embodiments 148-161, wherein the gas includes air, oxygen (O₂), nitrogen (N₂), and inert gasses such as Argon (Ar), Neon (Ne), Radon (Ra), Helium (He), Krypton (Kr), Nitric oxide (NO), or mixtures thereof.

163. The system of any of embodiments 148-162, wherein the gas has a higher than average oxygen content.

164. The system of any of embodiments 148-162, wherein the gas has a lower than average oxygen content.

165. The system of embodiment 164, wherein the lower than average oxygen content is 1-5% oxygen.
166. The system of any of embodiments 148-165, wherein the liquid includes a cell culture media.
167. The system of any of embodiments 148-165, wherein the liquid includes phosphate buffered saline (PBS).
168. The system of any of embodiments 148-165, wherein the liquid includes serum, Lactated Ringer's solution, 0.9% sodium chloride, sterile water, or 5% dextrose solution.
169. The system of any of embodiments 148-168, wherein the liquid includes a cytokine.
170. The system of any of embodiments 148-169, wherein the liquid includes fms-like tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO).
171. The system of any of embodiments 148-170, wherein the liquid includes IL-2, IL-7, and IL-15.
172. The system of any of embodiments 149-171, wherein the vector is within the liquid.
173. The system of any of embodiments 148-172, wherein the foam precursor is within the liquid.
174. The system of any of embodiments 149-173, wherein the vector and the foam precursor are within the liquid.
175. The system of any of embodiments 148-174, wherein the formed foam has an initial mean bubble count of 80/mm² to 110/mm².
176. The system of any of embodiments 148-174, wherein the formed foam has an initial mean bubble count of 90/mm² to 100/mm².
177. The system of any of embodiments 148-174, wherein the formed foam has an initial mean bubble count of or 95/mm².
178. The system of any of embodiments 148-174, wherein the formed foam has an initial mean bubble count of 8,000 μ m² to 13,000 μ m².
179. The system of any of embodiments 148-174, wherein the formed foam has an initial mean bubble count of 9,000 μ m² to 12,000 μ m².
180. The system of any of embodiments 148-174, wherein the formed foam has an initial mean bubble count of 10,000 μ m² to 11,000 μ m².
181. The system of any of embodiments 148-174, wherein the formed foam has an initial mean bubble count of 10,528 μ m².
182. The system of any of embodiments 148-174, wherein after 10 hours at room temperature the formed foam has a bubble count of 15/mm² to 35/mm².
183. The system of any of embodiments 148-174, wherein after 10 hours at room temperature the formed foam has a bubble count of 20/mm² to 32/mm².

184. The system of any of embodiments 148-174, wherein after 10 hours at room temperature the formed foam has a bubble count of $26/\text{mm}^2 \pm 6.7/\text{mm}^2$.
185. The system of any of embodiments 148-174, wherein after 10 hours at room temperature the formed foam has a mean bubble area of $30,000\ \mu\text{m}^2$ to $55,000\ \mu\text{m}^2$.
186. The system of any of embodiments 148-174, wherein after 10 hours at room temperature the formed foam has a mean bubble area of $32,000\ \mu\text{m}^2$ to $52,000\ \mu\text{m}^2$.
187. The system of any of embodiments 148-174, wherein after 10 hours at room temperature the formed foam has a mean bubble area of $41,597\ \mu\text{m}^2$.
188. The system of any of embodiments 148-187, wherein the nucleic acid encodes a therapeutic molecule.
189. The system of embodiment 188, wherein the therapeutic molecule is cytotoxic.
190. The system of embodiment 188, wherein the therapeutic molecule restores a physiological function of a cell.
191. The system of embodiment 188, wherein the therapeutic molecule includes a recombinant protein.
192. The system of any of embodiments 148-187, wherein the nucleic acid encodes a vaccine antigen, a genome editing agent, a cytosolic protein, a transmembrane protein, or a secreted protein.
193. The system of embodiment 192, wherein the cytosolic protein includes a transcription factor or a suicide gene.
194. The system of embodiment 192, wherein the transmembrane protein includes a T cell receptor (TCR), a chimeric antigen receptor (CAR), a cytokine receptor, a costimulatory ligand, or a death receptor.
195. The system of embodiment 192, wherein the secreted protein includes a chemoattractant, an antibody, a multispecific binding molecule or a growth factor.
196. The system of embodiment 195, wherein the multispecific binding molecule activates an immune cell.
197. The system of any of embodiments 148-187, wherein the nucleic acid encodes IRF5, HOXA3, beta-globin, CXCR4, CCR5, IL-10, or siRNA targeting GATA4.
198. The system of any of embodiments 148-187, wherein the nucleic acid encodes PTEN, TRAIL, SMAC, TNF- α , P53, or PLAA.
199. The system of any of embodiments 148-187, wherein the nucleic acid encodes a bacterial toxin.
200. The system of embodiment 199, wherein the bacterial toxin is produced by *Neisseria*

meningitidis, Escherichia coli (E.coli), Pseudomonas, Proteus, Enterobacter, or Klebsiella.

201. The system of embodiment 199, wherein the bacterial toxin includes Pseudomonas exotoxin A.

202. The system of any of embodiments 148-201, wherein the system further includes a sugar.

203. The system of embodiment 202, wherein the sugar includes sucrose, maltose, maltodextrin, or trehalose.

204. The system of any of embodiments 148-203, wherein the system further includes a binding domain that binds that binds an antigen expressed by a cell.

205. The system of embodiment 204, wherein the cell is an immune cell.

206. The system of embodiment 205, wherein the immune cell is a lymphocyte.

207. The system of embodiment 206, wherein the lymphocyte is a T cell or an NK cell.

208. The system of embodiment 207, wherein the antigen expressed by the T cell includes CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, 4-1BB (CD137), OX40, lymphocyte function-associated antigen-1 (LFA-1), LIGHT, NKG2C, or B7-H3

209. The system of embodiment 204, wherein the binding domain is a binding fragment of OKT3, orelizumab, teplizumab, visilizumab, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, or TR66.

210. The system of embodiment 204, wherein the cell is a stem cell.

211. The system of embodiment 210, wherein the antigen expressed by the stem cell is CD34, CD90, or CD117.

212. The system of embodiment 210 or 211, wherein the stem cell is a hematopoietic stem cell or an induced pluripotent stem cell (iPSC).

213. The system of embodiment 204, wherein the cell is a cell that has been differentiated from a hematopoietic stem cell or an iPSC.

214. The system of embodiment 204, wherein the cell is a cell that has been differentiated from a hematopoietic stem cell or an iPSC ex vivo.

215. The system of any of embodiments 204-214, wherein the cell is a cancer cell or an infected cell.

216. The system of embodiment 215, wherein the antigen expressed by the cancer cell include A33, BAGE, Bcl-2, β -catenin, CA125, CA19-9, CD5, CD19, CD20, CD21, CD22, CD33, CD37, CD45, CD123, CEA, c-Met, CS-1, cyclin B1, DAGE, EBNA, EGFR, ephrinB2, estrogen receptor, FAP, ferritin, folate-binding protein, GAGE, G250, GD-2, GM2, gp75, gp100 (Pmel 17), HER-2/neu, HPV E6, HPV E7, Ki-67, LRP, mesothelin, p53, PRAME, progesterone receptor, PSA, PSMA, MAGE, MART, mesothelin, MUC, MUM-1-B, myc, NYESO-1, ras, RORI, survivin, tenascin, TSTA tyrosinase, VEGF, or WT1.

217. The system of any of embodiments 204-216, wherein the binding domain is a binding fragment of R11, R12, 2A2, Y31, FMC63, SJ25C1, HD37, Rituximab, Ofatumumab, 2B8, 4D5, 3G10, 32716, or 32703.
218. The system of any of embodiments 204-217, wherein the binding domain is linked to a sugar and/or a methylcellulose or derivative thereof.
219. The system of any of embodiments 204-218, wherein the binding domain is linked to xanthum.
220. The system of any of embodiments 204-219, wherein the binding domain is linked to a vector.
221. The system of embodiment 220, wherein the vector is a viral vector or a nanoparticle.
222. The system of any of embodiments 148-221, further including a transduction enhancer.
223. The system of embodiment 222, wherein the transduction enhancer includes dimethyl sulfoxide (DMSO), polybrene, or protamine sulfate.
224. The system of any of embodiments 148-223, wherein the foam is self-expanding.
225. The system of embodiment 224, wherein the self-expanding foam includes an oxygen releasing biomaterial.
226. The system of embodiment 224 or 225, wherein the self-expanding foam includes a perfluorocarbon.
227. The system of any of embodiments 224-226, wherein the self-expanding foam includes sodium percarbonate, calcium peroxide, magnesium peroxide, hydrogen peroxide, or a fluorinated compound.
228. The system of any of embodiments 224-227, wherein the self-expanding foam includes 1-15% hydrogen peroxide.
229. The system of any of embodiments 224-227, wherein the self-expanding foam includes 1-6% hydrogen peroxide.
230. The system of any of embodiments 224-227, wherein the self-expanding foam includes 2-5% hydrogen peroxide.
231. The system of any of embodiments 224-227, wherein the self-expanding foam includes 3.75% hydrogen peroxide.
232. The system of any of embodiments 224-231, wherein the self-expanding foam includes 0.0025 – 0.03% potassium iodide.
233. The system of any of embodiments 224-231, wherein the self-expanding foam includes 0.01 – 0.02% potassium iodide.
234. The system of any of embodiments 224-231, wherein the self-expanding foam includes

0.015% potassium iodide.

235. The system of any of embodiments 148-234, wherein the system further includes a syringe.

236. The system of any of embodiments 148-235, wherein the system further includes a first syringe and a second syringe.

237. The system of embodiment 236, wherein the foam precursor and gas are in the first syringe and the nucleic acid and liquid are in the second syringe.

238. The system of embodiment 236 or 237, wherein the first syringe and the second syringe are connected.

239. The system of any of embodiments 148-238, wherein the foam is edible or drinkable.

240. The system of any of embodiments 148-239, further including a flavoring agent.

241. The system of any of embodiments 148-240, wherein the nucleic acid includes DNA or RNA.

242. The system of any of embodiments 148-241, wherein the nucleic acid includes plasmid DNA, minicircle DNA, self-replicating RNA, in vitro transcribed RNA, circular RNA, dogpybone DNA (dbDNA), siRNA, or guide RNA.

243. The system of any of embodiments 148-242, further including an anti-CD3 binding domain and/or an anti-CD28 binding domain.

244. The system of any of embodiments 148-243, further including a cytokine.

245. The system of embodiment 244, wherein the cytokine includes IL-2, IL-7, and/or IL-15.

246. A kit including a foam precursor and a liquid and instructions for how to form a foam including a nucleic acid using the foam precursor, the liquid, and a gas.

247. The kit of embodiment 246, wherein the foam precursor includes cellulose, sodium caseinate, or albumin.

248. The kit of embodiment 247, wherein the cellulose includes methylcellulose or a derivative thereof.

249. The kit of embodiment 248, wherein the derivative thereof includes sodium carboxymethyl cellulose (SCMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), methyl hydroxyethyl cellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), carboxymethylcellulose, hydroxyethyl methyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose succinate (HPMCS), hydroxypropyl methylcellulose trimellitate (HPMCT), hydroxypropyl methylcellulose acetate phthalate (HPMCAP), or hydroxypropyl methylcellulose acetate maleate (HPMCAM)

250. The kit of embodiment 248, wherein the derivative thereof includes hydroxypropyl methylcellulose or carboxymethyl cellulose.

251. The kit of any of embodiments 246-250, wherein the gas includes air, oxygen (O₂), nitrogen (N₂), and inert gasses such as Argon (Ar), Neon (Ne), Radon (Ra), Helium (He), Krypton (Kr), Nitric oxide (NO), or mixtures thereof.
252. The kit of any of embodiments 246-251, wherein the gas has a higher than average oxygen content.
253. The kit of any of embodiments 246-251, wherein the gas has a lower than average oxygen content.
254. The kit of embodiment 253, wherein the lower than average oxygen content is 1-5% oxygen.
255. The kit of any of embodiments 246-254, wherein the liquid includes a cell culture media.
256. The kit of any of embodiments 246-254, wherein the liquid includes phosphate buffered saline (PBS).
257. The kit of any of embodiments 246-254, wherein the liquid includes serum, Lactated Ringer's solution, 0.9% sodium chloride, sterile water, or 5% dextrose solution.
258. The kit of any of embodiments 246-257, wherein the liquid includes a cytokine.
259. The kit of any of embodiments 246-258, wherein the liquid includes fms-like tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO).
260. The kit of any of embodiments 246-259, wherein the liquid includes IL-2, IL-7, and IL-15.
261. The kit of any of embodiments 246-260, further including the nucleic acid.
262. The kit of embodiment 261, wherein the nucleic acid includes plasmid DNA, minicircle DNA, self-replicating RNA, in vitro transcribed RNA, circular RNA, dogpybone DNA (dbDNA), siRNA, or guide RNA.
263. The kit of embodiment 261 or 262, wherein the nucleic acid encodes a molecule.
264. The kit of embodiment 263, wherein the molecule is cytotoxic.
265. The kit of embodiment 263, wherein the molecule restores a physiological function of a cell.
266. The kit of embodiment 263, wherein the molecule includes a recombinant protein.
267. The kit of embodiment 263, wherein the nucleic acid encodes a vaccine antigen, a genome editing agent, a cytosolic protein, a transmembrane protein, or a secreted protein.
268. The kit of embodiment 267, wherein the cytosolic protein includes a transcription factor or a suicide gene.
269. The kit of embodiment 267, wherein the transmembrane protein includes a T cell receptor (TCR), a chimeric antigen receptor (CAR), a cytokine receptor, a costimulatory ligand, or a death receptor.
270. The kit of embodiment 267, wherein the secreted protein includes a chemoattractant, an antibody, a multispecific binding molecule or a growth factor.

271. The kit of embodiment 270, wherein the multispecific binding molecule activates an immune cell.
272. The kit of embodiment 261, wherein the nucleic acid encodes IRF5, HOXA3, beta-globin, CXCR4, CCR5, IL-10, or siRNA targeting GATA4.
273. The kit of embodiment 261, wherein the nucleic acid encodes PTEN, TRAIL, SMAC, TNF- α , P53, or PLAA.
274. The kit of embodiment 261, wherein the nucleic acid encodes a bacterial toxin.
275. The kit of embodiment 274, wherein the bacterial toxin is produced by *Neisseria meningitidis*, *Escherichia coli* (E.coli), *Pseudomonas*, *Proteus*, *Enterobacter*, or *Klebsiella*.
276. The kit of embodiment 274, wherein the bacterial toxin includes *Pseudomonas* exotoxin A.
277. The kit of any of embodiments 246-276, further including a vector.
278. The kit of embodiment 277, wherein the vector includes a lipid.
279. The kit of embodiment 278, wherein the lipid includes 2-(Di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethan-1-ol, Dinonyl 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Nonyl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Di(heptadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(4-(nonyloxy)-4-oxobutyl)amino)octanoate, Henicosan-11-yl 6-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)hexanoate, or 8-((8-(Heptadecan-9-yloxy)-8-oxooctyl)(2-hydroxyethyl)amino)octanoic acid.
280. The kit of embodiment 278, wherein the lipid includes Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate.
281. The kit of embodiment 277, wherein the vector includes a viral vector.
282. The kit of embodiment 281, wherein the viral vector includes an oncolytic vector or a lentiviral vector.
283. The kit of embodiment 282, wherein the oncolytic vector is derived from HSV-1 or HSV-2.
284. The kit of any of embodiments 246-283, further including xanthum gum or guar gum.
285. The kit of any of embodiments 246-284, further including a flavoring agent.
286. The kit of any of embodiments 246-285, further including a sugar.
287. The kit of embodiment 286, wherein the sugar includes sucrose, maltose, maltodextrin, or trehalose.

288. The kit of any of embodiments 246-287, further including a transduction enhancer.
289. The kit of embodiment 288, wherein the transduction enhancer includes dimethyl sulfoxide (DMSO), polybrene, or protamine sulfate.
290. The kit of any of embodiments 246-289, further including a binding domain that binds an antigen expressed by a cell.
291. The kit of embodiment 290, wherein the binding domain is a binding fragment of OKT3, oteelixumab, teplizumab, visilizumab, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, or TR66.
292. The kit of embodiment 290, wherein the binding domain is a binding fragment of R11, R12, 2A2, Y31, FMC63, SJ25C1, HD37, Rituximab, Ofatumumab, 2B8, 4D5, 3G10, 32716, or 32703.
293. The kit of embodiment 290, wherein the binding domain is linked to a sugar and/or a methylcellulose or derivative thereof.
294. The kit of any of embodiments 290-293, wherein the binding domain is linked to xanthum gum.
295. The kit of any of embodiments 290-294, wherein the binding domain is linked to a vector.
296. The kit of embodiment 295, wherein the vector is a viral vector or a nanoparticle.
297. The kit of any of embodiments 246-296, further including an oxygen releasing biomaterial.
298. The kit of embodiment 297, wherein the oxygen releasing biomaterial includes a perfluorocarbon.
299. The kit of embodiment 297 or 298, wherein the oxygen releasing biomaterial includes sodium percarbonate, calcium peroxide, magnesium peroxide, hydrogen peroxide, or a fluorinated compound.
300. The kit of any of embodiments 297-299, wherein the oxygen releasing biomaterial includes hydrogen peroxide.
301. The kit of any of embodiments 297-300, wherein the oxygen releasing biomaterial includes potassium iodide.
302. The kit of any of embodiments 246-301, further including a syringe.
303. The kit of any of embodiments 246-302, further including a continuous flow microfluidics system.
304. The kit of any of embodiments 246-303, further including an anti-CD3 binding domain and/or an anti-CD28 binding domain.
305. The kit of any of embodiments 246-304, further including a cytokine.
306. The kit of embodiment 305, wherein the cytokine includes IL-2, IL-7, and/or IL-15.
307. The kit of embodiment 305, wherein the cytokine includes fms-like tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO).

308. A foam including methylcellulose or a derivative thereof, a gas, a T-cell targeting lentivirus that encodes a recombinant protein, and peripheral blood mononuclear cells.

309. The foam of embodiment 308, wherein the recombinant protein includes a chimeric antigen receptor.

[0373] (xiii) Experimental Examples. Example 1. Various compounds with foaming properties were tested for their ability to deliver nucleic acids (e.g., nucleic acid vectors) to human cells. Only compounds that are generally regarded as safe (GRAS) by the U.S. Food and Drug Administration (FDA) and are already manufactured in bulk, mostly for the food industry but also the pharmaceutical industry, were considered. This selection criteria ensured that the foam vehicle is biocompatible, scalable, and low-cost. One popular plant-based food additive that was tested is methylcellulose, a widely used foaming agent in foods like pudding, ice cream, or whipped toppings. The second candidate, sodium caseinate, the sodium salt of casein (a milk protein), is used in foods and cosmetics for its foaming and thickening properties. Human serum albumin was chosen as the starting material of the third foam formulation. This globular protein is routinely administered into patients with acute conditions such as trauma, cardiogenic shock, and sepsis, but it is also being explored by bioengineers for its foaming properties (Caironi et al., *N Engl J Med* 370, 1412-1421 (2014), Zhang et al., *Dermatol Surg* 46, 1030-1034 (2020)). To make the foam more stable for therapeutic purposes, xanthan gum was added to all three tested foam precursor solutions. Xanthan gum is an FDA-approved polysaccharide used as a foam stabilizer in foods, cosmetics and healthcare products (Re-evaluation of xanthan gum (E 415) as a food additive in foods for infants below 16 weeks of age and follow-up of its re-evaluation as a food additive for uses in foods for all population groups. EFSA J 21, e07951 (2023), Ostrowski et al., *Nat Microbiol* 7, 556-569 (2022), Hasnain & Nayak. *Natural polysaccharides in drug delivery and biomedical applications.* (Elsevier/Academic Press, London; 2019)).

[0374] An initial experimental protocol for in vitro transfection screening involved delivering nonviral vector. Lipid nanoparticles (LNPs) were fabricated to carry luciferase mRNA. Sabnis et al., *Mol Ther* 26, 1509-1519 (2018). This allowed for easy comparison of transfection efficiencies using bioluminescence, following addition of these LNPs to cultured cells either suspended in liquid or embedded in various foam formulations. Cells were incubated with LNP suspensions or LNP foam for two hours, during which the culture dish was placed in a horizontal or tilted position. The horizontal setup is designed to compare nucleic acid transfer efficiencies in the absence of any liquid drainage, whereas the angled transfection mimics the more realistic scenario of patient tissue that is shifting in position and lacks defined borders that would prevent drainage of the applied LNPs. In the horizontal transfections, embedding mRNA LNPs in foam made of

methylcellulose boosted their transfection efficiency by 2.9-fold ($P < 0.0001$) compared to LNP suspension (FIG. 4A, 4B). This effect was even more pronounced in the angled cell transfection, where this foam formulation achieved a robust 384-fold higher nucleic acid transfer (FIG. 4A, 4C). Importantly, in all in vitro transfection studies, the viability of cells following extended contact with foam was unaffected (FIG. 4D). Other tested foams did not improve the transfection potential of mRNA LNPs at the levels that were observed with methylcellulose (FIG. 4A-4C). As methylcellulose foam stays in place, the transfections were spatially well-defined. This is vividly illustrated by the ability to write text onto cells grown in a tissue culture plate while holding the dish vertically, which then translated into an identical pattern of gene expression 24 hours later (FIG. 4E). Based on these in vitro results, the methylcellulose foam formulation was selected as a preferred candidate for further analysis and studies.

[0375] To precisely measure the foam structure with regards to bubble size and distribution as well as decay over time, the Dynamic Foam Analyzer DFA100FSM (Krüss Scientific) was used. This instrument uses contact-free optical sensors and an LED light source to continuously record the height of the foam, liquid drainage, and the sizes and distributions of foam bubbles at a precision of 0.1 mm. Based on three independently manufactured foam batches, an initial mean bubble count of $95.3 \pm 4.2/\text{mm}^2$, and mean bubble area of $10,528 \pm 455 \mu\text{m}^2$ (FIG. 2A) were measured. As expected, the larger bubbles grew with time at the expense of smaller bubbles due to diffusion of air from the smaller to the larger, resulting in a decrease in bubble count ($25.7 \pm 6.7 /\text{mm}^2$) and an increase in bubble size (mean $41,597 \pm 9,387 \mu\text{m}^2$) after 10 hours at room temperature (FIG. 2A). Foam height (total height – liquid height) decayed to an average $78 \pm 9\%$ of the maximum value. LNPs embedded in foam were relatively homogeneously dispersed throughout the liquid lamellae as visualized by confocal microscopy (FIG. 2B, 2C). Based on twenty analyzed foam lamellae, a slight increase (1.1-fold, $P < 0.0001$) in LNP concentration in centers compared to edges facing gas bubbles (FIG. 2D) was measured.

[0376] Based on these in vitro studies, the benefit of foam as a nucleic acid delivery vehicle in relevant in vivo test systems was evaluated next. First, the intraperitoneal route of administration was tested, which could be applied to a variety of pathologies or organs in the peritoneal cavity, including cancers such as ovarian, pancreatic, liver, colorectal and gastric. Since the abdominal cavity is a closed anatomic space, the options for LNP drainage are limited to the abdominal lymphatics. This means that whether delivered by liquid or foam, the residence time of the nucleic acid vector is similar. Therefore, the question that was addressed in this first set of vivo studies was whether a foam formulation intrinsically achieves higher in situ transfection rates than a liquid suspension, i.e., independent of the foam's ability to stay in place at the application site. Mice

were divided into groups that received an equal dose of LNPs containing luciferase-encoding mRNA administered in PBS solution or foam, which was freshly prepared just prior to injection. Bioluminescence imaging was then used to quantify gene expression over a four-day period. It was found that foam injections resulted in an overall average 3.2-fold higher transfection rate compared to liquid-based gene therapy (FIG. 6A, 6B). As expected with mRNA-based vectors, luciferase was transiently expressed, showing the highest level of expression 24 hours post injection. A direct visualization of nucleic acid transfer into various organs collected from the peritoneal cavity confirmed substantially stronger transfection rates achieved with foam versus liquid (FIG. 7A, 7B). Notably, the use of foam resulted not only in highly efficient but also homogeneous nucleic acid transfer, which was most obvious in the small intestine, displaying consistent transgene expression along its entire length in all injected animals (FIG. 7A). In contrast, LNP suspensions manifested in low and irregular nucleic acid transfer, with most sections of the intestine untransfected.

[0377] Guided by this imaging data, a comprehensive toxicity assessment was conducted. Mice were injected intraperitoneally with foam or PBS and euthanized 48 hours later to collect blood for serum chemistry and to perform a complete gross necropsy. The following tissues were evaluated by a board-certified staff pathologist: intestine, liver, spleen, mesentery, pancreas, and stomach. Serum chemistry was unremarkable (FIG. 5A). The few histological lesions noted in foam-treated animals were minimal to mild. In four of the ten mice injected with foam, sections of the mesenteric fat were infiltrated by a mixture of neutrophils and histiocytes (FIG. 5B). Also, an expansion of the marginal zone and red pulp of the spleen was observed in all foam-treated mice (FIG. 5C). The pathologist analyzing the samples, explained that the marginal sinus ectasia is presumed to be a drainage response to the viscous liquid foam. Overall, this data establishes that the methylcellulose-based foam does not trigger clinically significant local or systemic toxicities and is a safe delivery vehicle of nucleic acid vectors.

[0378] Next, skin gene therapy was assessed as a second in situ test system of the foam technology. Skin is an easily accessible and attractive organ for gene therapy. This field has been explored in recent years for a variety of skin diseases, such as epidermolysis bullosa (a genetic disease that causes painful blisters and persistent wounds), cutaneous lymphoma, and malignant melanoma. To compare the foam preparation to a suspension formulation for nucleic acid delivery to the skin, LNPs containing luciferase-encoding mRNA in foam or as a liquid were applied topically to the skin of shaved mice. Since nucleic acid delivery and resulting therapy relies on the presence of living cells, the outermost epidermal skin layer (stratum corneum) consisting of dead cells as a major barrier to nucleic acid transfer through the skin was removed using dermabrasion

prior to foam or liquid application. Notably, even though the mice were extremely active, climbing and running in their cages, the LNP foam adhered to the skin and stayed in place. In sharp contrast to the foam, most administered liquid eventually ran off the skin, having moistened it for only a short time. Using bioluminescence as a readout, an average 13.2-fold higher nucleic acid transfer with foam compared to liquid was observed ($P=0.02$; FIG. 9A, 9B).

[0379] To confirm that this technology has relevance not only for nucleic acid delivery using nonviral, but also viral vectors, the benefit of foam as a delivery vehicle for Lentivirus (LV) was investigated. LVs have proven their safety and efficacy as flexible gene delivery vectors in clinical applications of gene therapy for genetic diseases. Traditionally, LV vectors have been used to transduce patient-derived somatic cells *ex vivo*, which are then re-delivered to the same patient after transduction. However, a rapidly growing number of trials are currently investigating LVs for *in vivo* gene therapy applications to treat monogenic diseases and chronic diseases, including neurological diseases, ophthalmological diseases, and metabolic diseases. In the presented studies, a commonly used broadly tropic vesicular stomatitis virus envelope glycoprotein (VSV-G) pseudotyped LV vector with the luciferase transgene driven by the Cytomegalovirus (CMV) promoter was employed. An equal dose of viral particles (4×10^6 transduction units) either suspended in PBS or embedded in methylcellulose foam was injected into the peritoneal cavity of mice and nucleic acid expression was serially quantified by bioluminescence imaging. Two days after nucleic acid vector administration it was found that the foam improved LV-mediated *in situ* nucleic acid transfer by an average 10.2-fold (FIG. 8A, 8B). Gene expression levels were tracked for four weeks, during which time mice inoculated with LV-foam maintained similarly high differences in luciferase levels over mice treated with LV suspended in PBS (average 9.8-fold increase over LV-PBS during weeks 1-4, FIG. 8B). Quantification of viral particles in the peritoneal cavity versus the peripheral blood by ELISA two hours post injections revealed that foam retained an 89.4-fold higher concentration of LV nucleic acid vector at the application site, compared to liquid suspension (FIG. 8C, left panel). Conversely, a 2.9-fold higher off-target viral count in the peripheral blood of animals treated with LV suspensions was measured (FIG. 8C, right panel). This data establishes that foam can mitigate unwanted systemic nucleic acid exposure and focus its action on therapeutically desired tissue.

[0380] Example 2. Engineering therapeutic cells within methylcellulose-based nucleic acid delivery foam. Here, a facile and generalizable strategy is used to robustly augment transfection/transduction efficiencies of nonviral or viral vector into cells, based on the use of specially formulated foam. Co-embedding vector and cells into foam lamellae, which include thin layers of continuous liquid separating the foam bubbles, enables multi-fold higher nucleic acid

transfer rates, compared to the conventional suspension setup (i.e., adding vector to cultured cells). Based on screening biocompatible compounds with foaming properties, methylcellulose-based foams were used as a lead formulation. For example, embedding therapeutic cells (human T lymphocytes) into methylcellulose-based foam does not compromise cell viability or cell yield. Using this method, 20 to 50-fold higher transfection rates of mRNA lipid nanoparticles (LNPs) into primary human T cells and hematopoietic progenitor cells can be achieved, compared to suspension controls, without causing cell damage or interfering with intrinsic cell functions (FIGs. 18A, 18B, FIGs. 19A-19E). Up to 4-fold higher lentiviral transduction efficiency occurs, using foam versus suspension, even at very low multiplicities of infection (MOI; FIGs. 15A-15F). Importantly, improvement in nucleic acid transfer efficiency can be achieved without addition of chemical transduction enhancers, centrifugation or washing steps. Accordingly, the foam platform described herein transforms the way therapists genetically manipulate cells to treat a wide range of disorders. Nucleic acid delivery foam is easy to prepare, and the starting materials are already available at pharmaceutical grade and at low cost. Given that co-incubating vector and target cells in foam can be a brief (less than 1 minute), uncomplicated procedure, it can be seamlessly integrated into established cell manufacturing protocols, substantially lowering the cost and infrastructure for cellular therapies and increasing access to treatments.

[0381] Example 3. Injectable methylcellulose-based foam for in situ programming of CAR T-cells. This example describes a rapid point-of-care CAR T-manufacturing strategy that does not require cell culture, specialized infrastructure or high doses of vector. More specifically, patient lymphocytes can be genetically reprogrammed within a nucleic acid delivery foam that is mixed with freshly isolated peripheral blood mononuclear cells (PBMCs) and then directly injected back into the patient by subcutaneous injection.

[0382] Co-embedding human T cells and nucleic acid vector in methylcellulose-based foam enhances nucleic acid transfer efficiencies of viral (lentivirus) and nonviral (mRNA liposome) vectors, compared to conventional suspension formulation (FIGs. 14A-14E, 15A-15F), without impacting key cellular functions (FIGs. 18A, 18B). Embedding PMBCs into methylcellulose nucleic acid delivery foam, followed by direct subcutaneous injection back into the patient, will result in efficient programming and engraftment of CAR-T cell. In particular, injection sites near lymph nodes (e.g., axilla or inguinal region) can facilitate rapid dissemination of CAR-T cells throughout the body via the lymphatics.

[0383] Key advantages include a multifold reduction in the required vector dose, which can substantially lower cost and improve safety. By co-embedding T cells and nucleic acid vector into methylcellulose-based foam, nucleic acid transfer efficiencies are augmented, even when using

a small amount of vector (FIGs. 15A-15F). Reducing the required vector dose substantially lowers the cost of therapy. It also prevents or reduces toxicity from high-dose systemic administration of vector. This disclosed approach does not expose patients to vector systemically as methylcellulose foam retains vector and therapeutic T cells at the injection site. As foam matures, gravity slowly drains lamellae (FIGs. 14A-14E), gradually releasing genetically programmed CAR T-cells. Additionally, or alternatively, key advantages to the techniques described herein include a lack of a need for a modified vector. For example, since methylcellulose foam drives colocalization of T cells with concentrated vector, complex re-engineering of CAR-programming vector to selectively target host T cells is not required. Instead of infusing patients with large quantities of re-tooled vector to target a small fraction of circulating T cells in whole blood, the techniques described herein use a small volume of blood (<50 mL), isolates PMBCs, and directly exposes cells to CAR-programming vector within the methylcellulose foam. This way, the same vectors currently used to ex vivo transduce patient T cells for any of the six CAR T-cell therapies approved by the Food and Drug Administration (FDA), may be used. This facilitates clinical translation as these nucleic acid vectors are already manufactured at large scale and at high titer under GMP.

[0384] Advantages also include easy and safe re-dosing. For example, the techniques described herein are simple to administer in an outpatient setting and comparably low in cost. This way, patients can be treated repeatedly if medically required, or a treatment strategy can be shifted as their condition evolves (e.g., target different tumor antigens if tumor escape variants develop). Further, vector-induced neutralizing antibodies in the patient blood may not compromise CAR nucleic acid transfer or therapeutic efficacy. Using the techniques described herein, vector and T cells can be co-embedded within methylcellulose foam outside the patient and then injected subcutaneously. This way, there is little to no direct contact of vector with patient blood. Further, immune-mediated infusion reactions associated with conventional systemic administration of nucleic acid vector do not pose a safety concern.

[0385] The foam platform described herein transforms the way therapists genetically manipulate patient T cells to treat a wide range of disorders. Nucleic acid delivery foam is easy to prepare at the bedside, and/or the starting materials are already available at pharmaceutical grade. The techniques described herein can substantially lower the cost and infrastructure for T-cell therapies and help meet the rising demand.

[0386] Example 4. Injectable methylcellulose-based foam for affordable bedside manufacturing of genetically modified stem-cell treatments. Hematopoietic stem cell (HSC)-targeted gene therapy can be curative for multiple genetic diseases, including primary immunodeficiencies,

hemoglobinopathies, and inherited metabolic disorders. To expand the therapeutic potential of HSC gene therapy to a broader set of patients, the techniques described herein include an immediately accessible, affordable, bedside HSC cell manufacturing strategy that does not require cell culture, specialized infrastructure or patient chemotherapy preconditioning. More specifically, HSCs can be genetically reprogrammed within a specially formulated nucleic acid delivery foam that is mixed with freshly isolated bone marrow aspirate concentrate (BMAC) (e.g., an injectable product derived from a patient's bone marrow and/or the like), and then directly injected back into the marrow. The collection process may be minimally invasive and involves extracting marrow from the patient's hip bone using a needle and syringe. The marrow may then be placed in a centrifuge and spun at high speeds to separate the cells from the fluid. The cells may then be concentrated into a solution that is ready for injection.

[0387] In some instances, co-embedding HSCs and nucleic acid vector in methycellulose-based foam enhances nucleic acid transfer efficiencies of viral (lentivirus) and nonviral (mRNA liposome) vectors, compared to conventional suspension formulation, without impacting key cellular functions. Further, embedding BMAC into methylcellulose nucleic acid delivery foam, followed by direct intra-marrow injection back into the patient, will result in efficient HSC reprogramming and engraftment. For example, the foam retains vector and therapeutic cells in the marrow and can locally boost nucleic acid transfer into CD34+ progenitor cells, while minimizing off-target events.

[0388] The foam platform described herein can be used to genetically manipulate HSCs to treat a wide range of genetic disorders.

[0389] To confirm the superiority of foam over liquid as a delivery vehicle for transgenes into HSCs in human Bone Marrow Aspirate Concentrate (BMAC), cells collected from bone marrow aspirates in nucleic acid delivery foam (using the lead foam formulation identified in preliminary studies), or in suspension, will be transfected. Nucleic acid transfer efficiencies into various bone marrow cell types, with focus on therapeutically desired CD34+ HSCs, will be compared and the effects of foam on viability, proliferative activity, phenotype and differentiation will be examined. Both, nonviral (mRNA liposomes) and viral (replication incompetent lentivirus) vectors will be tested.

[0390] In order to measure the therapeutic advantage of nucleic acid delivery foam compared to liquid-based gene delivery for HSC gene editing, a 3D model of perfused bone marrow will be employed (FIG. 22). More specifically, human cancellous bone grafts will be placed in a continuous perfusion chamber recapitulating blood flow through the marrow. Human BMAC suspended in nucleic acid vector or embedded in nucleic acid delivery foam will be injected directly into the grafts. In some instances, it will be tested whether foam better retains vector in

the marrow and locally boosts nucleic acid transfer into CD34+ progenitor cells, while minimizing off-target events (e.g., vector leaking out of the bone graft). Nonviral and viral vectors will be tested. In some instances, vector carrying a CRISPR-Cas9 gene editing system that inactivates the BCL11A gene (an FDA-approved treatment for sickle-cell disease and thalassemia) will be delivered and indel frequencies in HSCs mediated by foam will be compared to indel frequencies in HSCs mediated by suspension. Foam delivery will achieve the benefits described herein.

[0391] Example 5. Drinkable nucleic acid delivery foam as a minimally disruptive treatment for esophageal cancer (FIG. 23). In some examples, the techniques described herein are adapted to a drinkable nucleic acid delivery foam for the treatment of esophageal cancer (hereinafter “EC”) (FIG. 23). Patients diagnosed with EC currently rely on treatments given at specialist care centers (e.g., surgery, chemotherapy, radiation, etc.) that may be extremely life-disruptive, cause severe pain, and have strong side effects. Further, after decades of clinical and experimental research, treatment of EC still remains challenging, with a 5-year survival rate of only 20%. Dysphagia, the inability to swallow, is one of the most distressing and debilitating symptoms in patients with cancer-related esophageal obstruction. The nucleic acid delivery foam described herein provides EC patients with a treatment option that can be administered orally. Such treatment option can be administered by a physician and/or—self-administered and prepared at home by the patient or caregiver. This way, patients can save costs, and refrain from radical surgeries (including postoperative complications), lengthy hospital stays, rounds of chemotherapy, and expensive palliative care. Additionally, or alternatively, the drinkable foams described herein may maintain a patient’s ability to swallow and eat, as well as avoid tube feeding, which may produce other debilitations and/or life-threatening infections.

[0392] The following genes can preferentially act on malignant cells and cause their death without affecting normal cells:

PTEN (phosphatase and tensin homolog): Silencing of the tumor suppressor gene PTEN occurs in a substantial proportion of EC. This abrogation drives tumorigenesis and tumor progression. PTEN is also implicated in modulation of the DNA damage response and in shaping the tumor immune microenvironment.

TRAIL (tumor necrosis factor-related apoptosis-inducing ligand): Induces apoptosis in a wide variety of cancer cells, without killing normal cells.

SMAC (second mitochondria-derived activator of caspase): Overexpression sensitizes tumor cells to apoptosis.

TNF- α (tumor necrosis factor alpha): Can inhibit tumor growth by damaging the tumor vasculature and by directly inducing cancer cell killing.

[0393] As shown in FIG. 24, co-expression of SMAC with TRAIL or PTEN, or co-expression of TRAIL with TNF-alpha all achieved similar potent inhibition of EC growth in vitro.

[0394] Foam is mostly air (80-90% of total volume) and therefore cannot be dispensed in volumes of less than 500 μ L. It is therefore not possible to study the therapeutic potential of drinkable nucleic acid delivery foam in small animal models of EC, such as mice (esophagus volume: 10-20 μ L) or rats (esophagus volume: 150-200 μ L). Hence, a simplified ex vivo tissue model of a human esophagus narrowed by an ingrowing esophageal carcinoma (FIG. 26) was developed. A monolayer of human epithelial cells (HT29-MTX) was first cultured on the inner surface of a collagen tube to model the healthy section of the esophagus. Then, to model a tumorous obstruction, human OE19 esophageal carcinoma cells expressing luciferase were grown in three-dimensional collagen plugs for 4 weeks and placed into the distal part of the esophagus tubing to narrow its lumen (FIG. 25, right panel). To simulate lubrication and swallowing, artificial saliva (commercially available from Millipore Sigma) was pumped into the model esophagus at a flow rate similar to normal saliva production in humans (1 mL/min).

[0395] To directly visualize differences in fate and dynamics of foam versus liquid as they pass through the esophagus with a constricting lesion, time lapse videos were generated. Glow-in-the-dark UV black-light pigments were added to the foam precursor solution or the suspension to be able to easily track the carriers under black light. Foam initially passed through the esophageal tube relatively fast (within a few minutes), but then gradually accumulated at the therapeutically relevant tumor constriction and the junction between the esophagus and the tumor lesion FIG. 26). Importantly, foam was not “stuck” in the esophagus, but was cleared within 20 minutes from the healthy upper section and moved down the esophagus toward the constriction by the steady saliva stream. In sharp contrast to foam, liquid suspension quickly drained through the malignant constriction with no substantial accumulation (FIG. 26).

[0396] The following studies will demonstrate the superiority of drinkable-foam gene therapy over liquid-based gene therapy for treatment of EC.

[0397] Foam will boost nucleic acid transfer into obstructing EC lesions. To confirm that foam will boost nucleic acid transfer into obstructing EC lesions, as shown in FIG. 25, advanced EC will be modeled. 3 mL of foam or PBS suspension will be added to this test system (each containing 3×10^9 LNPs loaded with 50 μ g mRNA encoding the fluorescent protein mCherry). Control groups will be exposed to foam or suspension that does not contain nucleic acid vector. After 24 hours (under constant artificial saliva flow), the following assays will be performed:

In vitro fluorescence imaging: Esophageal tumor plugs (containing 3D cultures of OACM5.1C cells) will be removed from the tubing (coated with HT29-MTX epithelial cells to model

the healthy section of the esophagus). Nucleic acid transfer (mCherry signals) into each component (tumor vs. healthy epithelium) will be quantitated using an IVIS® imaging system.

Confocal microscopy: In parallel experiments, isolated esophageal tumor plugs will be snap-frozen for Cryostat sectioning. Using the automated TissueFAXS slide scanner (Fred Hutchinson Cancer Center Cellular Imaging core facility), differences in intensities and tissue-penetration depths of mCherry gene expression will be measured between the foam and suspension groups.

[0398] Nucleic acid delivery foam for the treatment of EC will have therapeutic effect. To confirm that nucleic acid delivery foam for the treatment of EC will have therapeutic effect, first, human esophageal carcinoma cells (OACM5.1C) expressing luciferase for serial bioluminescence imaging of tumor growth will be grown in three-dimensional collagen plugs for 4 weeks and placed into the distal part of the esophagus tubing (as shown in FIG. 25). Tumor lesions will then be treated with LNPs (delivered in 3 mL of foam or suspension) encoding a potent combination of the identified pro-apoptotic genes (FIG. 24) or a truncated form of Pseudomonas exotoxin A (PE38). Controls will receive no therapy or gene therapy-free foam/suspension. Treatments will be bi-weekly (Monday/Friday) for 3 weeks (3×10^9 LNPs per treatment loaded with 50 µg mRNA). To measure the therapeutic advantage of foam over suspension, the following assays will be conducted:

Serial bioluminescence tumor imaging: Once a week, therapeutic anti-tumor responses will be compared by bioluminescence imaging of the luciferase-expressing EC tumor cells. Specifically, the entire esophageal tube, including the tumor plugs in the distal part will be quickly removed from the incubator, submerged in a solution containing D-Luciferin at a concentration of 100 µg/mL, and tumor signals will be quantitated using an IVIS® instrument. Tubes will then be returned to the setup, shown in FIG. 25, to continue the experiment.

Microscopic evaluation: After the final day of treatment, isolated esophageal tumor plugs will be snap-frozen for Cryostat sectioning. Differences in tumor cell death between treatment groups will be microscopically evaluated by a pathologist in a blinded fashion.

[0399] Nucleic acid delivery foam treatments make radiation therapy more effective.

[0400] Image-guided radiation therapy has become an important treatment option for patients with EC¹; however, resistance leads to treatment failure and cancer relapse²². While the ultimate goal is to develop drinkable nucleic acid delivery foam as a monotherapy, combining it with radiotherapy will dramatically improve the survival of EC patients and represent a potential first path of the technology to clinical translation. One could envision EC patients drinking nucleic acid delivery foam one or two days prior to each radiation session (it takes mRNA 24 to 48 hours to

translate to protein) to enhance the lethal effect of radiation on cancer cells. Thus, the studies described below will show that nucleic acid delivery foam increases the sensitivity of EC to radiation.

[0401] The following administration protocols will be tested:

Pre-conditioning: EC will be transfected with nucleic acid delivery foam 2 days prior to irradiation.

Intra-: EC will be transfected with nucleic acid delivery foam on the day of irradiation.

Post-: EC will be transfected with nucleic acid delivery foam 2 days after irradiation.

Control 1: No treatment

Control 2: Nucleic acid delivery foam only

Control 3: Radiation only

[0402] To measure the therapeutic advantage of combination therapy, the following assays will be conducted:

Serial bioluminescence tumor imaging: Once a week, the esophageal tube, including the tumor plugs in the distal part, will be quickly removed from the incubator and tumor signals will be quantitated using an IVIS® instrument. Tubes will then be returned to the incubator setup.

Flow cytometry cell viability assay: On the final day of the experiment, EC tumor plugs will be digested into single-cell suspensions. The cells will be stained with APC Annexin V and propidium iodide and then analyzed by FACS to measure differences in tumor cell death stages between groups.

[0403] Combining nucleic acid delivery foam with radiation will greatly amplify the tumor-killing effects compared to either treatment alone.

[0404] Example 6. Rectally applied nucleic acid delivery foam as a minimally disruptive treatment for rectal cancer. Referring to FIG. 27, in additional examples, the foams described herein can be adapted to treat other constricting cancer types, for instance rectal cancer.

[0405] Patients with locally advanced rectal cancer are currently treated with neoadjuvant chemoradiation, total mesorectal excision, and adjuvant chemotherapy. While generally effective, this trimodal approach is arduous. For example, the boundaries of the bony pelvis, the proximity to the sphincter, and the need to preserve autonomic nerves may make surgical resection challenging and morbid. Rectally applied nucleic acid delivery foam ($\pm$ targeted radiation), will allow patients to continue their daily lives and keep all normal body functions that are lost following painful surgery (e.g., regular sphincter function, no need for colostomy bag, etc.).

[0406] (xiv) Closing Paragraphs. Unless otherwise indicated, the practice of the present disclosure can employ conventional techniques of chemistry, organic chemistry, biochemistry,

and molecular biology. These methods are described in, for example, Harcourt et al., Holt McDougal Modern Chemistry: Student Edition (2018); J. Karty, Organic Chemistry Principles and Mechanisms (2014); Nelson et al., Lehninger Principles of Biochemistry 5th edition (2008); Skoog et al., Fundamentals of Analytical Chemistry (8th Edition); Atkins et al., Atkins' Physical Chemistry (11th Edition); David P. Clark, et al., Molecular Biology 3rd Edition (2018); Thomas D. Pollard, et al., Cell Biology, 4th Edition (2023).

[0407] As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms “include” or “including” should be interpreted to recite: “comprise, consist of, or consist essentially of.” The transition term “comprise” or “comprises” means has, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase “consisting of” excludes any element, step, ingredient or component not specified. The transition phrase “consisting essentially of” limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause a statistically significant reduction in the ability to obtain a claimed effect according to a relevant parameter described in the current disclosure.

[0408] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term “about” has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e., denoting somewhat more or somewhat less than the stated value or range, to within a range of $\pm 20\%$ of the stated value; $\pm 19\%$ of the stated value; $\pm 18\%$ of the stated value; $\pm 17\%$ of the stated value; $\pm 16\%$ of the stated value; $\pm 15\%$ of the stated value; $\pm 14\%$ of the stated value; $\pm 13\%$ of the stated value; $\pm 12\%$ of the stated value; $\pm 11\%$ of the stated value; $\pm 10\%$ of the stated value; $\pm 9\%$ of the stated value; $\pm 8\%$ of the stated value; $\pm 7\%$ of the stated value; $\pm 6\%$ of the stated value; $\pm 5\%$ of the stated value; $\pm 4\%$ of the stated value; $\pm 3\%$ of the stated value; $\pm 2\%$ of the stated value; or $\pm 1\%$ of the stated value.

[0409] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0410] The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0411] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0412] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0413] Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

[0414] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0415] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0416] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology (Eds. Attwood T et al., Oxford University Press, Oxford, 2006).

CLAIMS

What is claimed is:

1. A gas-bubble based degrading liquid foam comprising a nucleic acid.
2. The gas-bubble based degrading liquid foam of claim 1, wherein the nucleic acid is within a vector.
3. The gas-bubble based degrading liquid foam of claim 2, wherein the vector comprises a lipid nanoparticle or a viral vector.
4. The gas-bubble based degrading liquid foam of claim 3, wherein the lipid nanoparticle comprises 2-(Di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethan-1-ol, Dinonyl 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Nonyl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Di(heptadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(4-(nonyloxy)-4-oxobutyl)amino)octanoate, Henicosan-11-yl 6-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)hexanoate, or 8-((8-(Heptadecan-9-yloxy)-8-oxooctyl)(2-hydroxyethyl)amino)octanoic acid.
5. The gas-bubble based degrading liquid foam of claim 3, wherein the lipid nanoparticle comprises Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate.
6. The gas-bubble based degrading liquid foam of claim 2, wherein the vector comprises an oncolytic vector.
7. The gas-bubble based degrading liquid foam of claim 6, wherein the oncolytic vector is derived from HSV-1 or HSV-2.
8. The gas-bubble based degrading liquid foam of claim 3, wherein the viral vector comprises a lentiviral vector.
9. The gas-bubble based degrading liquid foam of claim 1, wherein the foam comprises a material generally recognized as safe (GRAS).
10. The gas-bubble based degrading liquid foam of claim 9, wherein the GRAS material comprises cellulose, sodium caseinate, or albumin.
11. The gas-bubble based degrading liquid foam of claim 10, wherein the cellulose comprises methylcellulose or a derivative thereof.
12. The gas-bubble based degrading liquid foam of claim 11, wherein the derivative thereof comprises sodium carboxymethyl cellulose (SCMC), hydroxyethyl cellulose (HEC),

hydroxypropyl cellulose (HPC), methyl hydroxyethyl cellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), carboxymethylcellulose, hydroxyethyl methyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose succinate (HPMCS), hydroxypropyl methylcellulose trimellitate (HPMCT), hydroxypropyl methylcellulose acetate phthalate (HPMCAP), or hydroxypropyl methylcellulose acetate maleate (HPMCAM)

13. The gas-bubble based degrading liquid foam of claim 11, wherein the derivative thereof comprises hydroxypropyl methylcellulose or carboxymethyl cellulose.
14. The gas-bubble based degrading liquid foam of claim 1, further comprising xanthum gum or guar gum.
15. The gas-bubble based degrading liquid foam of claim 1, wherein gas within the gas-bubble based degrading liquid foam comprises air, oxygen (O₂), nitrogen (N₂), and inert gasses such as Argon (Ar), Neon (Ne), Radon (Ra), Helium (He), Krypton (Kr), Nitric oxide (NO), or mixtures thereof.
16. The gas-bubble based degrading liquid foam of claim 1, wherein gas within the gas-bubble based degrading liquid foam has a higher than average oxygen content.
17. The gas-bubble based degrading liquid foam of claim 1, wherein gas within the gas-bubble based degrading liquid foam has a lower than average oxygen content.
18. The gas-bubble based degrading liquid foam of claim 1, wherein the lower than average oxygen content is 1-5% oxygen.
19. The gas-bubble based degrading liquid foam of claim 1, wherein the liquid within the gas-bubble based degrading liquid foam comprises a cell culture media.
20. The gas-bubble based degrading liquid foam of claim 1, wherein the liquid within the gas-bubble based degrading liquid foam comprises phosphate buffered saline (PBS).
21. The gas-bubble based degrading liquid foam of claim 1, wherein the liquid within the gas-bubble based degrading liquid foam comprises serum, Lactated Ringer's solution, 0.9% sodium chloride, sterile water, or 5% dextrose solution.
22. The gas-bubble based degrading liquid foam of claim 1, wherein the liquid within the gas-bubble based degrading liquid foam comprises a cytokine.
23. The gas-bubble based degrading liquid foam of claim 1, wherein the liquid within the gas-bubble based degrading liquid foam comprises fms-like tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO).
24. The gas-bubble based degrading liquid foam of claim 1, wherein the liquid within the gas-bubble based degrading liquid foam comprises IL-2, IL-7, and IL-15.

25. The gas-bubble based degrading liquid foam of claim 1, wherein the foam has an initial mean bubble count of 8,000 μm^2 to 13,000 μm^2 .
26. The gas-bubble based degrading liquid foam of claim 1, wherein the foam has an initial mean bubble count of 9,000 μm^2 to 12,000 μm^2 .
27. The gas-bubble based degrading liquid foam of claim 1, wherein the foam has an initial mean bubble count of 10,000 μm^2 to 11,000 μm^2 .
28. The gas-bubble based degrading liquid foam of claim 1, wherein the foam has an initial mean bubble count of 10,528 μm^2 .
29. The gas-bubble based degrading liquid foam of claim 1, wherein after 10 hours at room temperature the foam has a bubble count of 15/mm² to 35/mm².
30. The gas-bubble based degrading liquid foam of claim 1, wherein after 10 hours at room temperature the foam has a bubble count of 20/mm² to 32/mm².
31. The gas-bubble based degrading liquid foam of claim 1, wherein after 10 hours at room temperature the foam has a bubble count of 26/mm² $\pm$ 6.7 /mm².
32. The gas-bubble based degrading liquid foam of claim 1, wherein after 10 hours at room temperature the foam has a mean bubble area of 30,000 μm^2 to 55,000 μm^2 .
33. The gas-bubble based degrading liquid foam of claim 1, wherein after 10 hours at room temperature the foam has a mean bubble area of 32,000 μm^2 to 52,000 μm^2 .
34. The gas-bubble based degrading liquid foam of claim 1, wherein after 10 hours at room temperature the foam has a mean bubble area of 41,597 μm^2 .
35. The gas-bubble based degrading liquid foam of claim 1, wherein the nucleic acid encodes a therapeutic molecule.
36. The gas-bubble based degrading liquid foam of claim 35, wherein the therapeutic molecule is cytotoxic.
37. The gas-bubble based degrading liquid foam of claim 35, wherein the therapeutic molecule restores a physiological function of a cell.
38. The gas-bubble based degrading liquid foam of claim 35, wherein the therapeutic molecule comprises a recombinant protein.
39. The gas-bubble based degrading liquid foam of claim 1, wherein the nucleic acid encodes a vaccine antigen, a genome editing agent, a cytosolic protein, a transmembrane protein, or a secreted protein.
40. The gas-bubble based degrading liquid foam of claim 39, wherein the cytosolic protein comprises a transcription factor or a suicide gene.
41. The gas-bubble based degrading liquid foam of claim 39, wherein the transmembrane protein

comprises a T cell receptor (TCR), a chimeric antigen receptor (CAR), a cytokine receptor, a costimulatory ligand, or a death receptor.

42. The gas-bubble based degrading liquid foam of claim 39, wherein the secreted protein comprises a chemoattractant, an antibody, a multispecific binding molecule or a growth factor.
43. The gas-bubble based degrading liquid foam of claim 42, wherein the multispecific binding molecule activates an immune cell.
44. The gas-bubble based degrading liquid foam of claim 1, wherein the nucleic acid encodes IRF5, HOXA3, beta-globin, CXCR4, CCR5, IL-10, or siRNA targeting GATA4.
45. The gas-bubble based degrading liquid foam of claim 1, wherein the nucleic acid encodes PTEN, TRAIL, SMAC, TNF- α , P53, or PLAA.
46. The gas-bubble based degrading liquid foam of claim 1, wherein the nucleic acid encodes a bacterial toxin.
47. The gas-bubble based degrading liquid foam of claim 46, wherein the bacterial toxin is produced by *Neisseria meningitidis*, *Escherichia coli* (E.coli), *Pseudomonas*, *Proteus*, *Enterobacter*, or *Klebsiella*.
48. The gas-bubble based degrading liquid foam of claim 46, wherein the bacterial toxin comprises *Pseudomonas* exotoxin A.
49. The gas-bubble based degrading liquid foam of claim 1, wherein the foam further comprises a sugar.
50. The gas-bubble based degrading liquid foam of claim 49, wherein the sugar comprises sucrose, maltose, maltodextrin, or trehalose.
51. The gas-bubble based degrading liquid foam of claim 1, wherein the foam further comprises a binding domain that binds an antigen expressed by a cell.
52. The gas-bubble based degrading liquid foam of claim 51, wherein the cell is a lymphocyte.
53. The gas-bubble based degrading liquid foam of claim 52, wherein the lymphocyte is a T cell or an NK cell.
54. The gas-bubble based degrading liquid foam of claim 53, wherein the antigen expressed by the T cell comprises CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, 4-1BB (CD137), OX40, lymphocyte function-associated antigen-1 (LFA-1), LIGHT, NKG2C, or B7-H3
55. The gas-bubble based degrading liquid foam of claim 51, wherein the binding domain is a binding fragment of OKT3, otilixizumab, teplizumab, visilizumab, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, or TR66.
56. The gas-bubble based degrading liquid foam of claim 51, wherein the cell is a stem cell.
57. The gas-bubble based degrading liquid foam of claim 56, wherein the antigen expressed by

the stem cell is CD34, CD90, or CD117.

58. The gas-bubble based degrading liquid foam of claim 56, wherein the stem cell is a hematopoietic stem cell or an induced pluripotent stem cell (iPSC).
59. The gas-bubble based degrading liquid foam of claim 51, wherein the cell has been differentiated from a hematopoietic stem cell or an iPSC.
60. The gas-bubble based degrading liquid foam of claim 51, wherein the cell has been differentiated from a hematopoietic stem cell or an iPSC ex vivo.
61. The gas-bubble based degrading liquid foam of claim 51, wherein the cell is a cancer cell or an infected cell.
62. The gas-bubble based degrading liquid foam of claim 61, wherein the antigen expressed by the cancer cell comprises A33, BAGE, Bcl-2, β -catenin, CA125, CA19-9, CD5, CD19, CD20, CD21, CD22, CD33, CD37, CD45, CD123, CEA, c-Met, CS-1, cyclin B1, DAGE, EBNA, EGFR, ephrinB2, estrogen receptor, FAP, ferritin, folate-binding protein, GAGE, G250, GD-2, GM2, gp75, gp100 (Pmel 17), HER-2/neu, HPV E6, HPV E7, Ki-67, LRP, mesothelin, p53, PRAME, progesterone receptor, PSA, PSMA, MAGE, MART, mesothelin, MUC, MUM-1-B, myc, NYESO-1, ras, RORI, survivin, tenascin, TSTA tyrosinase, VEGF, or WT1.
63. The gas-bubble based degrading liquid foam of claim 51, wherein the binding domain is a binding fragment of R11, R12, 2A2, Y31, FMC63, SJ25C1, HD37, Rituximab, Ofatumumab, 2B8, 4D5, 3G10, 32716, or 32703.
64. The gas-bubble based degrading liquid foam of claim 51, wherein the binding domain is linked to a sugar and/or a methylcellulose or derivative thereof within the foam.
65. The gas-bubble based degrading liquid foam of claim 51, wherein the binding domain is linked to xanthum gum within the foam.
66. The gas-bubble based degrading liquid foam of claim 51, wherein the binding domain is linked to a vector within the foam.
67. The gas-bubble based degrading liquid foam of claim 66, wherein the vector is a viral vector or a nanoparticle.
68. The gas-bubble based degrading liquid foam of claim 1, wherein the foam is self-expanding.
69. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises an oxygen releasing biomaterial.
70. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises a perfluorocarbon.
71. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises sodium percarbonate, calcium peroxide, magnesium peroxide, hydrogen peroxide,

or a fluorinated compound.

72. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises 1-15% hydrogen peroxide.
73. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises 1-6% hydrogen peroxide.
74. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises 2-5% hydrogen peroxide.
75. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises 3.75% hydrogen peroxide.
76. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises 0.0025 – 0.03% potassium iodide.
77. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises 0.01 – 0.02% potassium iodide.
78. The gas-bubble based degrading liquid foam of claim 68, wherein the self-expanding foam comprises 0.015% potassium iodide.
79. The gas-bubble based degrading liquid foam of claim 1, wherein the foam is edible or drinkable.
80. The gas-bubble based degrading liquid foam of claim 79, wherein the edible or drinkable foam comprises a flavoring agent.
81. The gas-bubble based degrading liquid foam of claim 1, wherein the nucleic acid comprises DNA or RNA.
82. The gas-bubble based degrading liquid foam of claim 1, wherein the nucleic acid comprises plasmid DNA, minicircle DNA, self-replicating RNA, in vitro transcribed RNA, circular RNA, dogpybone DNA (dbDNA), siRNA, or guide RNA.
83. The gas-bubble based degrading liquid foam of claim 1, further comprising a cell.
84. The gas-bubble based degrading liquid foam of claim 83, wherein the cell is an immune cell.
85. The gas-bubble based degrading liquid foam of claim 84, wherein the immune cell is a lymphocyte or a stem cell.
86. The gas-bubble based degrading liquid foam of claim 85, wherein the lymphocyte is a T cell or a natural killer (NK) cell.
87. The gas-bubble based degrading liquid foam of claim 86, further comprising an anti-CD3 binding domain and/or an anti-CD28 binding domain.
88. The gas-bubble based degrading liquid foam of claim 1, further comprising a transduction enhancer.

89. The gas-bubble based degrading liquid foam of claim 88, wherein the transduction enhancer comprises dimethyl sulfoxide (DMSO), polybrene, or protamine sulfate.
90. Use of a foam of any of claim 1, to treat a condition in a subject in need thereof.
91. The use of claim 90, wherein the condition is cancer.
92. The use of claim 91, wherein the cancer is esophageal cancer, rectal cancer, colon cancer, or ovarian cancer.
93. The use of claim 90, wherein the condition is a genetic disorder.
94. The use of claim 93, wherein the genetic disorder is a genetic blood disorder.
95. The use of claim 90, wherein the condition comprises an immunodeficiency, a hemoglobinopathy, or an inherited metabolic disorder.
96. A method of forming a gas-bubble based degrading liquid foam of claim 1, the method comprising adding a population of cells within a liquid to a foam precursor.
97. A method of forming a gas-bubble based degrading liquid foam of claim 1, the method comprising turbulently mixing a foam precursor, a nucleic acid, a liquid, and a gas, thereby forming the gas-bubble based degrading liquid foam.
98. The method of claim 97, wherein the turbulently mixing is automatic.
99. The method of claim 97, wherein the turbulently mixing is within a continuous flow static mixer.
100. The method of claim 97, wherein the foam precursor, the liquid, and the nucleic acid are within a first syringe and the gas is within a second syringe, and the turbulently mixing comprises drawing a plunger of the first syringe and a plunger of the second syringe back and forth.
101. The method of claim 100, wherein drawing the syringe plunger of the first syringe and the second syringe back and forth comprises drawing the syringe plunger of the first syringe and the second syringe back and forth 10 – 50 times.
102. The method of claim 100, wherein drawing the syringe plunger of the first syringe and the second syringe back and forth comprises drawing the syringe plunger of the first syringe and the second syringe back and forth 20 – 40 times.
103. The method of claim 100, wherein drawing the syringe plunger of the first syringe and the second syringe back and forth comprises drawing the syringe plunger of the first syringe and the second syringe back and forth 30 times.
104. The method claim 100, wherein the drawing is automatic.
105. A method of delivering a nucleic to a cell, the method comprising applying a foam of claim 1 to an area in proximity to the cell.
106. The method of claim 105, wherein the area in proximity is within 10 centimeters of the cell.

- 107. The method of claim 105, wherein the cell is in vivo.
- 108. The method of claim 105, wherein the cell is ex vivo.
- 109. The method of claim 105, wherein the cell is a lymphocyte.
- 110. The method of claim 109, wherein the lymphocyte is a T cell or NK cell.
- 111. The method of claim 105, wherein the cell is a stem cell.
- 112. The method of claim 111, wherein the stem cell is a hematopoietic stem cell or an induced pluripotent stem cell (iPSC).
- 113. The method of claim 105, wherein the cell is a cell that has been differentiated from a hematopoietic stem cell or an iPSC.
- 114. The method of claim 105, wherein the cell is a cell that has been differentiated from a hematopoietic stem cell or an iPSC ex vivo.
- 115. The method of claim 105, wherein the cell is a cancer cell or an infected cell.
- 116. The method of claim 105, wherein the cell is a cancer cell within a solid tumor.
- 117. The method of claim 105, wherein the cell is within a tumor resection bed.
- 118. The method of claim 115, wherein the cancer cell is an ovarian cancer cell within an abdominal cavity.
- 119. The method of claim 105, wherein the cell lines a body cavity.
- 120. The method of claim 119, wherein the body cavity is oral, thoracic, abdominal, or bladder.
- 121. The method of claim 105, wherein the cell is within an intestinal tract, a female genital lumen, a muscle, bone marrow, or a lymph node.
- 122. The method of claim 105, wherein the cell is within skin.
- 123. The method of claim 105, wherein the cell is within a mouth, throat, or upper gastrointestinal tract.
- 124. A method of modifying cells, the method comprising obtaining a population of cells and applying a foam of claim 1 to the population of cells, thereby modifying the population of cells.
- 125. The method of claim 124, wherein the obtaining is from a cell culture device.
- 126. The method of claim 124, wherein the obtaining is from a subject.
- 127. The method of claim 124, wherein the obtaining is from blood of a subject.
- 128. The method of claim 124, wherein the obtaining is from peripheral blood of a subject.
- 129. The method of claim 128, wherein the obtaining is from non-mobilized peripheral blood of a subject.
- 130. The method of claim 128, wherein the obtaining is from mobilized peripheral blood of a subject.
- 131. The method of claim 124, wherein the obtaining is from bone marrow of a subject.

132. The method of claim 124, wherein the obtaining further comprises separating the population of cells from an initial environment.
133. The method of claim 132, wherein the separating comprises density gradient separation or magnetic separation.
134. The method of claim 132, wherein the population of cells comprises lymphocytes and the separating comprises density gradient separation.
135. The method of claim 134, wherein the lymphocytes comprise T cells or NK cells.
136. The method of claim 135, wherein the lymphocytes comprise T cells and the method further comprises incubating the T cells with at least one of stimulatory anti-CD3 antibodies, stimulatory anti-CD28 antibodies, interleukin (IL)-2, IL-7, and IL-15.
137. The method of claim 132, wherein the population of cells comprises stem cells and the separating comprises magnetic separation.
138. The method of claim 124, further comprising administering the modified population of cells to a subject.
139. The method of claim 138, wherein the administering is at least one of subcutaneous, intraperitoneal, or intratumoral.
140. The method of claim 139, wherein the modified population of cells comprises lymphocytes.
141. The method of claim 138, wherein the administering is into bone marrow.
142. The method of claim 141, wherein the modified population of cells comprises stem cells.
143. A method of treating a gastrointestinal cancer, the method comprising administering a foam of claim 1 to a gastrointestinal area of a subject who has the gastrointestinal cancer.
144. The method of claim 143, wherein the administering is by providing an edible or drinkable foam of claim 79 to the subject and directing that the subject ingest the foam.
145. The method of claim 143, wherein the administering is rectal administering.
146. The method of claim 143, wherein the nucleic acid within the foam encodes phosphatase and tensin homolog (PTEN), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), second mitochondria-derived activator of caspase (SMAC), or tumor necrosis factor alpha (TNF- α).
147. The method of claim 143, further comprising administering a radiotherapy to the subject.
148. A system to form a gas-bubble based degrading liquid foam comprising a nucleic acid, the system comprising a foam precursor, a gas, a nucleic acid, and a liquid.
149. The system of claim 148, wherein the nucleic acid is within a vector.
150. The system of claim 149, wherein the vector comprises a lipid nanoparticle or a viral vector.

151. The system of claim 150, wherein the lipid nanoparticle comprises 2-(Di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethan-1-ol, Dinonyl 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Nonyl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Di(heptadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(4-(nonyloxy)-4-oxobutyl)amino)octanoate, Henicosan-11-yl 6-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)hexanoate, or 8-((8-(Heptadecan-9-yloxy)-8-oxooctyl)(2-hydroxyethyl)amino)octanoic acid.
152. The system of claim 150, wherein the lipid nanoparticle comprises Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate.
153. The system of claim 150, wherein the viral vector comprises an oncolytic vector.
154. The system of claim 153, wherein the oncolytic vector is derived from HSV-1 or HSV-2.
155. The system of claim 150, wherein the viral vector comprises a lentiviral vector.
156. The system of claim 148, wherein the foam precursor comprises a material generally recognized as safe (GRAS).
157. The system of claim 156, wherein the GRAS material comprises cellulose, sodium caseinate, or albumin.
158. The system of claim 157, wherein the cellulose comprises methylcellulose or a derivative thereof.
159. The system of claim 158, wherein the derivative thereof comprises sodium carboxymethyl cellulose (SCMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), methyl hydroxyethyl cellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), carboxymethylcellulose, hydroxyethyl methyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose succinate (HPMCS), hydroxypropyl methylcellulose trimellitate (HPMCT), hydroxypropyl methylcellulose acetate phthalate (HPMCAP), or hydroxypropyl methylcellulose acetate maleate (HPMCAM).
160. The system of claim 158, wherein the derivative thereof comprises hydroxypropyl methylcellulose or carboxymethyl cellulose.
161. The system of claim 148, further comprising xanthum gum or guar gum.
162. The system of claim 148, wherein the gas comprises air, oxygen (O₂), nitrogen (N₂), and

inert gasses such as Argon (Ar), Neon (Ne), Radon (Ra), Helium (He), Krypton (Kr), Nitric oxide (NO), or mixtures thereof.

- 163. The system of claim 148, wherein the gas has a higher than average oxygen content.
- 164. The system of claim 148, wherein the gas has a lower than average oxygen content.
- 165. The system of claim 164, wherein the lower than average oxygen content is 1-5% oxygen.
- 166. The system of claim 148, wherein the liquid comprises a cell culture media.
- 167. The system of claim 148, wherein the liquid comprises phosphate buffered saline (PBS).
- 168. The system of claim 148, wherein the liquid comprises serum, Lactated Ringer's solution, 0.9% sodium chloride, sterile water, or 5% dextrose solution.
- 169. The system of claim 148, wherein the liquid comprises a cytokine.
- 170. The system of claim 148, wherein the liquid comprises fms-like tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO).
- 171. The system of claim 148, wherein the liquid comprises IL-2, IL-7, and IL-15.
- 172. The system of claim 149, wherein the vector is within the liquid.
- 173. The system of claim 148, wherein the foam precursor is within the liquid.
- 174. The system of claim 149, wherein the vector and the foam precursor are within the liquid.
- 175. The system of claim 148, wherein the formed foam has an initial mean bubble count of 80/mm² to 110/mm².
- 176. The system of claim 148, wherein the formed foam has an initial mean bubble count of 90/mm² to 100/mm².
- 177. The system of claim 148, wherein the formed foam has an initial mean bubble count of or 95/mm².
- 178. The system of claim 148, wherein the formed foam has an initial mean bubble count of 8,000 μ m² to 13,000 μ m².
- 179. The system of claim 148, wherein the formed foam has an initial mean bubble count of 9,000 μ m² to 12,000 μ m².
- 180. The system of claim 148, wherein the formed foam has an initial mean bubble count of 10,000 μ m² to 11,000 μ m².
- 181. The system of claim 148, wherein the formed foam has an initial mean bubble count of 10,528 μ m².
- 182. The system of claim 148, wherein after 10 hours at room temperature the formed foam has a bubble count of 15/mm² to 35/mm².
- 183. The system of claim 148, wherein after 10 hours at room temperature the formed foam has a bubble count of 20/mm² to 32/mm².

184. The system of claim 148, wherein after 10 hours at room temperature the formed foam has a bubble count of $26/\text{mm}^2 \pm 6.7/\text{mm}^2$.
185. The system of claim 148, wherein after 10 hours at room temperature the formed foam has a mean bubble area of $30,000\ \mu\text{m}^2$ to $55,000\ \mu\text{m}^2$.
186. The system of claim 148, wherein after 10 hours at room temperature the formed foam has a mean bubble area of $32,000\ \mu\text{m}^2$ to $52,000\ \mu\text{m}^2$.
187. The system of claim 148, wherein after 10 hours at room temperature the formed foam has a mean bubble area of $41,597\ \mu\text{m}^2$.
188. The system of claim 148, wherein the nucleic acid encodes a therapeutic molecule.
189. The system of claim 188, wherein the therapeutic molecule is cytotoxic.
190. The system of claim 188, wherein the therapeutic molecule restores a physiological function of a cell.
191. The system of claim 188, wherein the therapeutic molecule comprises a recombinant protein.
192. The system of claim 148, wherein the nucleic acid encodes a vaccine antigen, a genome editing agent, a cytosolic protein, a transmembrane protein, or a secreted protein.
193. The system of claim 192, wherein the cytosolic protein comprises a transcription factor or a suicide gene.
194. The system of claim 192, wherein the transmembrane protein comprises a T cell receptor (TCR), a chimeric antigen receptor (CAR), a cytokine receptor, a costimulatory ligand, or a death receptor.
195. The system of claim 192, wherein the secreted protein comprises a chemoattractant, an antibody, a multispecific binding molecule or a growth factor.
196. The system of claim 195, wherein the multispecific binding molecule activates an immune cell.
197. The system of claim 148, wherein the nucleic acid encodes IRF5, HOXA3, beta-globin, CXCR4, CCR5, IL-10, or siRNA targeting GATA4.
198. The system of claim 148, wherein the nucleic acid encodes PTEN, TRAIL, SMAC, TNF- α , P53, or PLAA.
199. The system of claim 148, wherein the nucleic acid encodes a bacterial toxin.
200. The system of claim 199, wherein the bacterial toxin is produced by *Neisseria meningitidis*, *Escherichia coli* (E.coli), *Pseudomonas*, *Proteus*, *Enterobacter*, or *Klebsiella*.
201. The system of claim 199, wherein the bacterial toxin comprises *Pseudomonas* exotoxin A.

202. The system of claim 148, wherein the system further comprises a sugar.
203. The system of claim 202, wherein the sugar comprises sucrose, maltose, maltodextrin, or trehalose.
204. The system of claim 148, wherein the system further comprises a binding domain that binds that binds an antigen expressed by a cell.
205. The system of claim 204, wherein the cell is an immune cell.
206. The system of claim 205, wherein the immune cell is a lymphocyte.
207. The system of claim 206, wherein the lymphocyte is a T cell or an NK cell.
208. The system of claim 207, wherein the antigen expressed by the T cell comprises CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, 4-1BB (CD137), OX40, lymphocyte function-associated antigen-1 (LFA-1), LIGHT, NKG2C, or B7-H3
209. The system of claim 204, wherein the binding domain is a binding fragment of OKT3, otelixizumab, teplizumab, visilizumab, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, or TR66.
210. The system of claim 204, wherein the cell is a stem cell.
211. The system of claim 210, wherein the antigen expressed by the stem cell is CD34, CD90, or CD117.
212. The system of claim 210, wherein the stem cell is a hematopoietic stem cell or an induced pluripotent stem cell (iPSC).
213. The system of claim 204, wherein the cell is a cell that has been differentiated from a hematopoietic stem cell or an iPSC.
214. The system of claim 204, wherein the cell is a cell that has been differentiated from a hematopoietic stem cell or an iPSC ex vivo.
215. The system of claim 204, wherein the cell is a cancer cell or an infected cell.
216. The system of claim 215, wherein the antigen expressed by the cancer cell comprise A33, BAGE, Bcl-2, β -catenin, CA125, CA19-9, CD5, CD19, CD20, CD21, CD22, CD33, CD37, CD45, CD123, CEA, c-Met, CS-1, cyclin B1, DAGE, EBNA, EGFR, ephrinB2, estrogen receptor, FAP, ferritin, folate-binding protein, GAGE, G250, GD-2, GM2, gp75, gp100 (Pmel 17), HER-2/neu, HPV E6, HPV E7, Ki-67, LRP, mesothelin, p53, PRAME, progesterone receptor, PSA, PSMA, MAGE, MART, mesothelin, MUC, MUM-1-B, myc, NYESO-1, ras, RORI, survivin, tenascin, TSTA tyrosinase, VEGF, or WT1.
217. The system of claim 204, wherein the binding domain is a binding fragment of R11, R12, 2A2, Y31, FMC63, SJ25C1, HD37, Rituximab, Ofatumumab, 2B8, 4D5, 3G10, 32716, or 32703.
218. The system of claim 204, wherein the binding domain is linked to a sugar and/or a

methycellulose or derivative thereof.

- 219. The system of claim 204, wherein the binding domain is linked to xanthum.
- 220. The system of claim 204, wherein the binding domain is linked to a vector.
- 221. The system of claim 220, wherein the vector is a viral vector or a nanoparticle.
- 222. The system of claim 148, further comprising a transduction enhancer.
- 223. The system of claim 222, wherein the transduction enhancer comprises dimethyl sulfoxide (DMSO), polybrene, or protamine sulfate.
- 224. The system of claim 148, wherein the foam is self-expanding.
- 225. The system of claim 224, wherein the self-expanding foam comprises an oxygen releasing biomaterial.
- 226. The system of claim 224, wherein the self-expanding foam comprises a perfluorocarbon.
- 227. The system of claim 224, wherein the self-expanding foam comprises sodium percarbonate, calcium peroxide, magnesium peroxide, hydrogen peroxide, or a fluorinated compound.
- 228. The system of claim 224, wherein the self-expanding foam comprises 1-15% hydrogen peroxide.
- 229. The system of claim 224, wherein the self-expanding foam comprises 1-6% hydrogen peroxide.
- 230. The system of claim 224, wherein the self-expanding foam comprises 2-5% hydrogen peroxide.
- 231. The system of claim 224, wherein the self-expanding foam comprises 3.75% hydrogen peroxide.
- 232. The system of claim 224, wherein the self-expanding foam comprises 0.0025 – 0.03% potassium iodide.
- 233. The system of claim 224, wherein the self-expanding foam comprises 0.01 – 0.02% potassium iodide.
- 234. The system of claim 224, wherein the self-expanding foam comprises 0.015% potassium iodide.
- 235. The system of claim 148, wherein the system further comprises a syringe.
- 236. The system of claim 148, wherein the system further comprises a first syringe and a second syringe.
- 237. The system of claim 236, wherein the foam precursor and gas are in the first syringe and the nucleic acid and liquid are in the second syringe.
- 238. The system of claim 236, wherein the first syringe and the second syringe are connected.

239. The system of claim 148, wherein the foam is edible or drinkable.
240. The system of claim 148, further comprising a flavoring agent.
241. The system of claim 148, wherein the nucleic acid comprises DNA or RNA.
242. The system of claim 148, wherein the nucleic acid comprises plasmid DNA, minicircle DNA, self-replicating RNA, in vitro transcribed RNA, circular RNA, dogpybone DNA (dbDNA), siRNA, or guide RNA.
243. The system of claim 148, further comprising an anti-CD3 binding domain and/or an anti-CD28 binding domain.
244. The system of claim 148, further comprising a cytokine.
245. The system of claim 244, wherein the cytokine comprises IL-2, IL-7, and/or IL-15.
246. A kit comprising a foam precursor and a liquid and instructions for how to form a foam comprising a nucleic acid using the foam precursor, the liquid, and a gas.
247. The kit of claim 246, wherein the foam precursor comprises cellulose, sodium caseinate, or albumin.
248. The kit of claim 247, wherein the cellulose comprises methylcellulose or a derivative thereof.
249. The kit of claim 248, wherein the derivative thereof comprises sodium carboxymethyl cellulose (SCMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), methyl hydroxyethyl cellulose (MHEC), hydroxypropylmethyl cellulose (HPMC), carboxymethylcellulose, hydroxyethyl methyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose acetate succinate (HPMCAS), hydroxypropyl methylcellulose phthalate (HPMCP), hydroxypropyl methylcellulose succinate (HPMCS), hydroxypropyl methylcellulose trimellitate (HPMCT), hydroxypropyl methylcellulose acetate phthalate (HPMCAP), or hydroxypropyl methylcellulose acetate maleate (HPMCAM)
250. The kit of claim 248, wherein the derivative thereof comprises hydroxypropyl methylcellulose or carboxymethyl cellulose.
251. The kit of claim 246, wherein the gas comprises air, oxygen (O₂), nitrogen (N₂), and inert gasses such as Argon (Ar), Neon (Ne), Radon (Ra), Helium (He), Krypton (Kr), Nitric oxide (NO), or mixtures thereof.
252. The kit of claim 246, wherein the gas has a higher than average oxygen content.
253. The kit of claim 246, wherein the gas has a lower than average oxygen content.
254. The kit of claim 253, wherein the lower than average oxygen content is 1-5% oxygen.
255. The kit of claim 246, wherein the liquid comprises a cell culture media.
256. The kit of claim 246, wherein the liquid comprises phosphate buffered saline (PBS).

257. The kit of claim 246, wherein the liquid comprises serum, Lactated Ringer's solution, 0.9% sodium chloride, sterile water, or 5% dextrose solution.
258. The kit of claim 246, wherein the liquid comprises a cytokine.
259. The kit of claim 246, wherein the liquid comprises fms-like tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO).
260. The kit of claim 246, wherein the liquid comprises IL-2, IL-7, and IL-15.
261. The kit of claim 246, further comprising the nucleic acid.
262. The kit of claim 261, wherein the nucleic acid comprises plasmid DNA, minicircle DNA, self-replicating RNA, in vitro transcribed RNA, circular RNA, dogpybone DNA (dbDNA), siRNA, or guide RNA.
263. The kit of claim 261, wherein the nucleic acid encodes a molecule.
264. The kit of claim 263, wherein the molecule is cytotoxic.
265. The kit of claim 263, wherein the molecule restores a physiological function of a cell.
266. The kit of claim 263, wherein the molecule comprises a recombinant protein.
267. The kit of claim 263, wherein the nucleic acid encodes a vaccine antigen, a genome editing agent, a cytosolic protein, a transmembrane protein, or a secreted protein.
268. The kit of claim 267, wherein the cytosolic protein comprises a transcription factor or a suicide gene.
269. The kit of claim 267, wherein the transmembrane protein comprises a T cell receptor (TCR), a chimeric antigen receptor (CAR), a cytokine receptor, a costimulatory ligand, or a death receptor.
270. The kit of claim 267, wherein the secreted protein comprises a chemoattractant, an antibody, a multispecific binding molecule or a growth factor.
271. The kit of claim 270, wherein the multispecific binding molecule activates an immune cell.
272. The kit of claim 261, wherein the nucleic acid encodes IRF5, HOXA3, beta-globin, CXCR4, CCR5, IL-10, or siRNA targeting GATA4.
273. The kit of claim 261, wherein the nucleic acid encodes PTEN, TRAIL, SMAC, TNF- α , P53, or PLAA.
274. The kit of claim 261, wherein the nucleic acid encodes a bacterial toxin.
275. The kit of claim 274, wherein the bacterial toxin is produced by *Neisseria meningitidis*, *Escherichia coli* (E.coli), *Pseudomonas*, *Proteus*, *Enterobacter*, or *Klebsiella*.
276. The kit of claim 274, wherein the bacterial toxin comprises *Pseudomonas* exotoxin A.
277. The kit of claim 246, further comprising a vector.
278. The kit of claim 277, wherein the vector comprises a lipid.

279. The kit of claim 278, wherein the lipid comprises 2-(Di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethan-1-ol, Dinonyl 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Nonyl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Di(heptadecan-9-yl) 8,8'-((2-hydroxyethyl)azanediyl)dioctanoate, Heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate, Heptadecan-9-yl 8-((2-hydroxyethyl)(4-(nonyloxy)-4-oxobutyl)amino)octanoate, Henicosan-11-yl 6-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)hexanoate, or 8-((8-(Heptadecan-9-yloxy)-8-oxooctyl)(2-hydroxyethyl)amino)octanoic acid.
280. The kit of claim 278, wherein the lipid comprises Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate.
281. The kit of claim 277, wherein the vector comprises a viral vector.
282. The kit of claim 281, wherein the viral vector comprises an oncolytic vector or a lentiviral vector.
283. The kit of claim 282, wherein the oncolytic vector is derived from HSV-1 or HSV-2.
284. The kit of claim 246, further comprising xanthum gum or guar gum.
285. The kit of claim 246, further comprising a flavoring agent.
286. The kit of claim 246, further comprising a sugar.
287. The kit of claim 286, wherein the sugar comprises sucrose, maltose, maltodextrin, or trehalose.
288. The kit of claim 246, further comprising a transduction enhancer.
289. The kit of claim 288, wherein the transduction enhancer comprises dimethyl sulfoxide (DMSO), polybrene, or protamine sulfate.
290. The kit of claim 246, further comprising a binding domain that binds an antigen expressed by a cell.
291. The kit of claim 290, wherein the binding domain is a binding fragment of OKT3, oteixizumab, teplizumab, visilizumab, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, or TR66.
292. The kit of claim 290, wherein the binding domain is a binding fragment of R11, R12, 2A2, Y31, FMC63, SJ25C1, HD37, Rituximab, Ofatumumab, 2B8, 4D5, 3G10, 32716, or 32703.
293. The kit of claim 290, wherein the binding domain is linked to a sugar and/or a methylcellulose or derivative thereof.
294. The kit of claim 290, wherein the binding domain is linked to xanthum gum.

- 295. The kit of claim 290, wherein the binding domain is linked to a vector.
- 296. The kit of claim 295, wherein the vector is a viral vector or a nanoparticle.
- 297. The kit of claim 246, further comprising an oxygen releasing biomaterial.
- 298. The kit of claim 297, wherein the oxygen releasing biomaterial comprises a perfluorocarbon.
- 299. The kit of claim 297, wherein the oxygen releasing biomaterial comprises sodium percarbonate, calcium peroxide, magnesium peroxide, hydrogen peroxide, or a fluorinated compound.
- 300. The kit of claim 297, wherein the oxygen releasing biomaterial comprises hydrogen peroxide.
- 301. The kit of claim 297, wherein the oxygen releasing biomaterial comprises potassium iodide.
- 302. The kit of claim 246, further comprising a syringe.
- 303. The kit of claim 246, further comprising a continuous flow microfluidics system.
- 304. The kit of claim 246, further comprising an anti-CD3 binding domain and/or an anti-CD28 binding domain.
- 305. The kit of claim 246, further comprising a cytokine.
- 306. The kit of claim 305, wherein the cytokine comprises IL-2, IL-7, and/or IL-15.
- 307. The kit of claim 305, wherein the cytokine comprises fms-like tyrosine kinase 3 ligand (Flt3L), stem cell factor (SCF), IL-3, IL-6, and thrombopoietin (TPO).
- 308. A foam comprising methylcellulose or a derivative thereof, a gas, a T-cell targeting lentivirus that encodes a recombinant protein, and peripheral blood mononuclear cells.
- 309. The foam of claim 308, wherein the recombinant protein comprises a chimeric antigen receptor.

1/54

FIG. 1

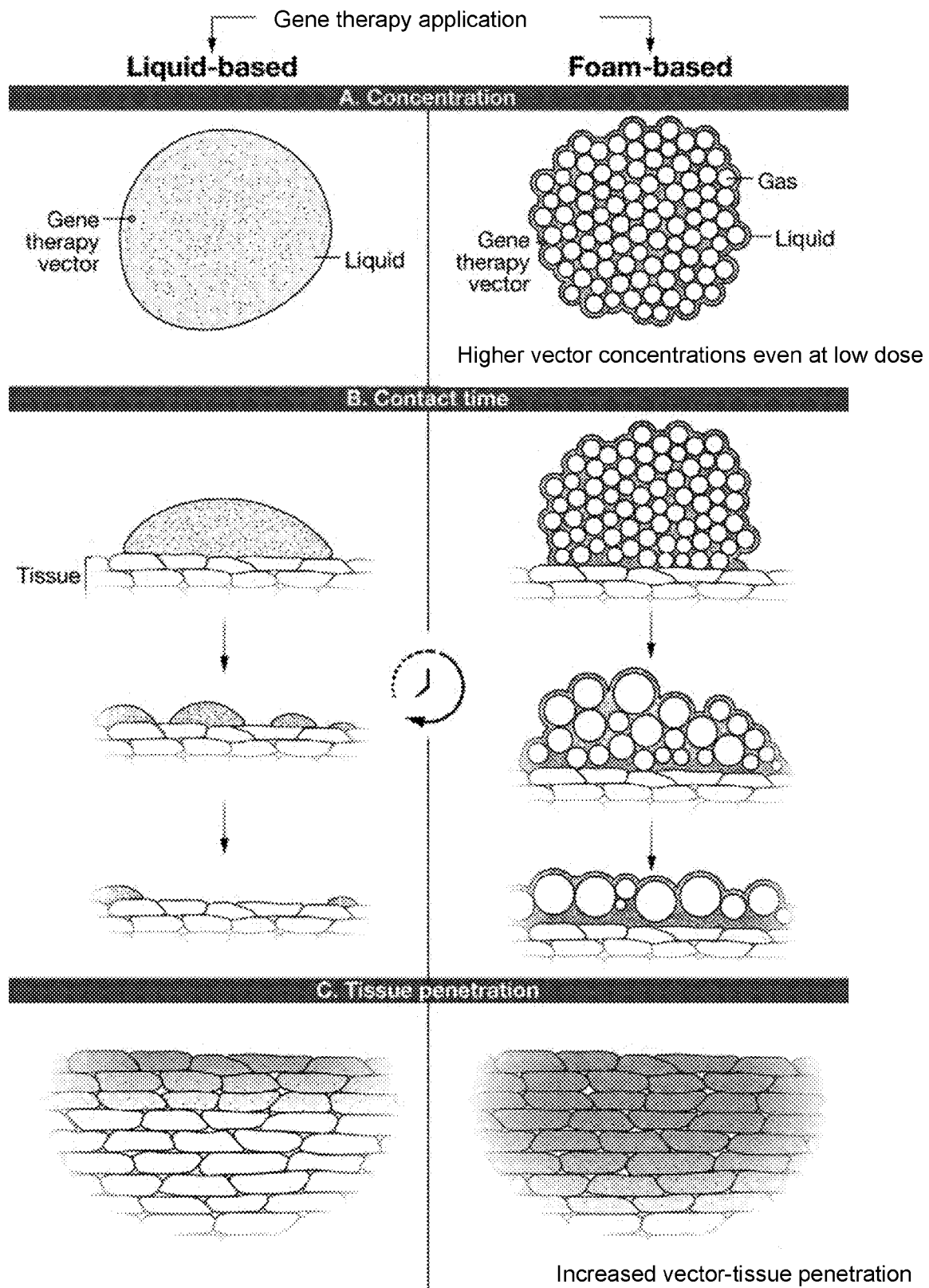
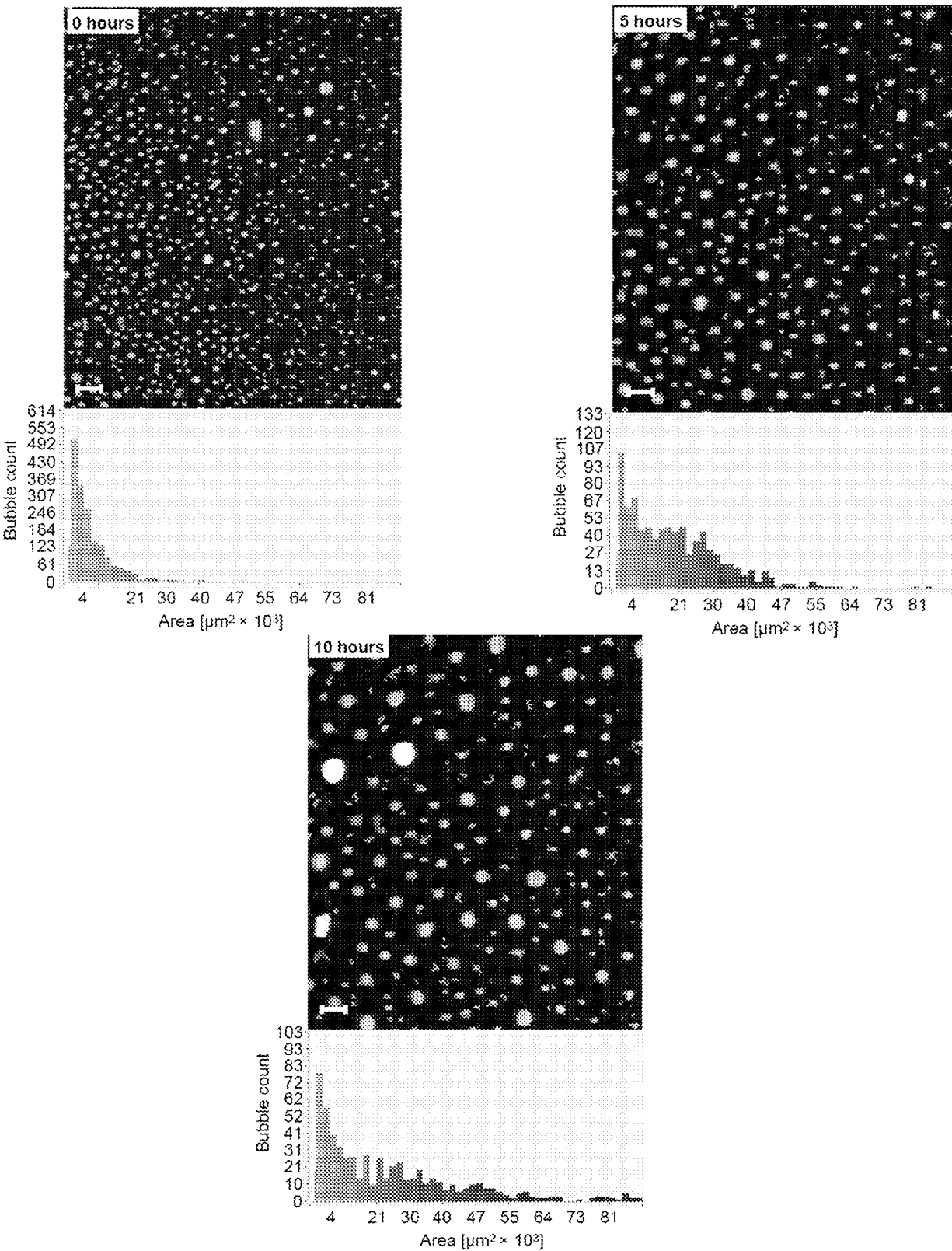


FIG. 2A



3/54

FIG. 2B

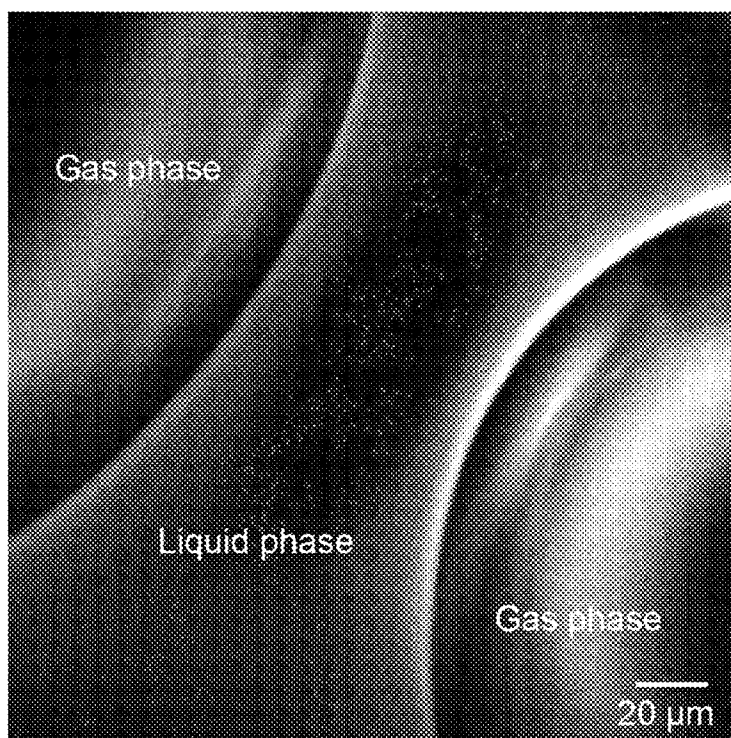


FIG. 2C

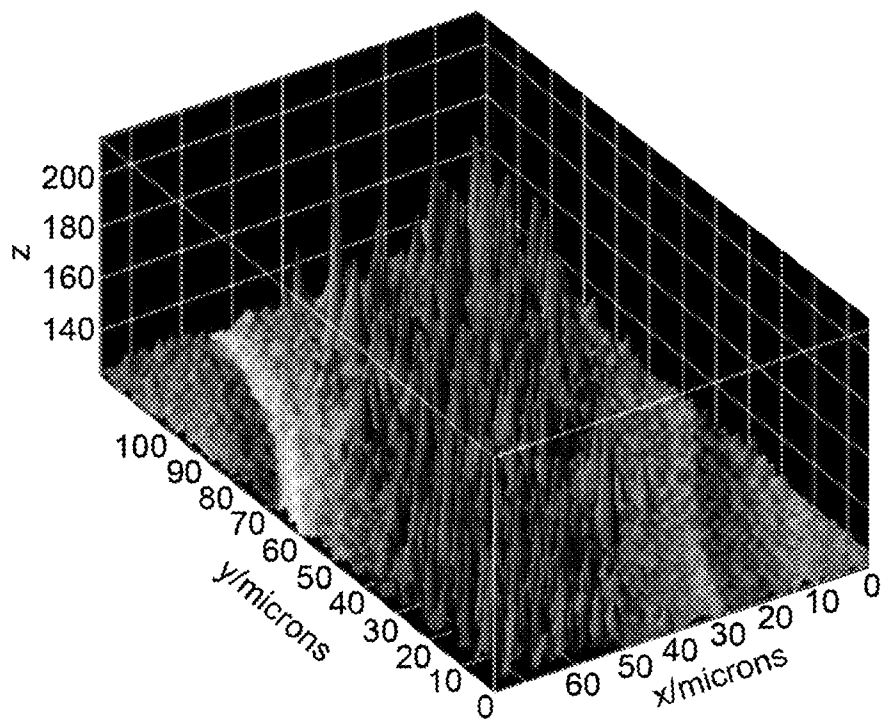


FIG. 2D

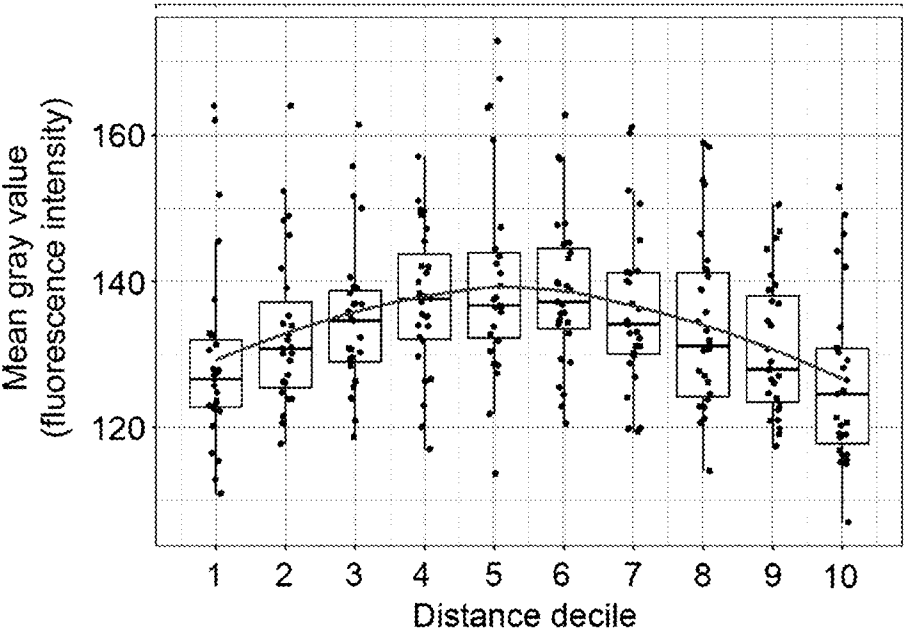


FIG. 3A

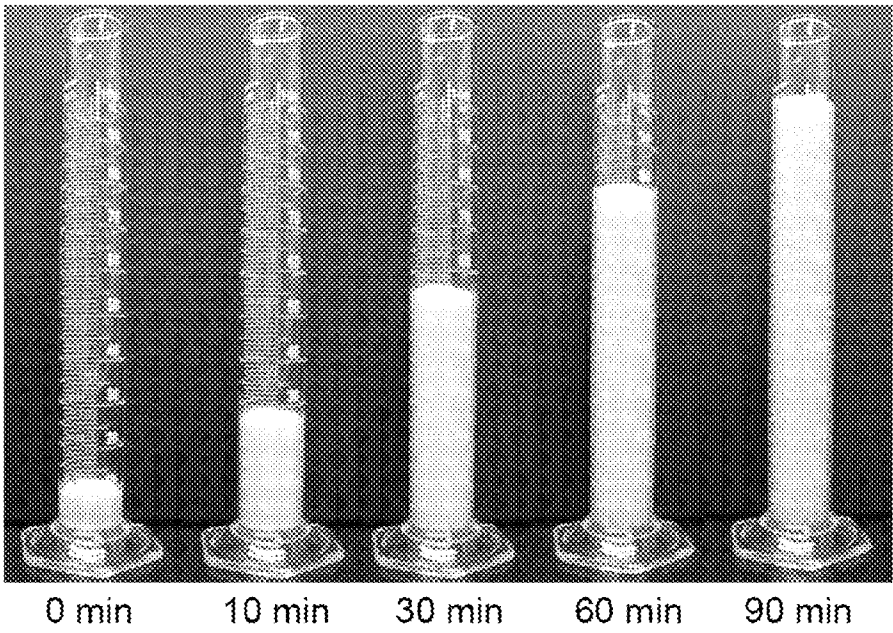


FIG. 3B

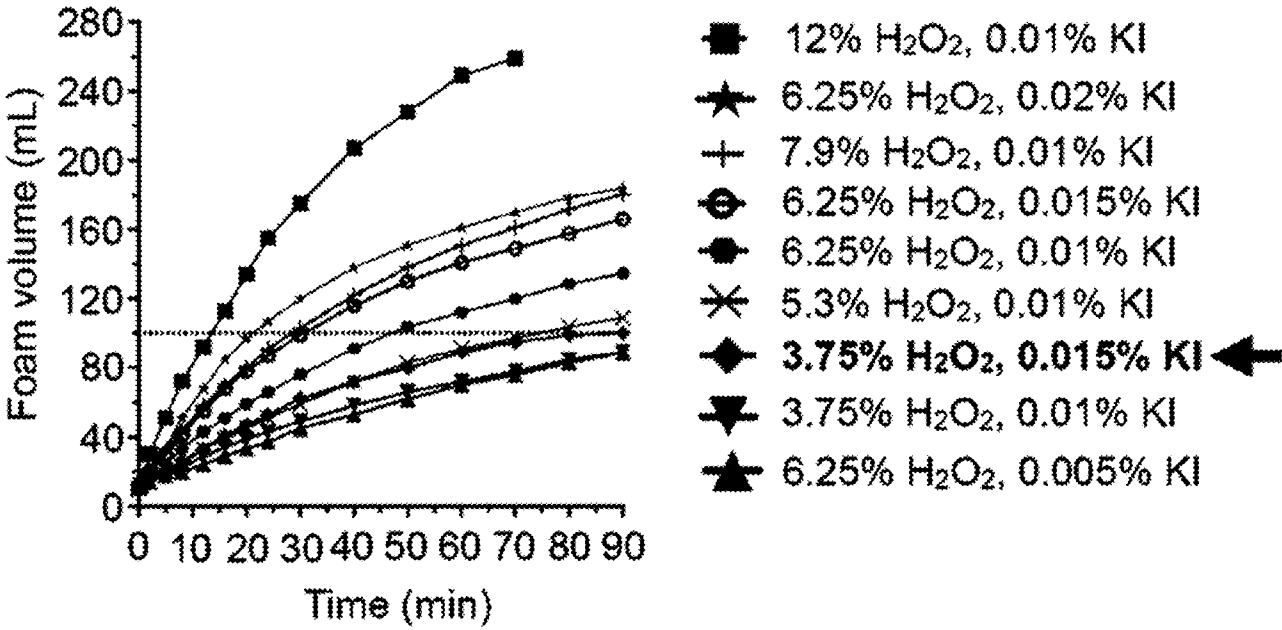


FIG. 4A

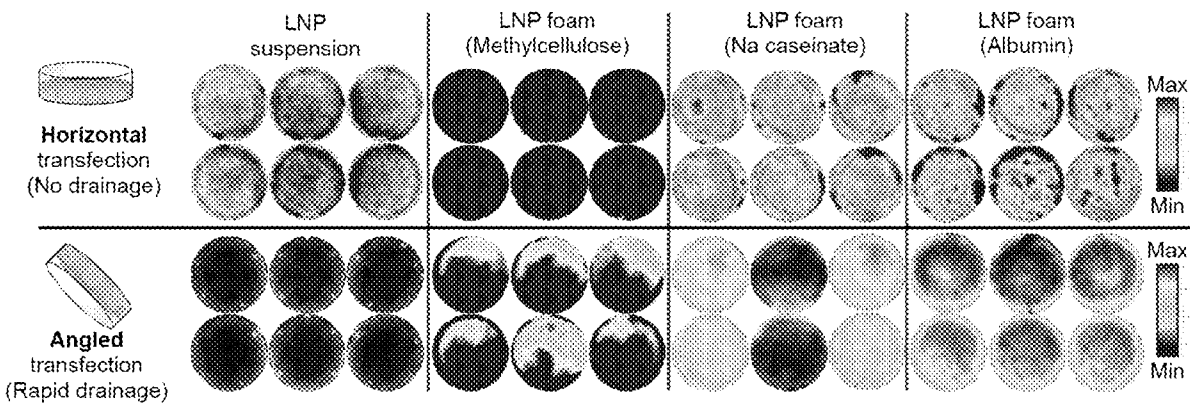
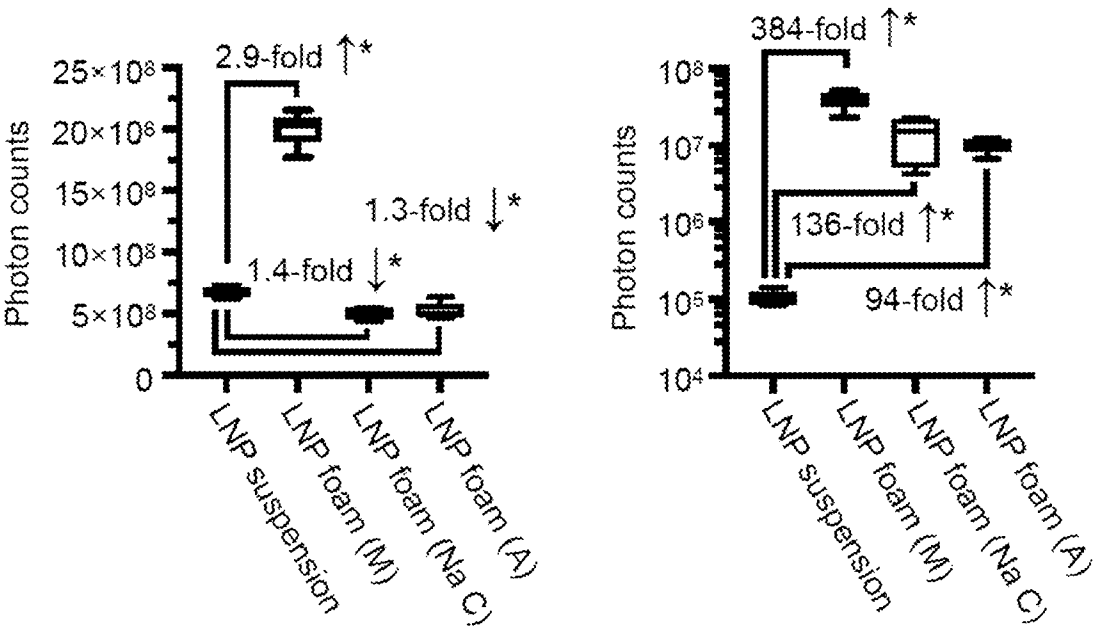


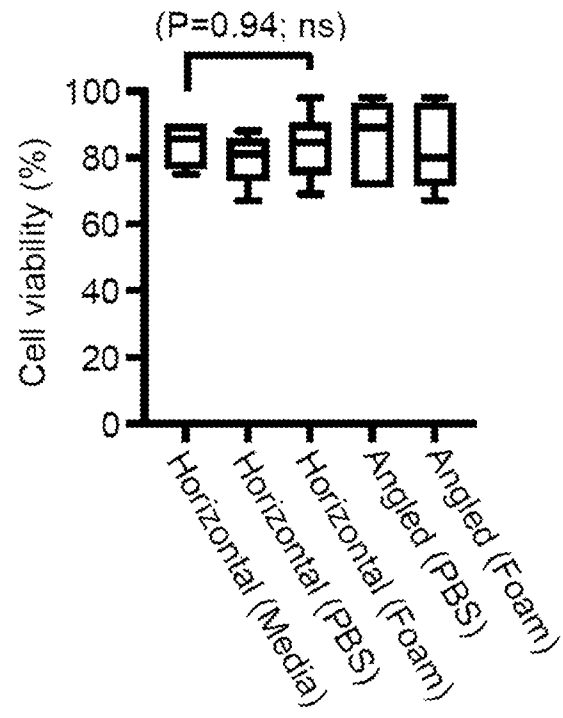
FIG. 4B

FIG. 4C



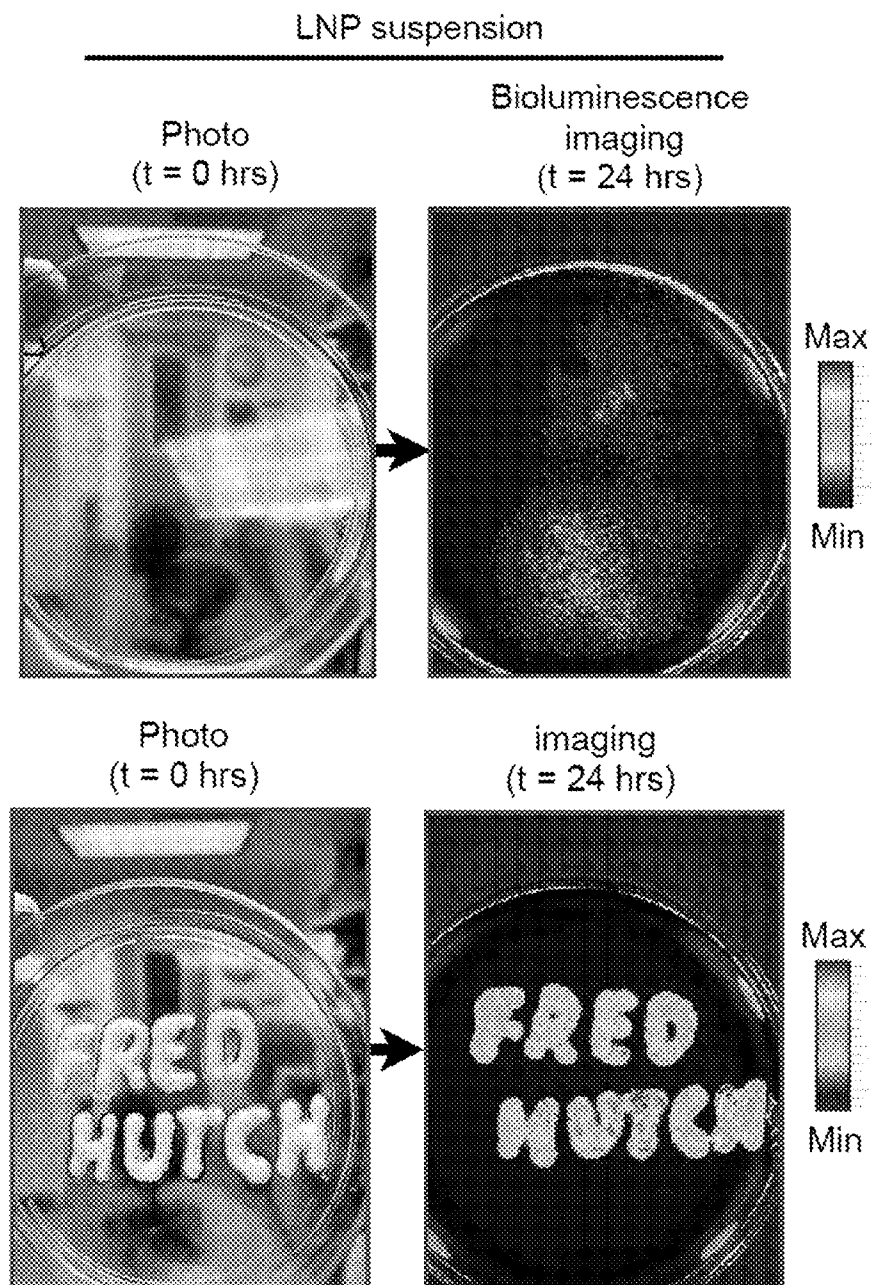
7/54

FIG. 4D



8/54

FIG. 4E



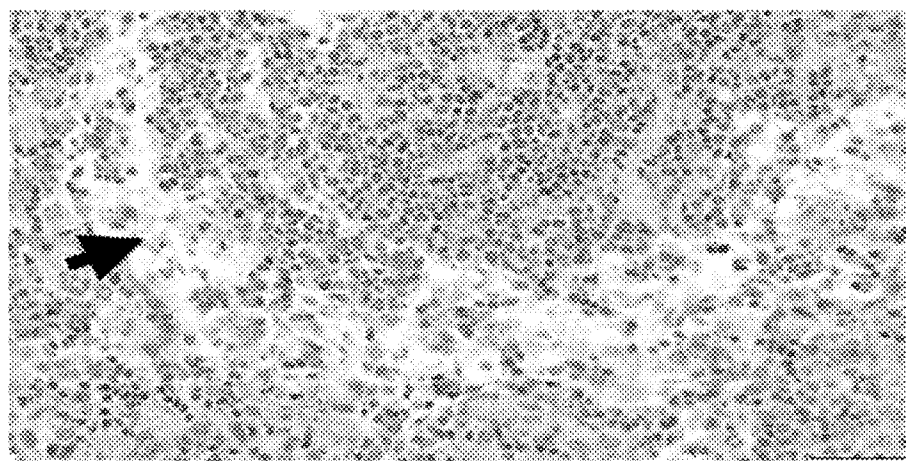
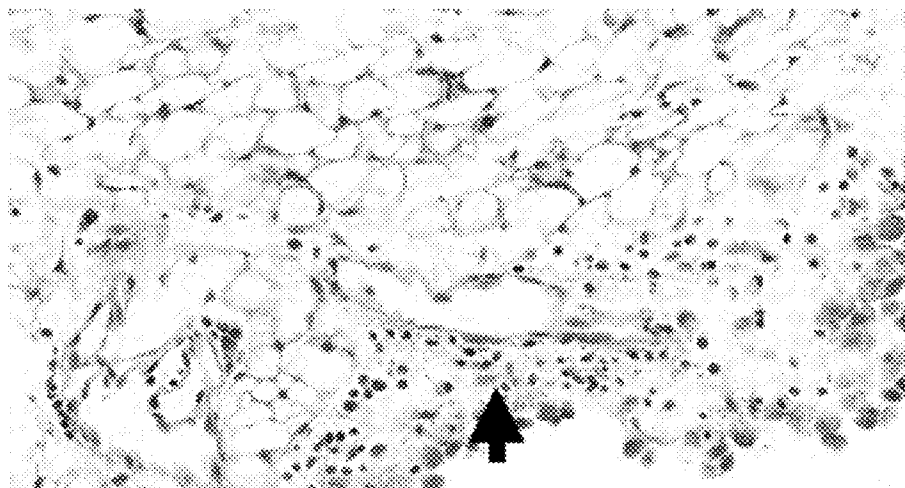
9/54

FIG. 5A

Parameter	Unit	PBS control	Foam
PCV	%	43.8±6.6	46.6±3.2
WBC	K/ μ L	4.2±0.6	5.2±2.0
NEUCT	/ μ L	460±95	667±183
LYMCT	K/ μ L	3.6±0.6	3.8±1.0
MONCT	/ μ L	180±50	317±102
BASCT	/ μ L	43±36	37±22
EOSCT	/ μ L	70±46	60±39
ALP	U/L	113±40	41±6.4
ALT	U/L	38±17	63±55
AST	U/L	212±106	377±281
BUN	mg/dL	21±5	15±3
CREA	mg/dL	0.4±0.1	0.3±0.1
GLU	mg/dL	204±46	166±45

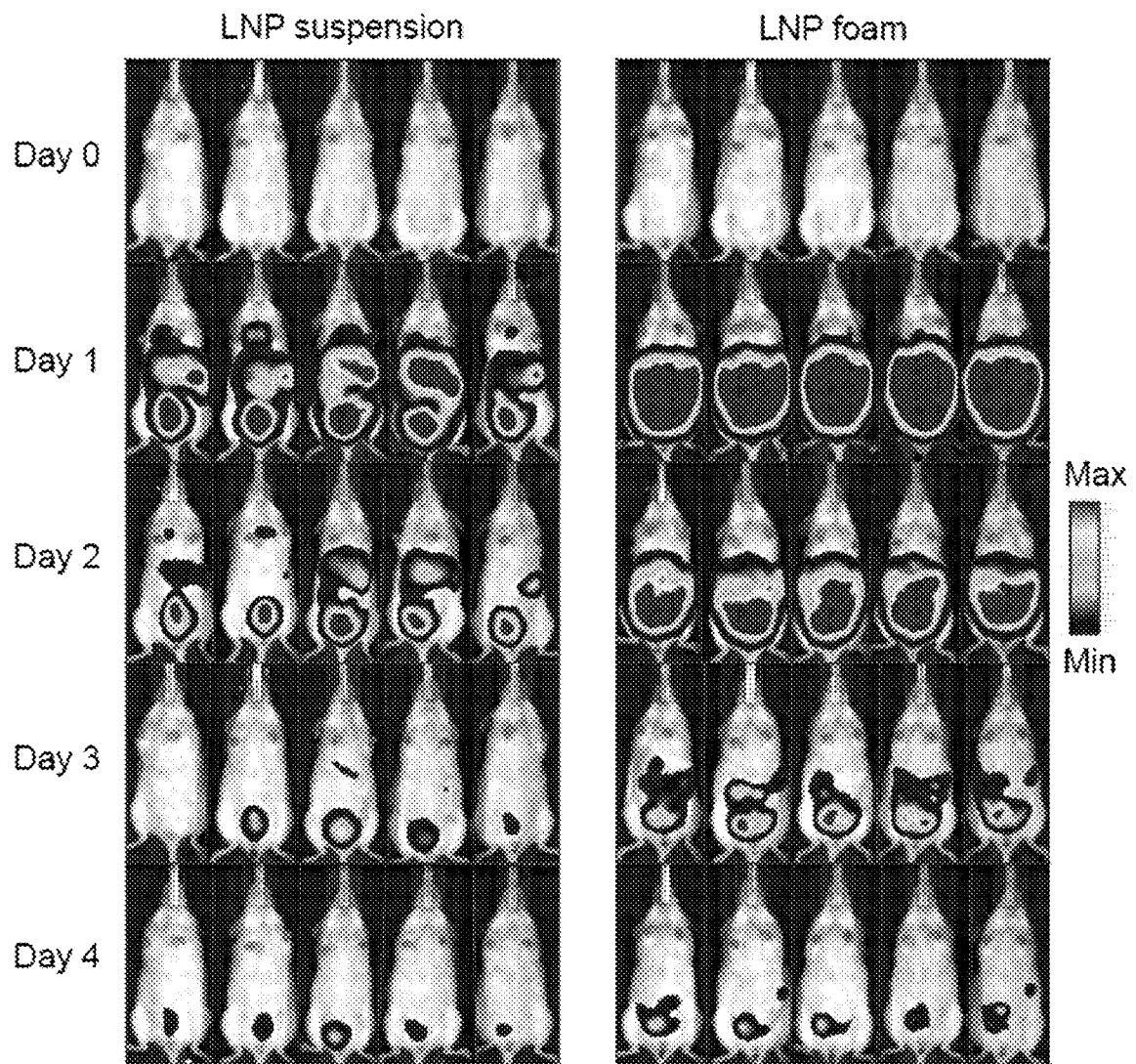
10/54

FIG. 5B



11/54

FIG. 6A



12/54

FIG. 6B

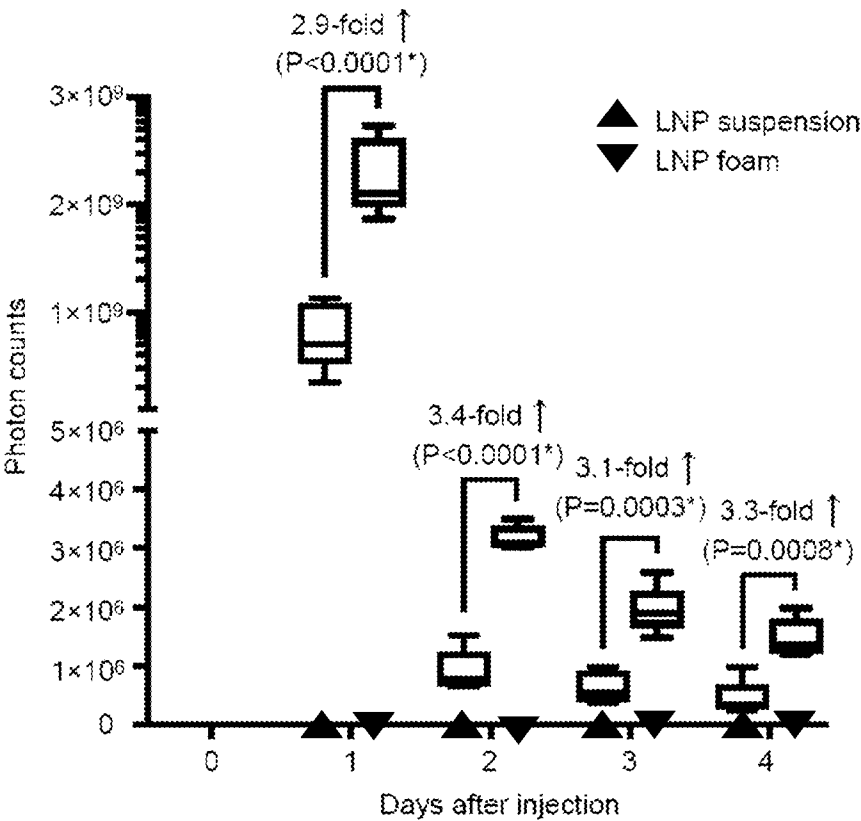


FIG. 7A

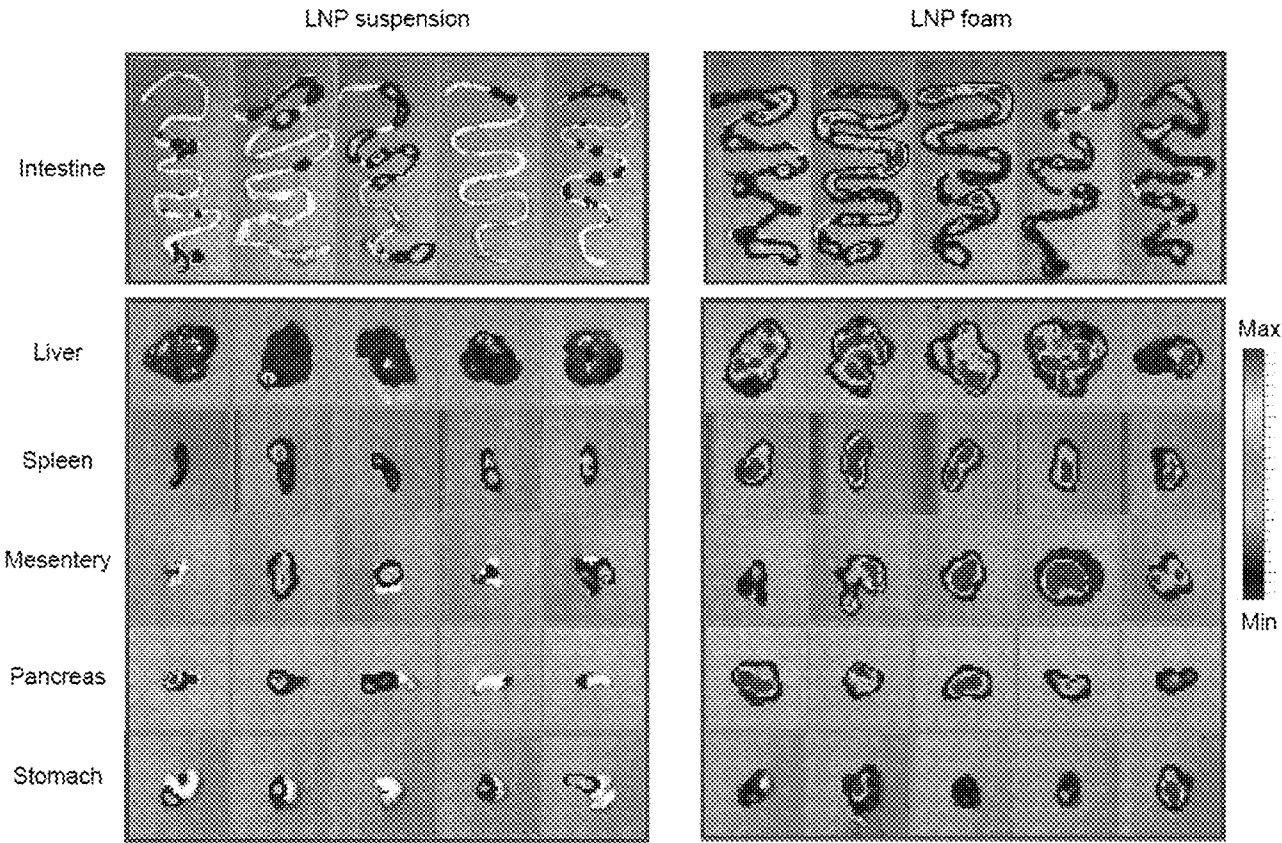
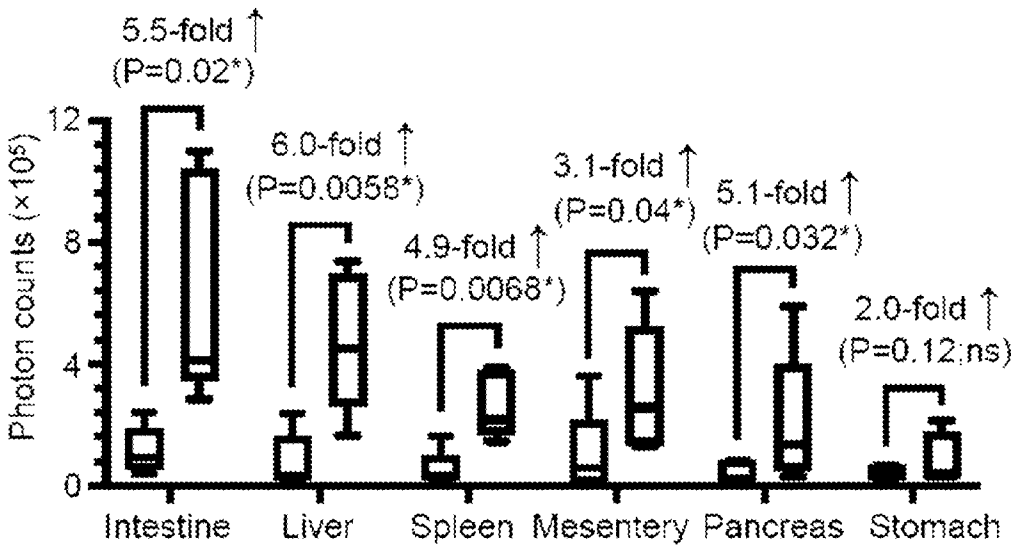


FIG. 7B



14/54

FIG. 8A

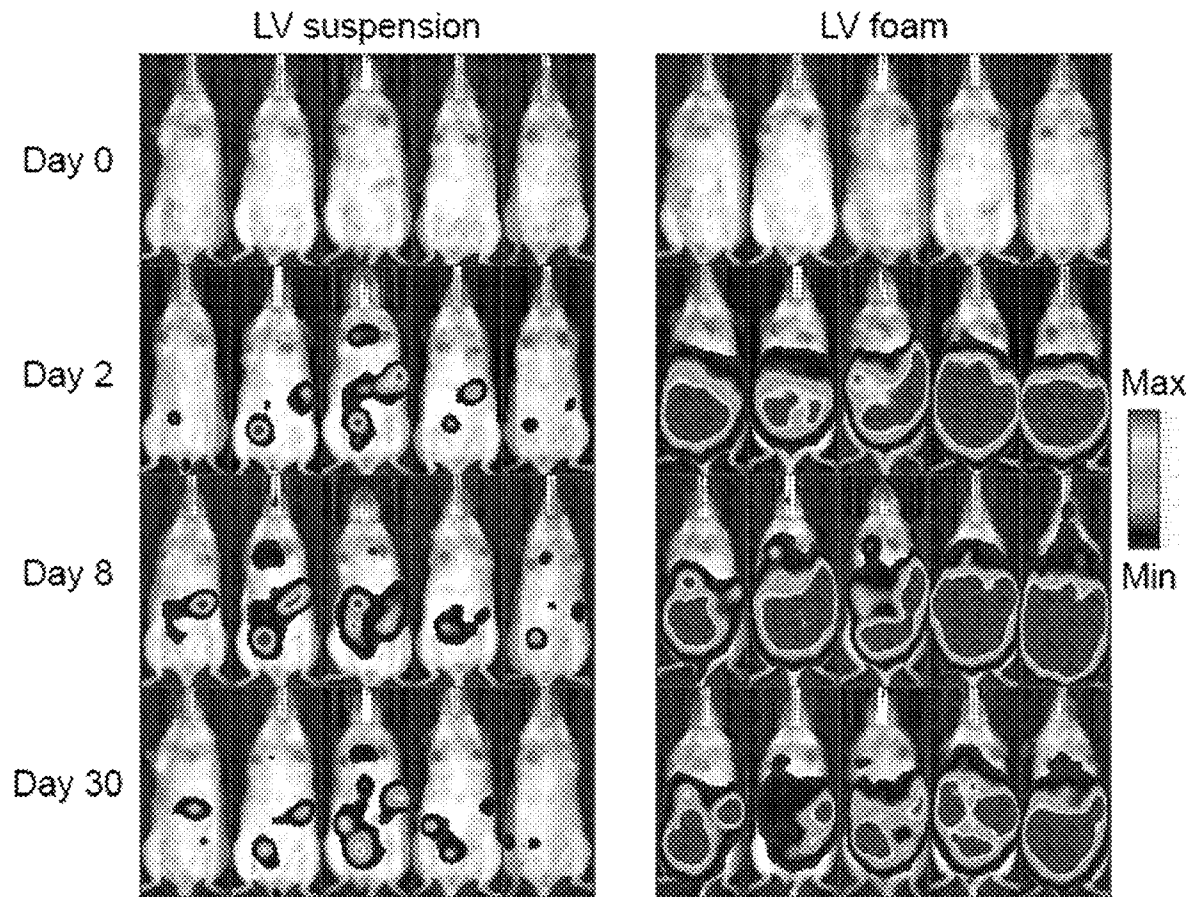
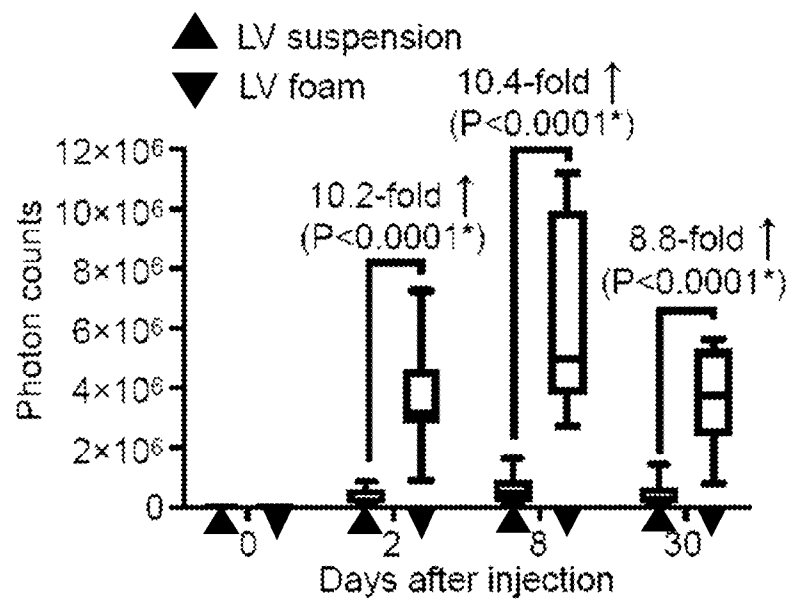
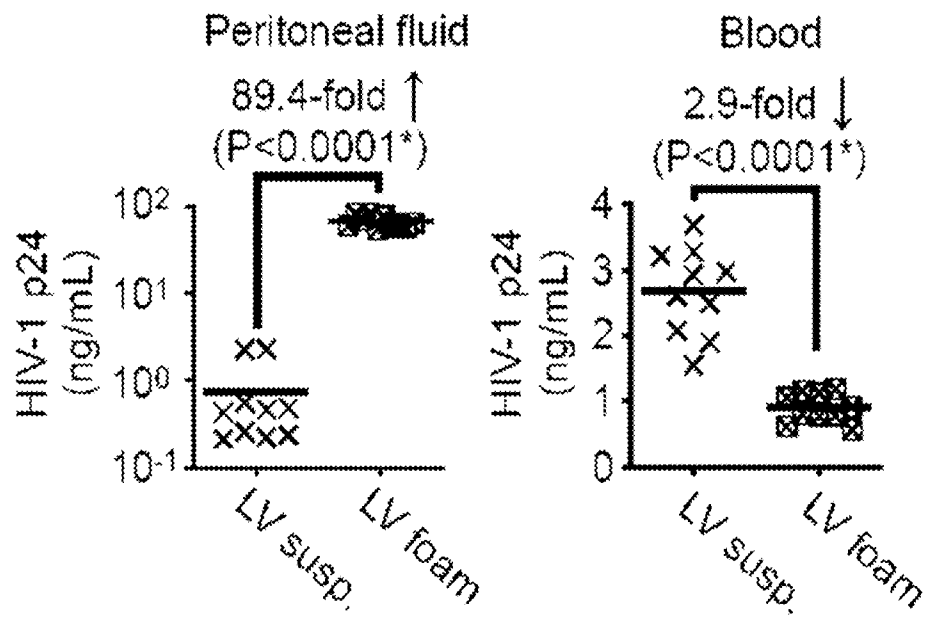


FIG. 8B



15/54

FIG. 8C



16/54

FIG. 9A

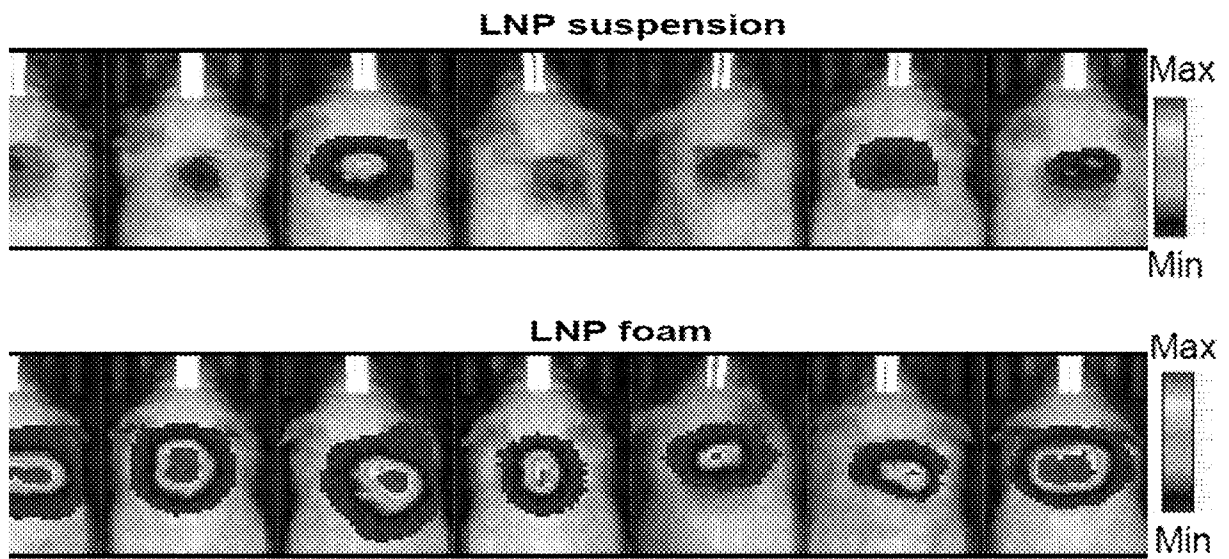
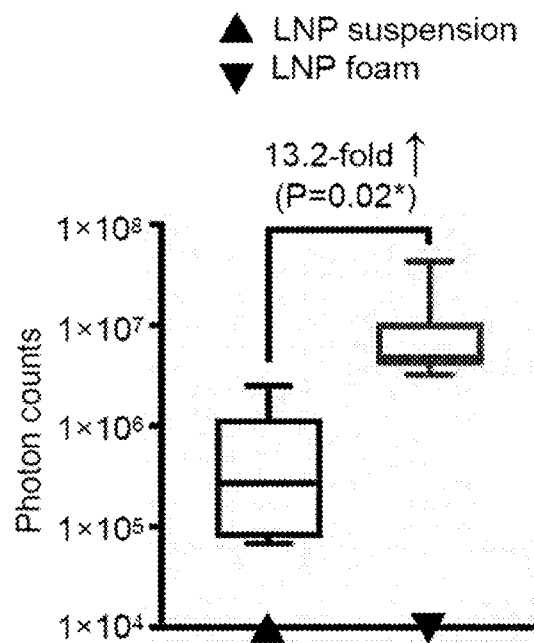
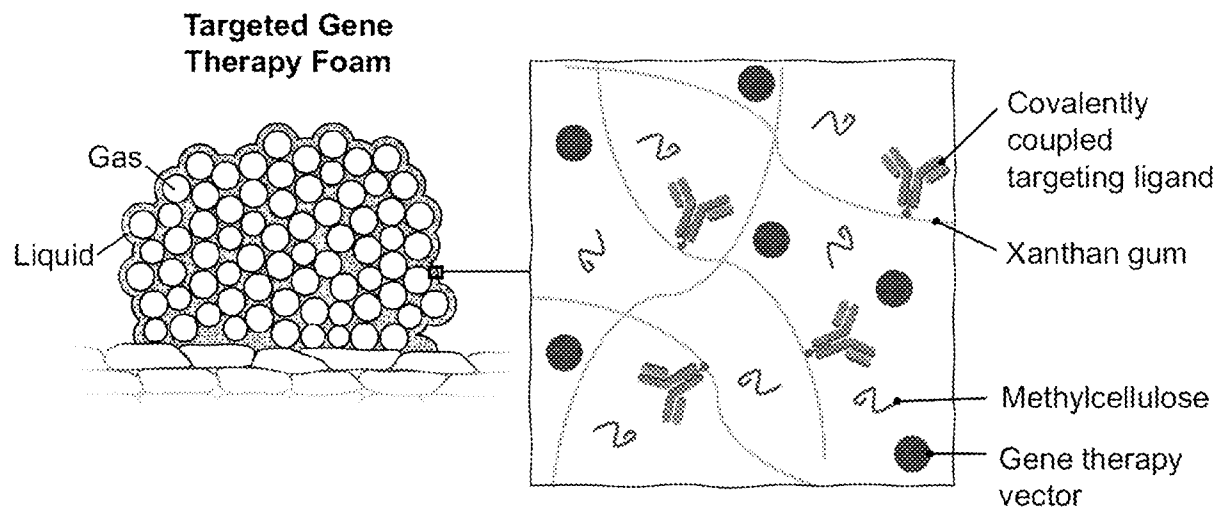


FIG. 9B



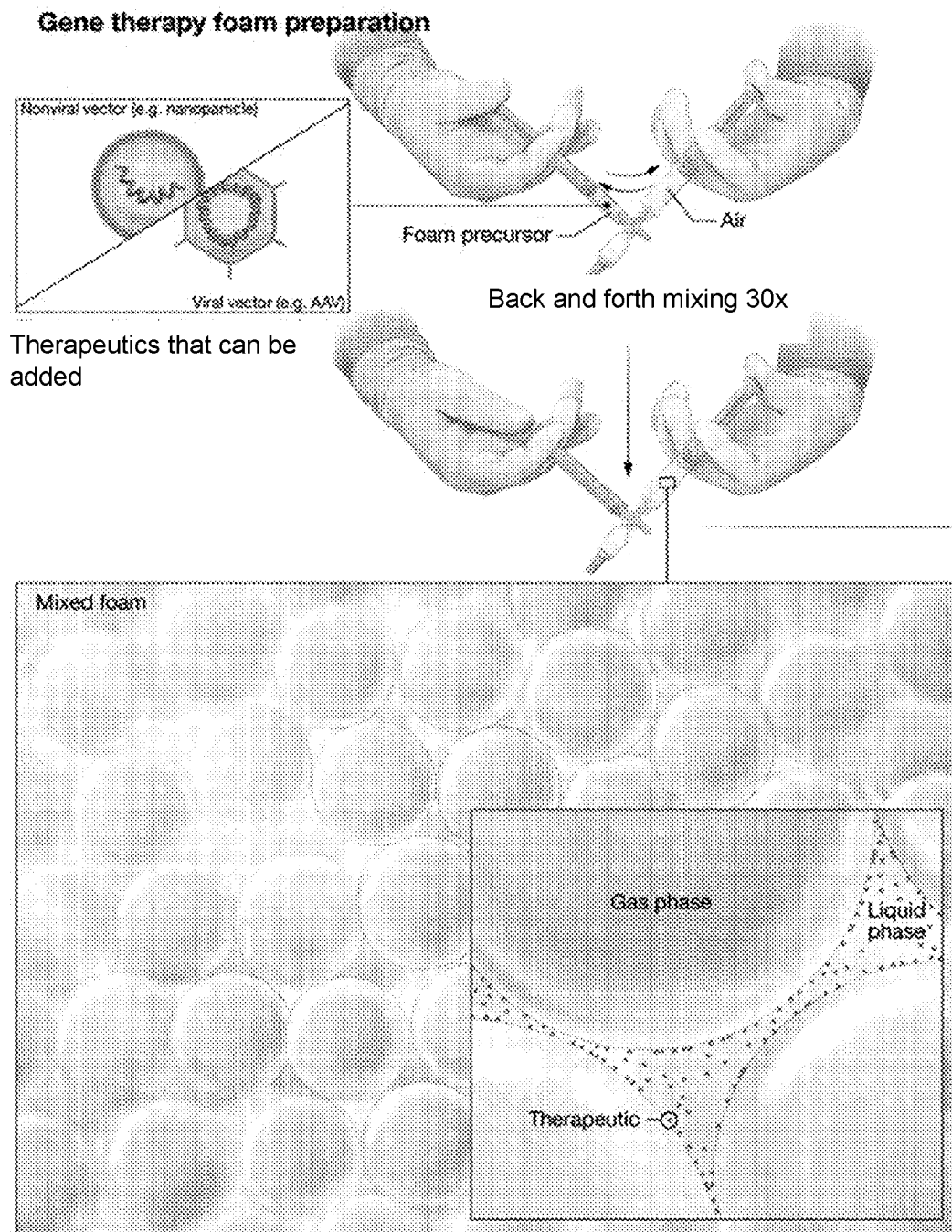
17/54

FIG. 10



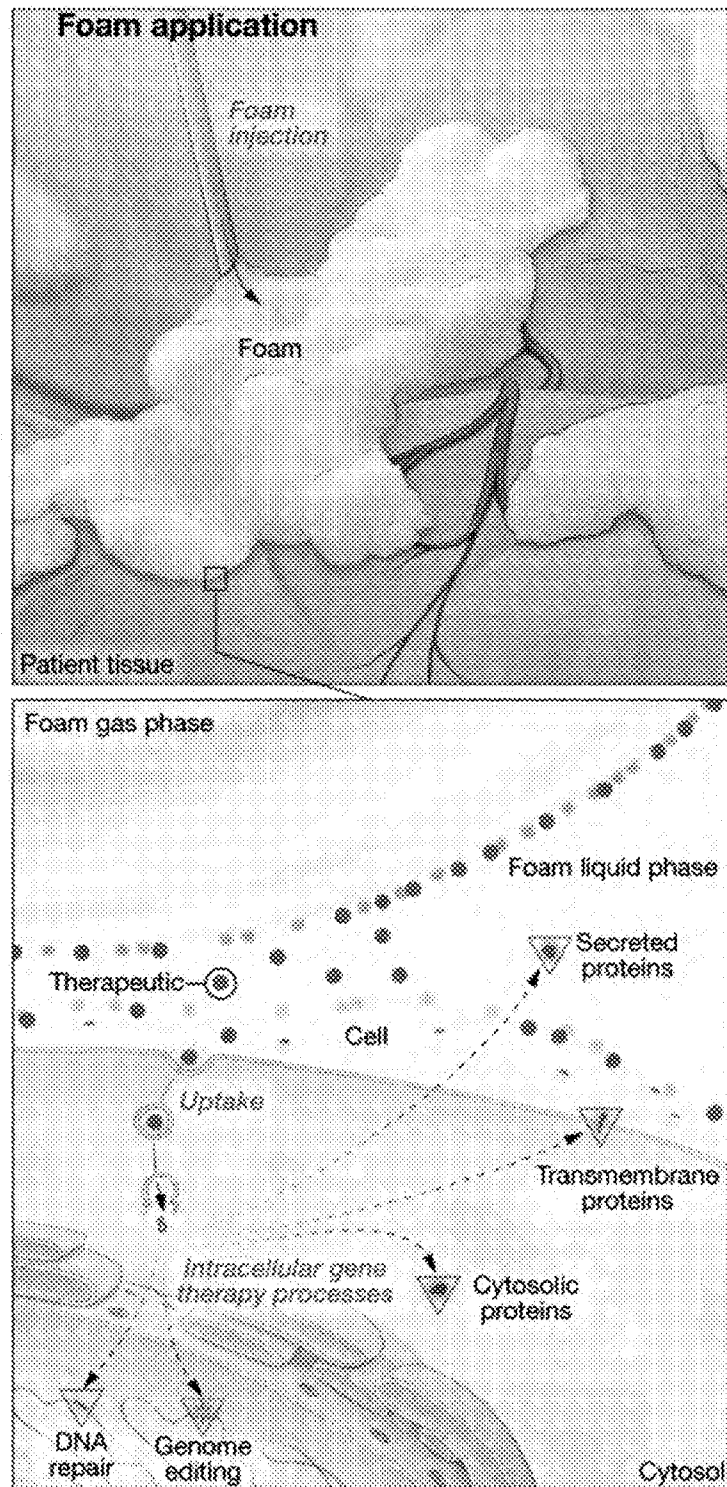
18/54

FIG. 11A



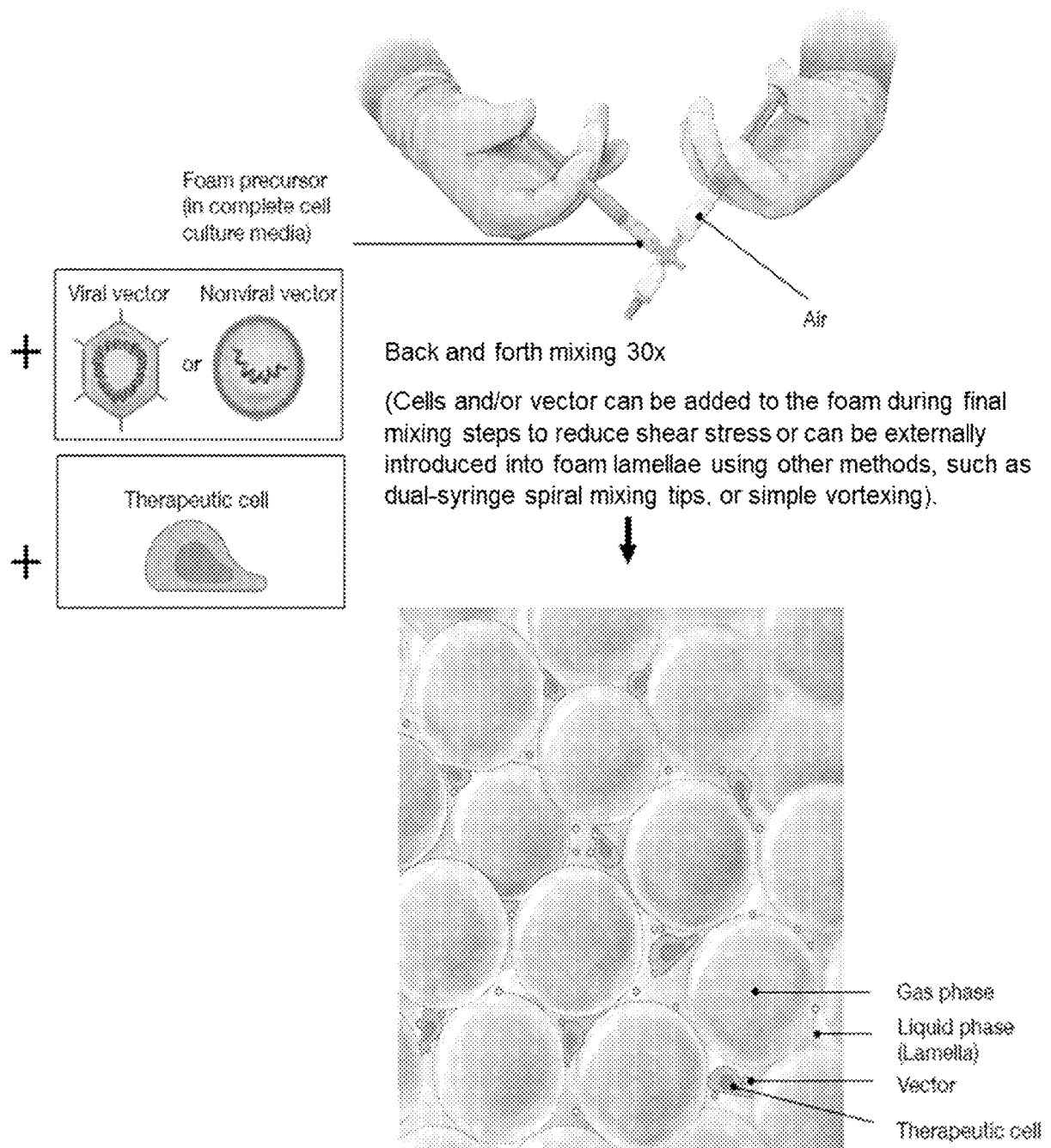
19/54

FIG. 11B



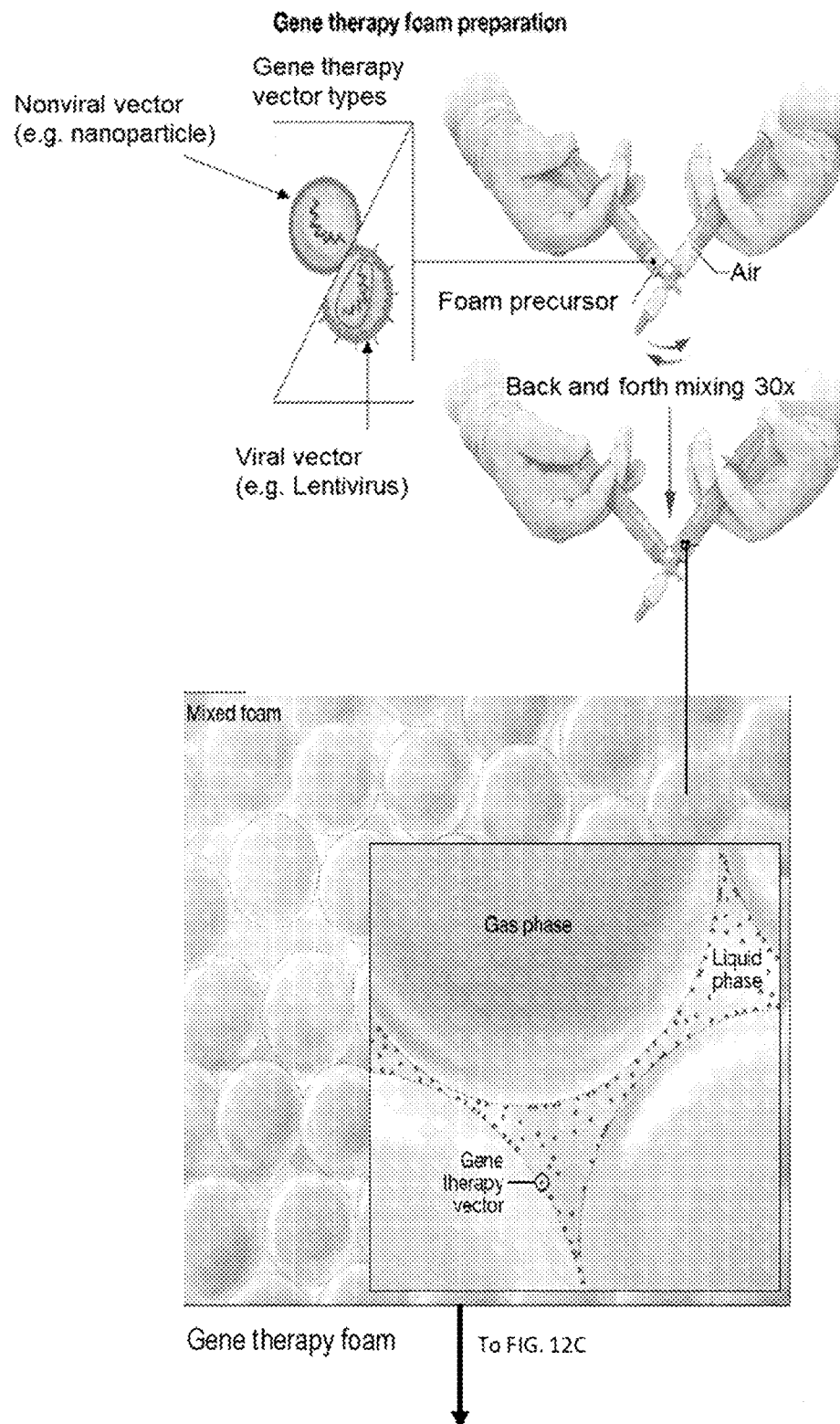
20/54

FIG. 12A



21/54

FIG. 12B



22/54

FIG. 12C

1. Extraction of ex vivo cultured therapeutic cells

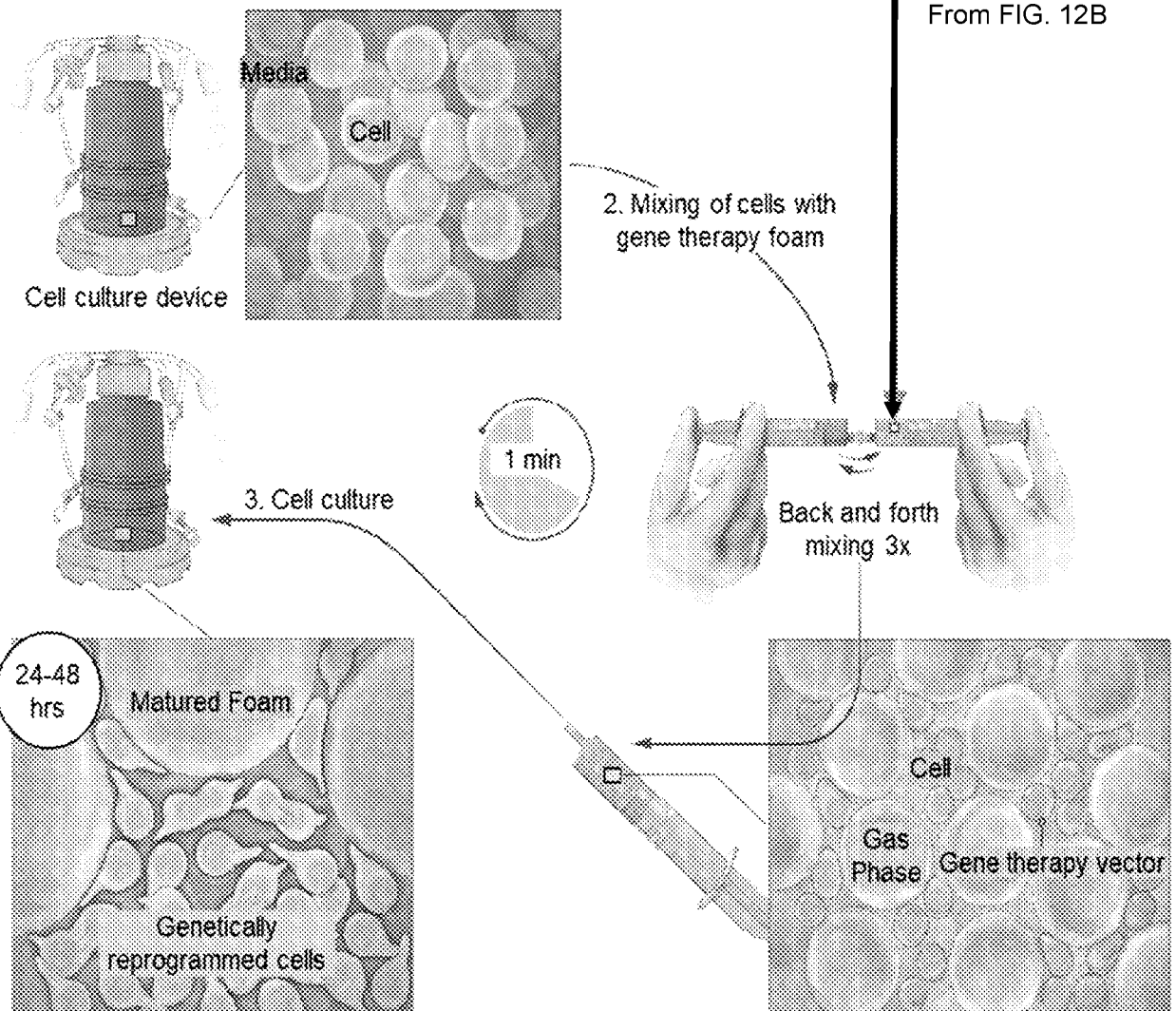
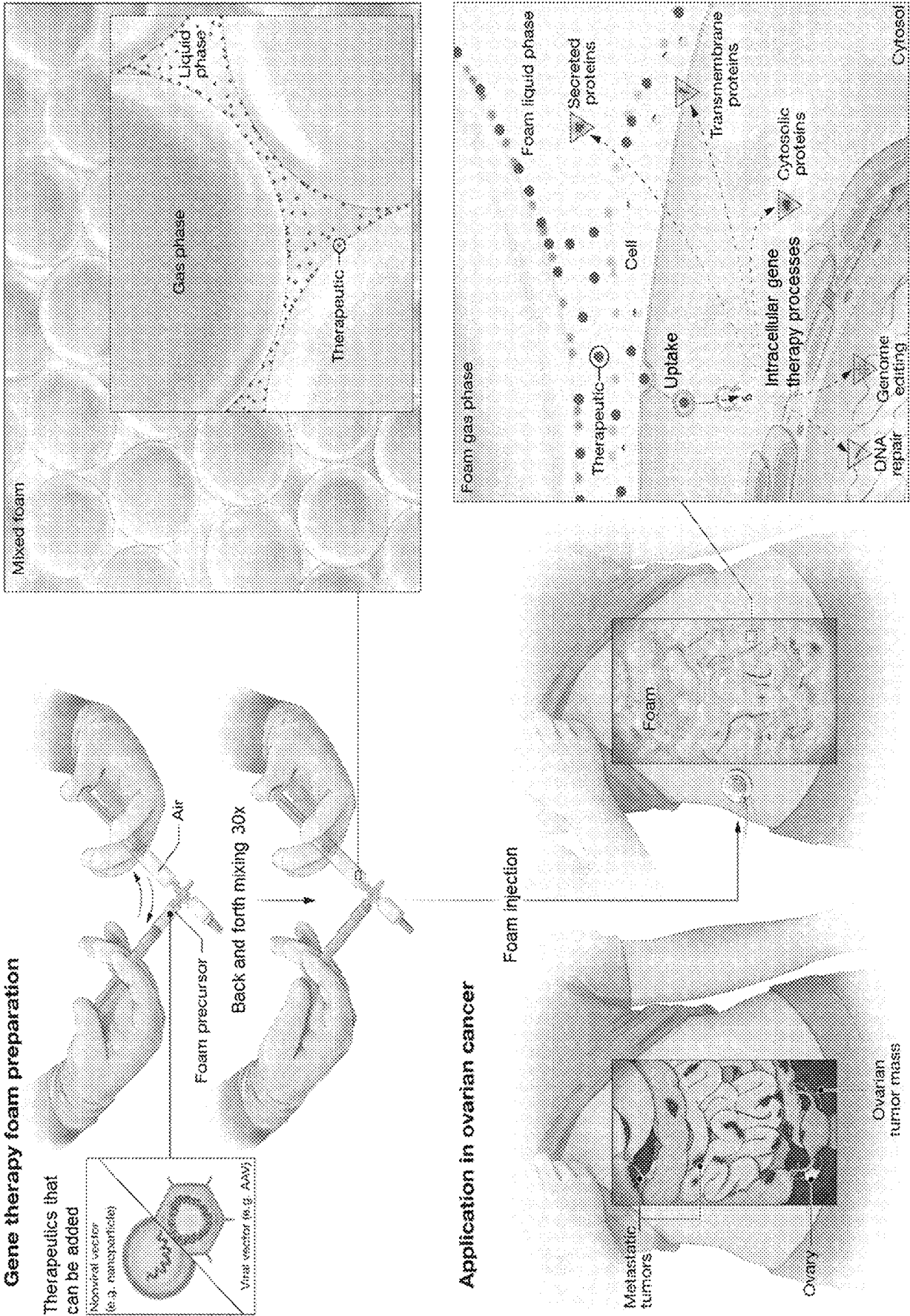


FIG. 13



24/54

FIG. 14A

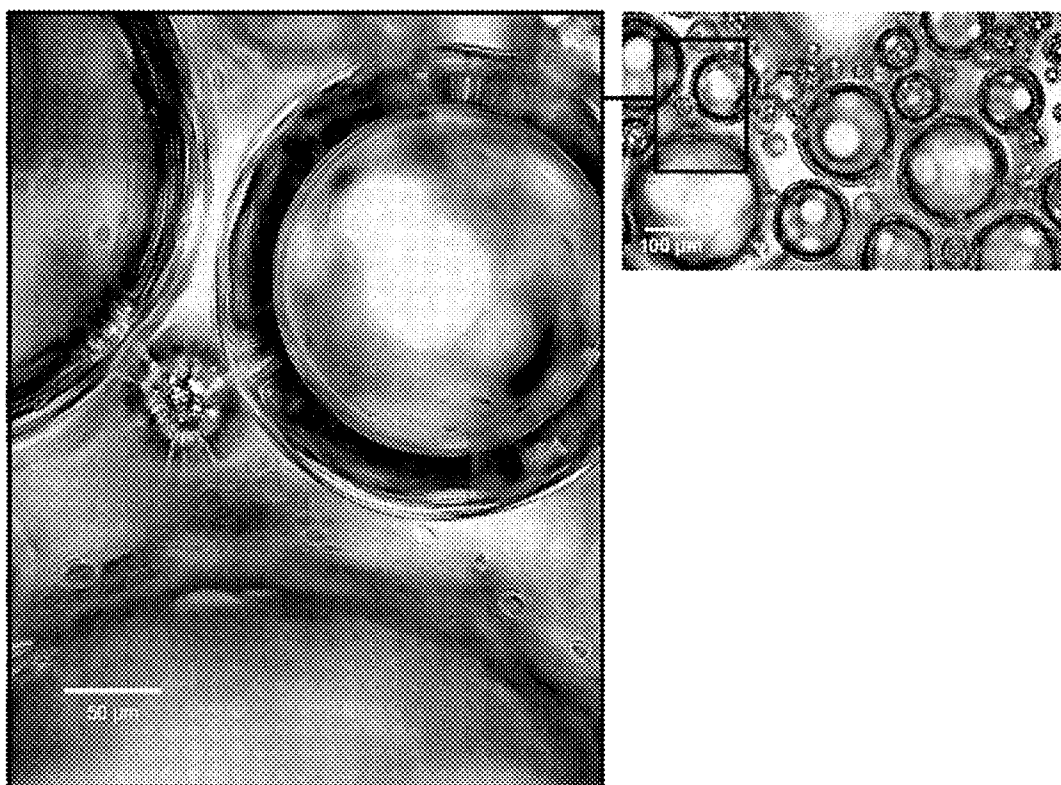
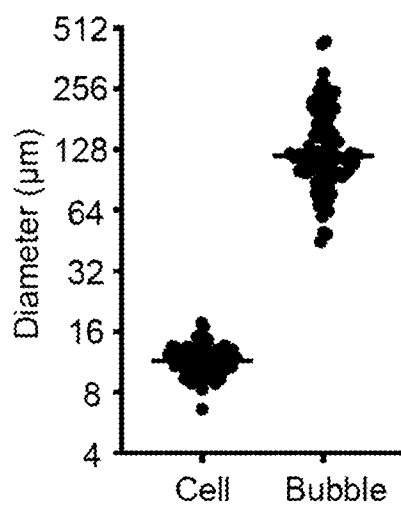


FIG. 14B



25/54

FIG. 14C

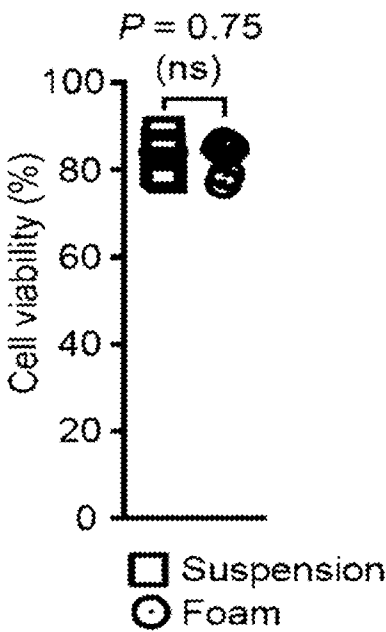
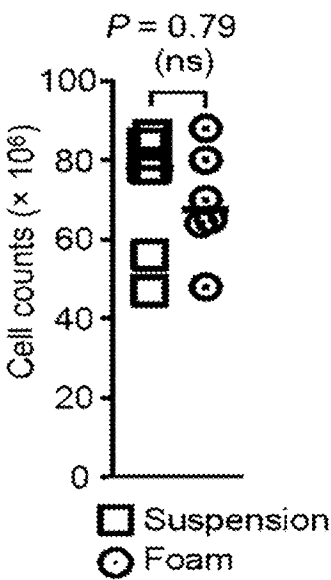
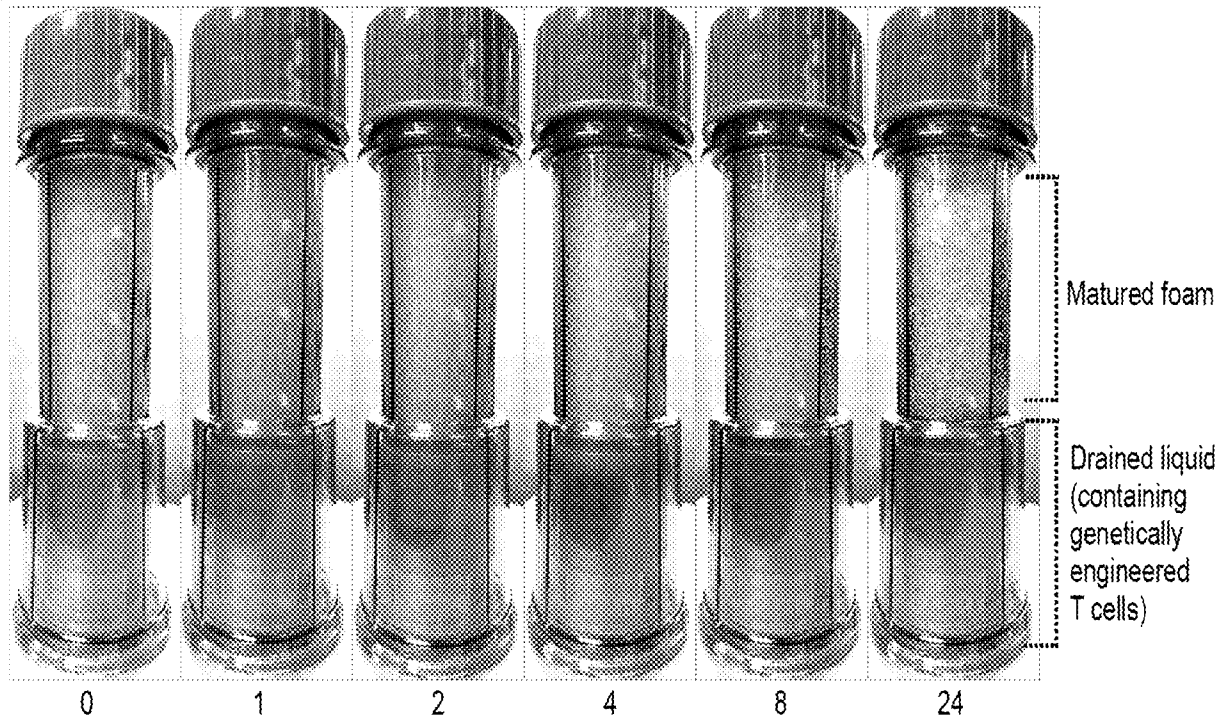


FIG. 14D



26/54

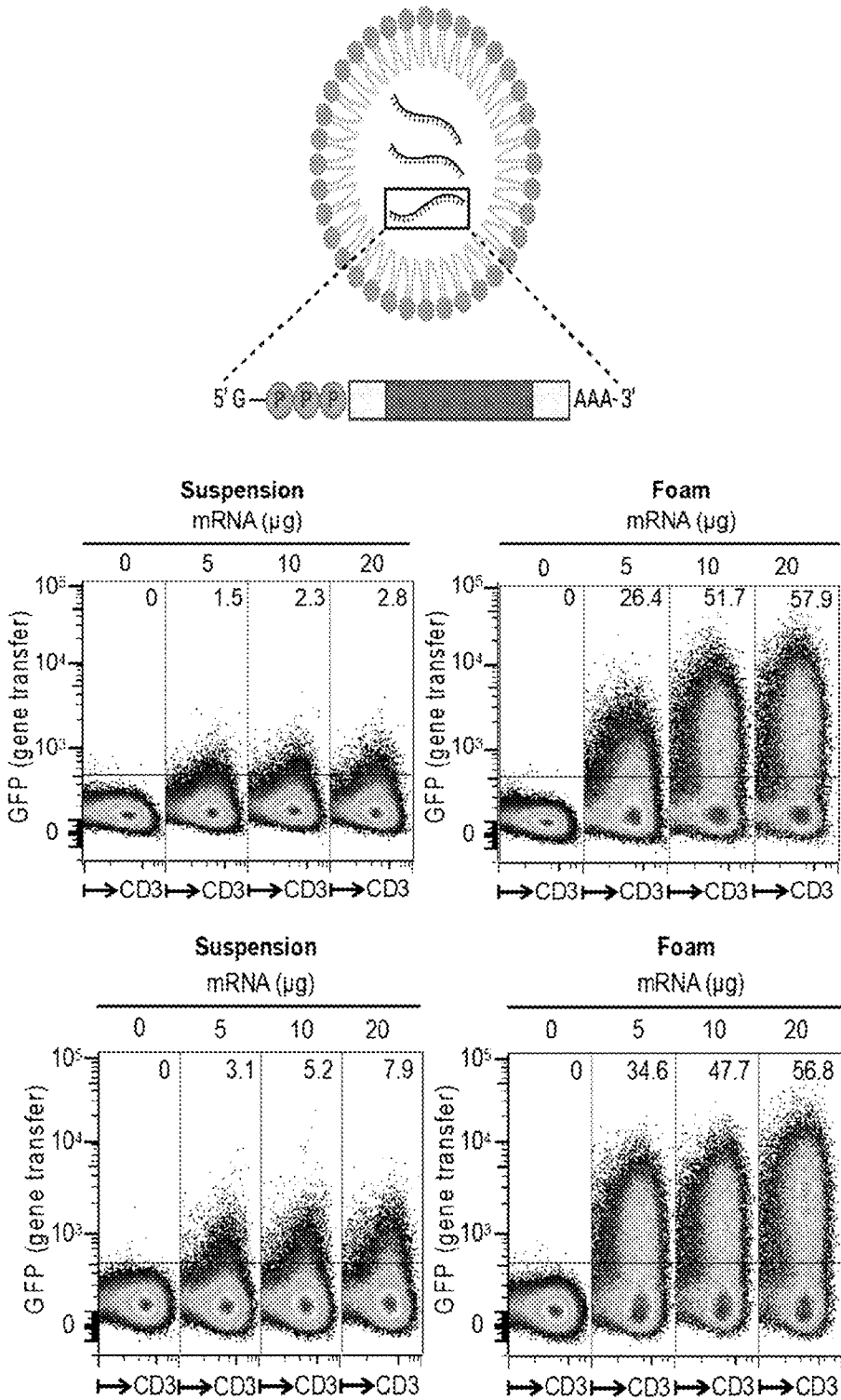
FIG. 14E



27/54

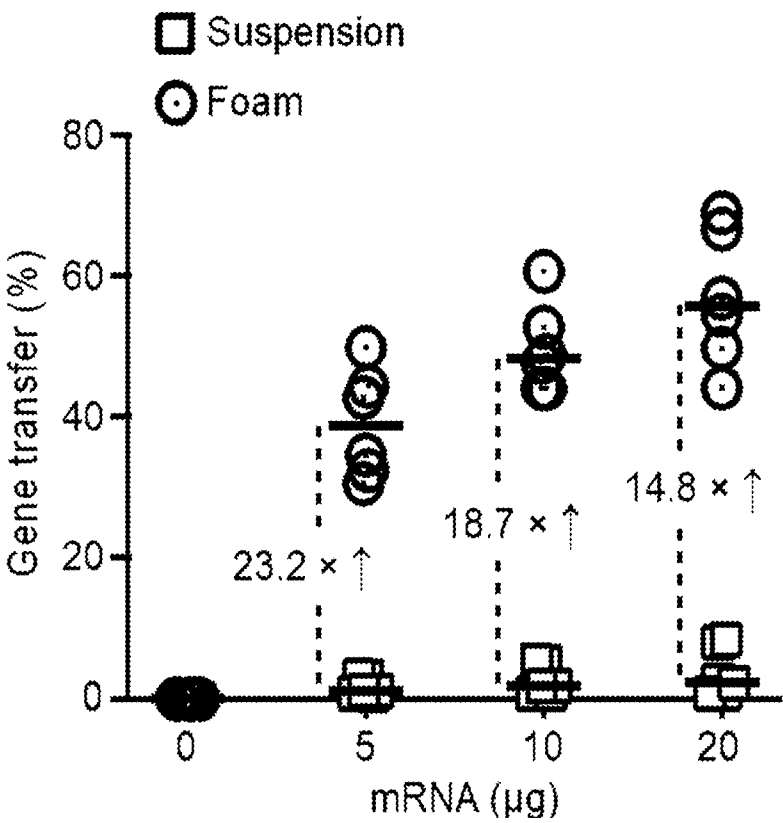
FIG. 15A

mRNA LNP
formulation 1



28/54

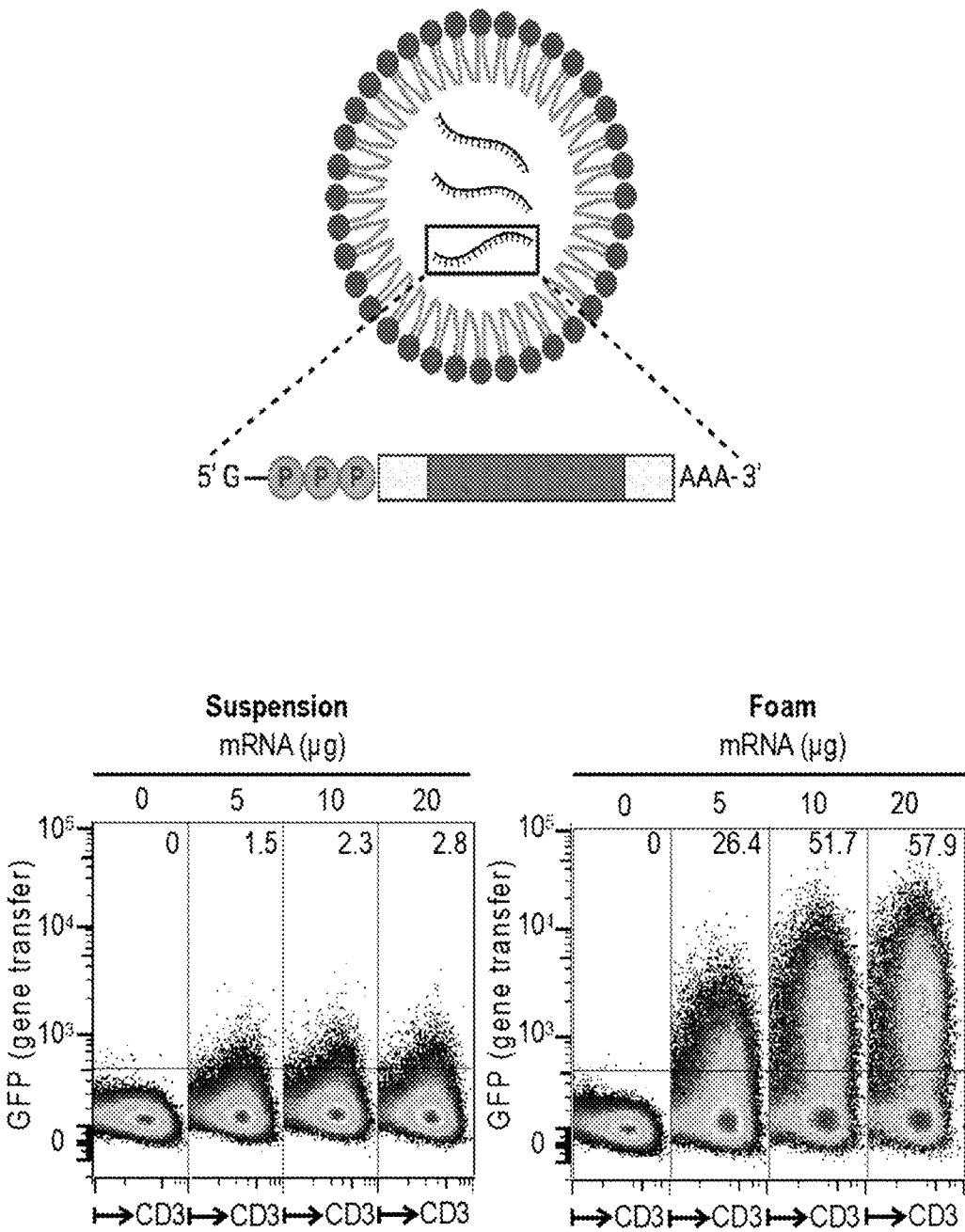
FIG. 15B



29/54

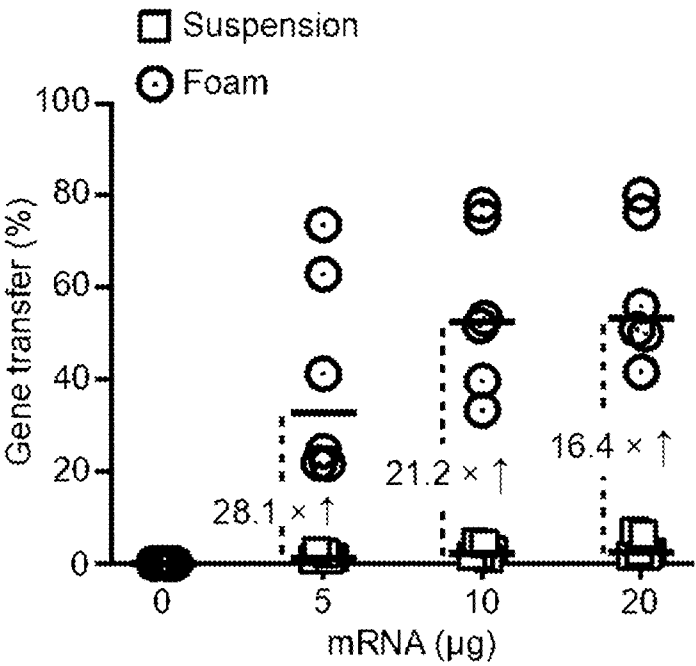
FIG. 15C

mRNA LNP
formulation 2



30/54

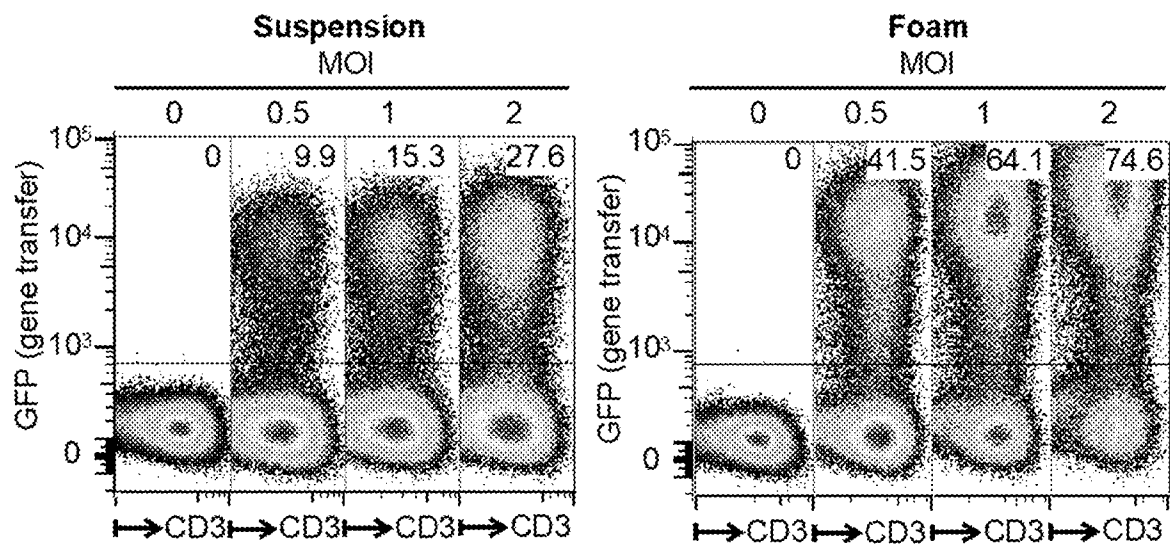
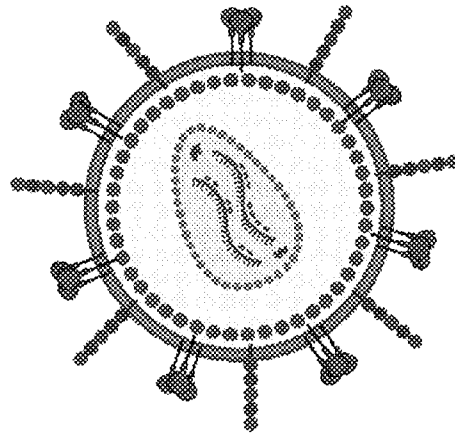
FIG. 15D



31/54

FIG. 15E

Lentivirus



32/54

FIG. 15F

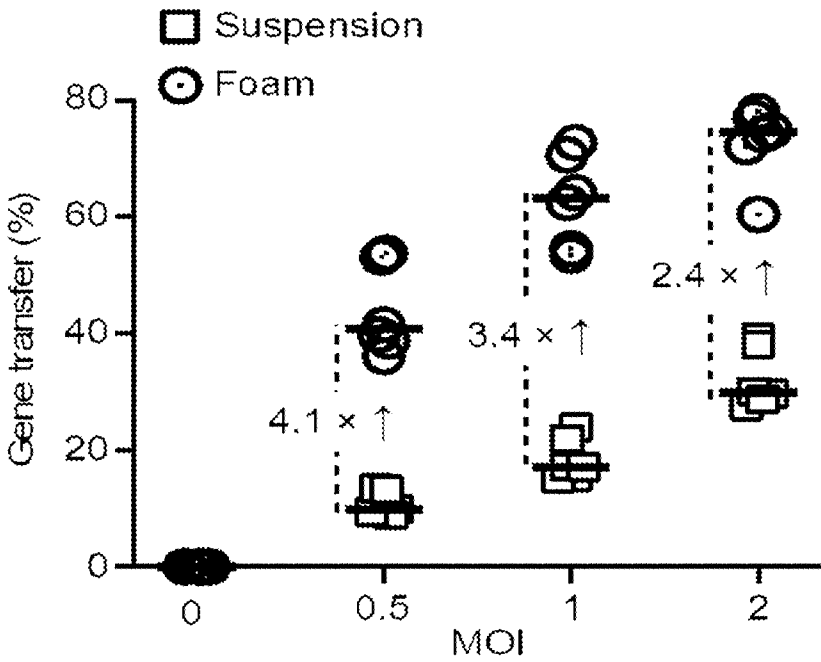


FIG. 16A

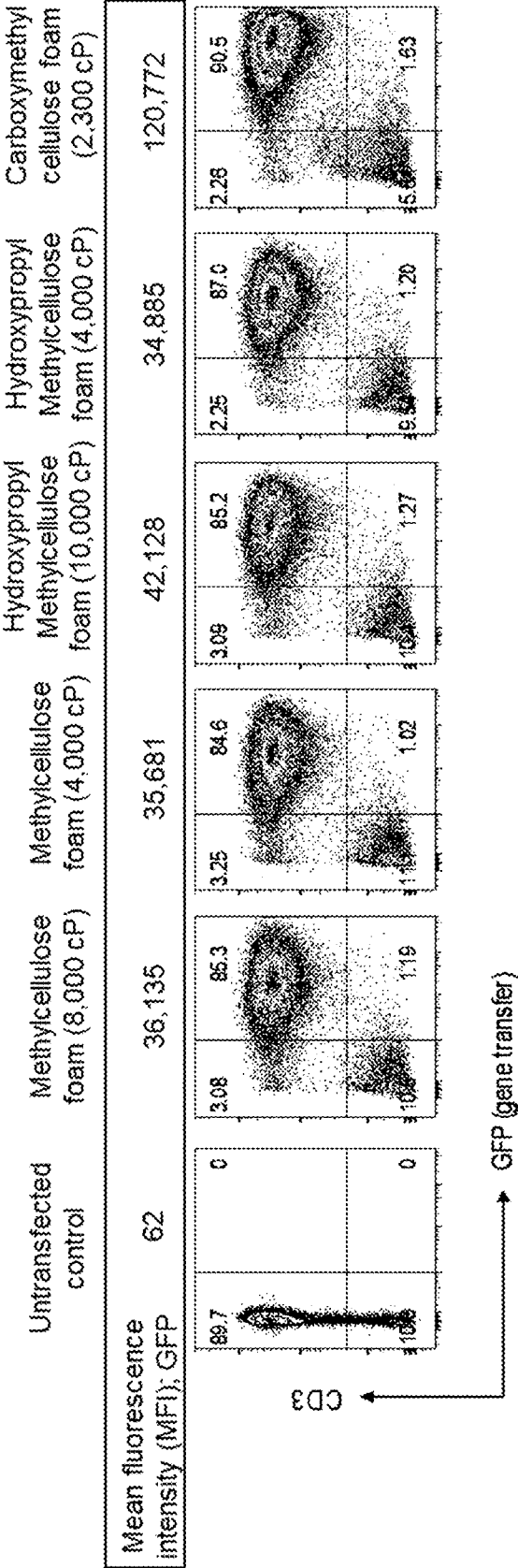
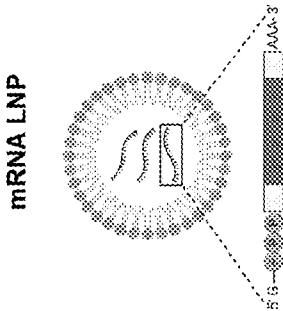
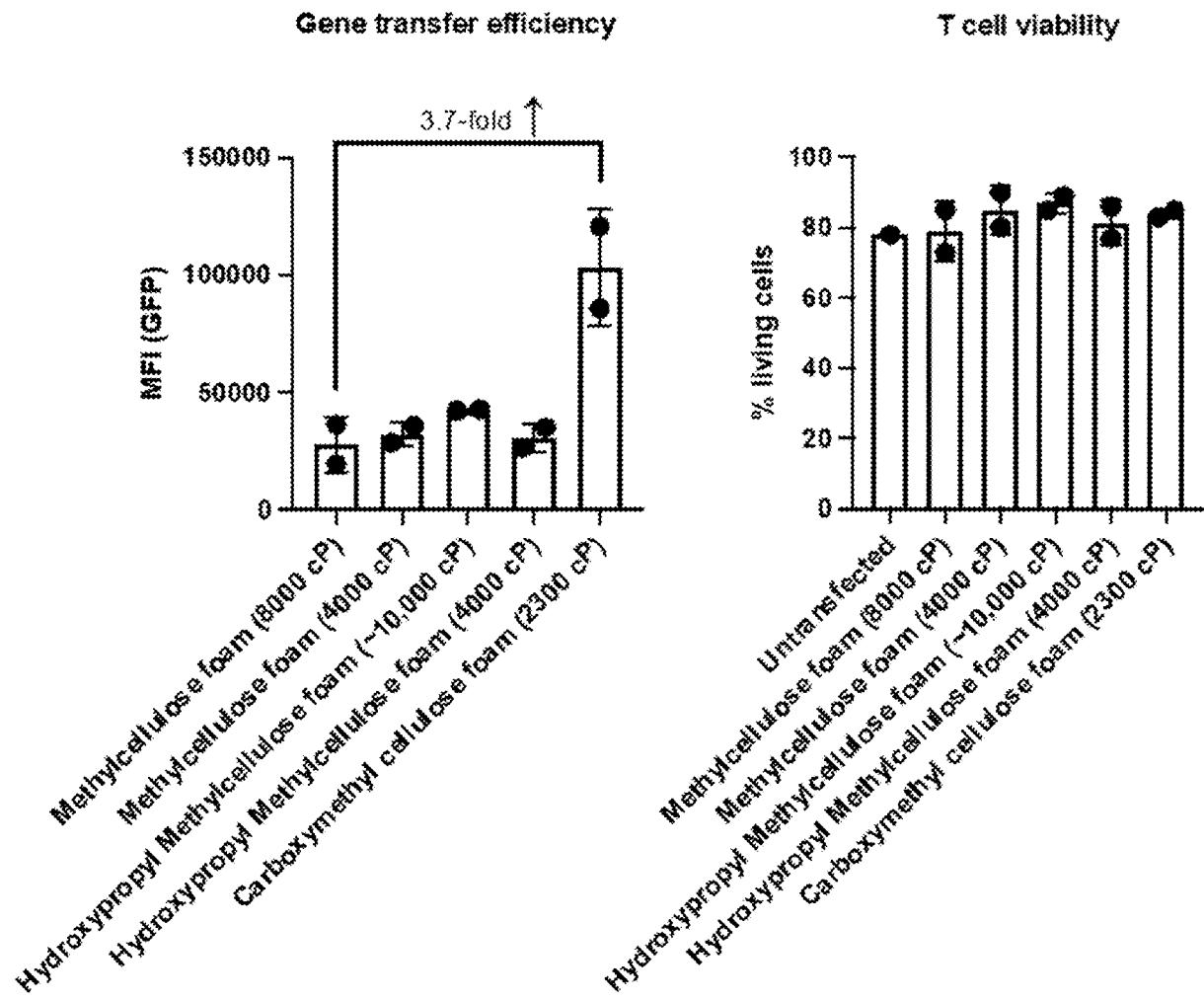


FIG. 16B



35/54

FIG. 16C

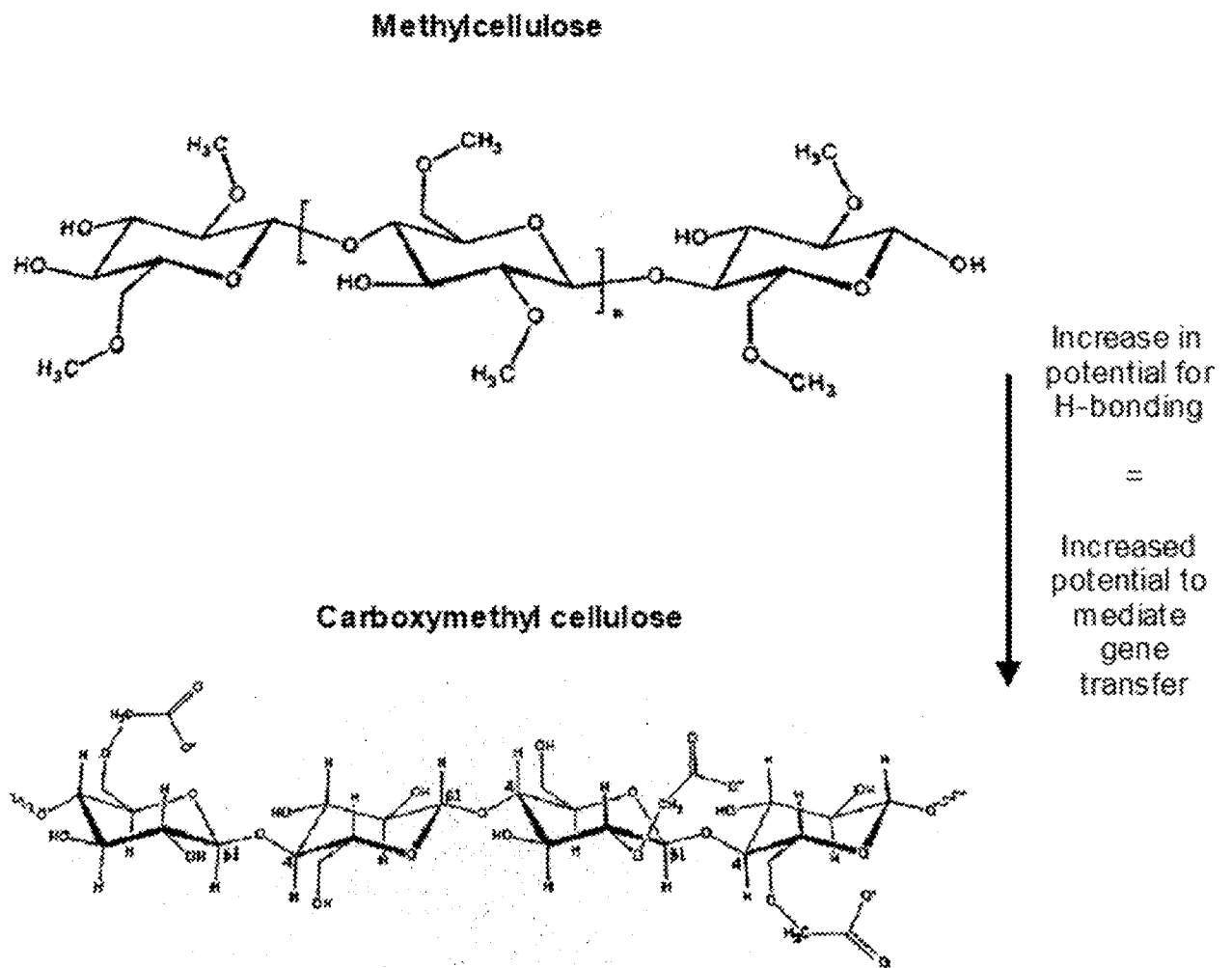
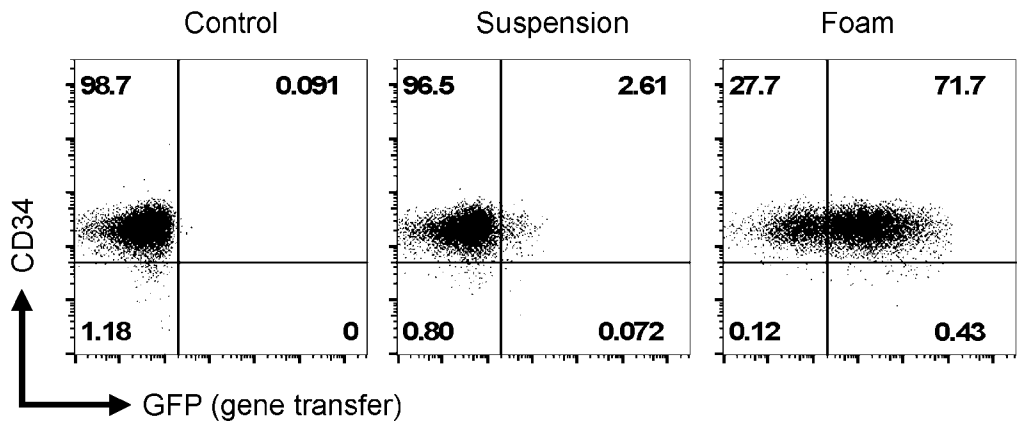
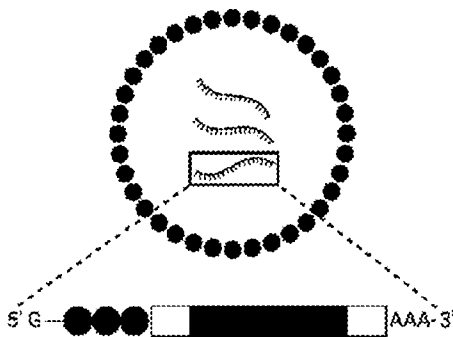


FIG. 17A

mRNA LNP



37/54

FIG. 17B

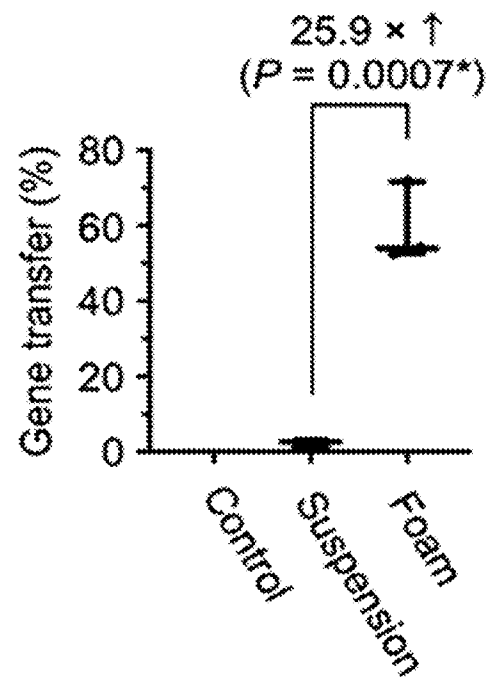
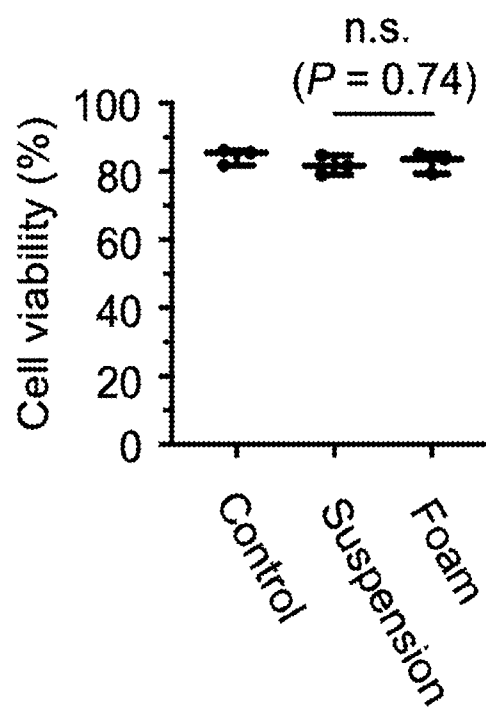


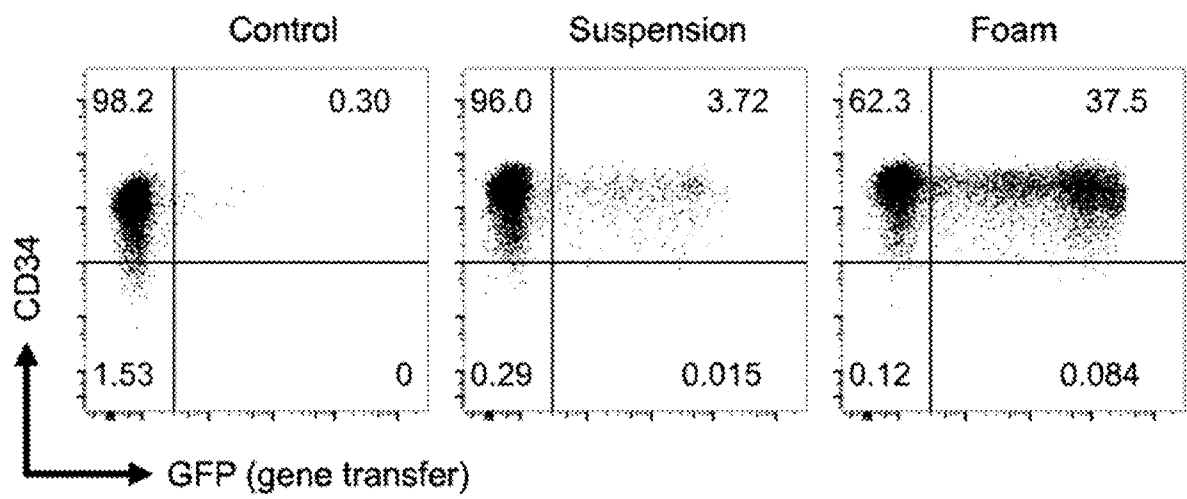
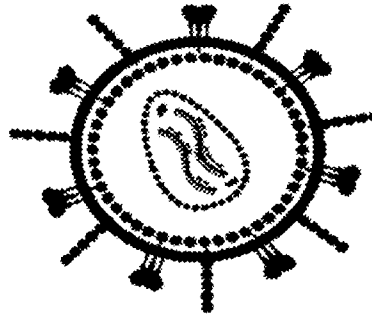
FIG. 17C



38/54

FIG. 17D

Lentivirus



39/54

FIG. 17E

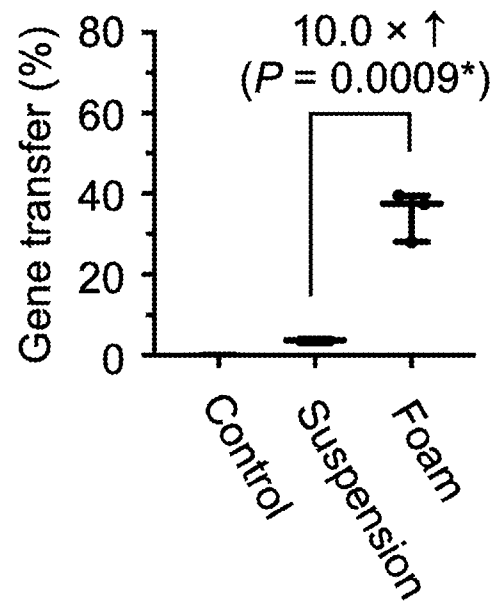
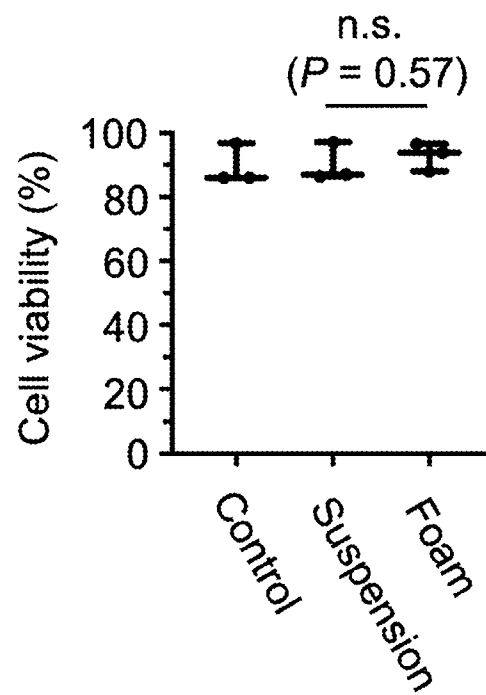


FIG. 17F



40/54

FIG. 18A

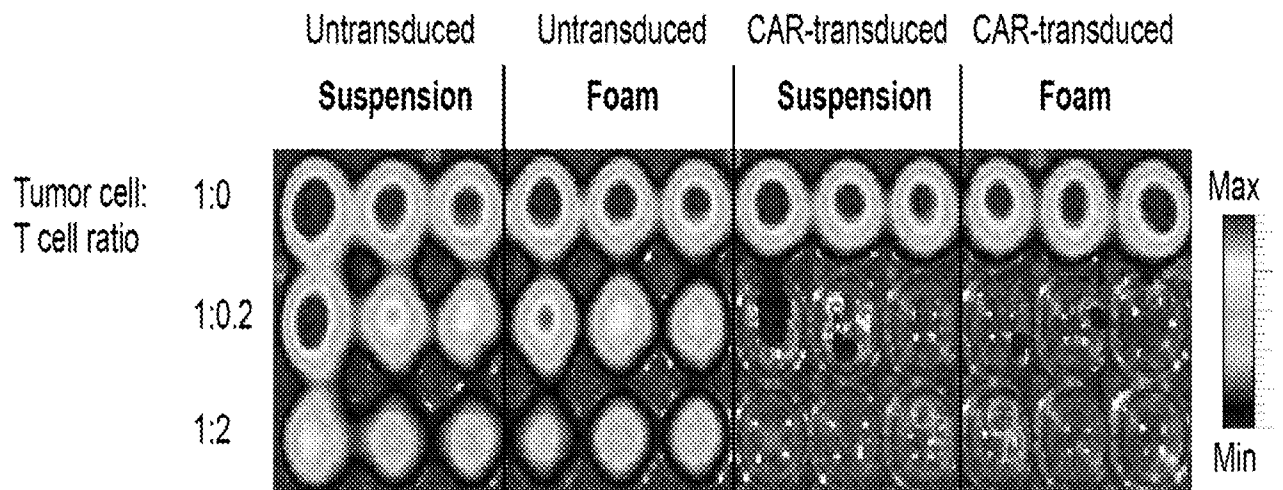
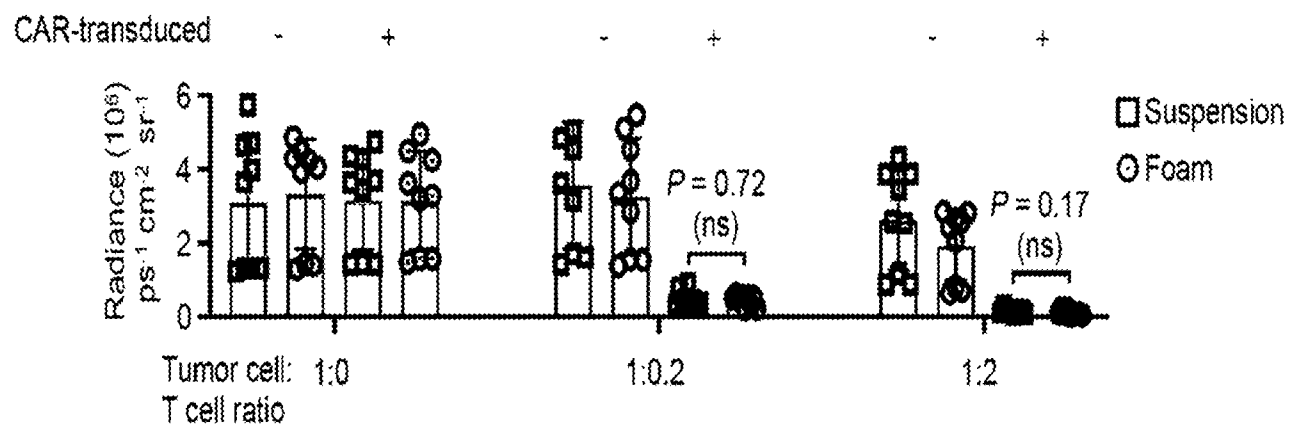


FIG. 18B



41/54

FIG. 19A

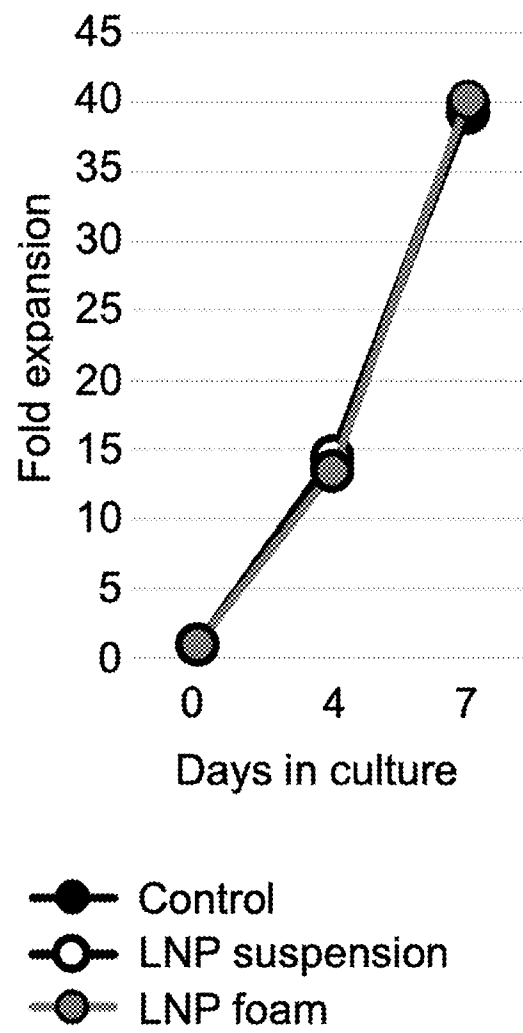
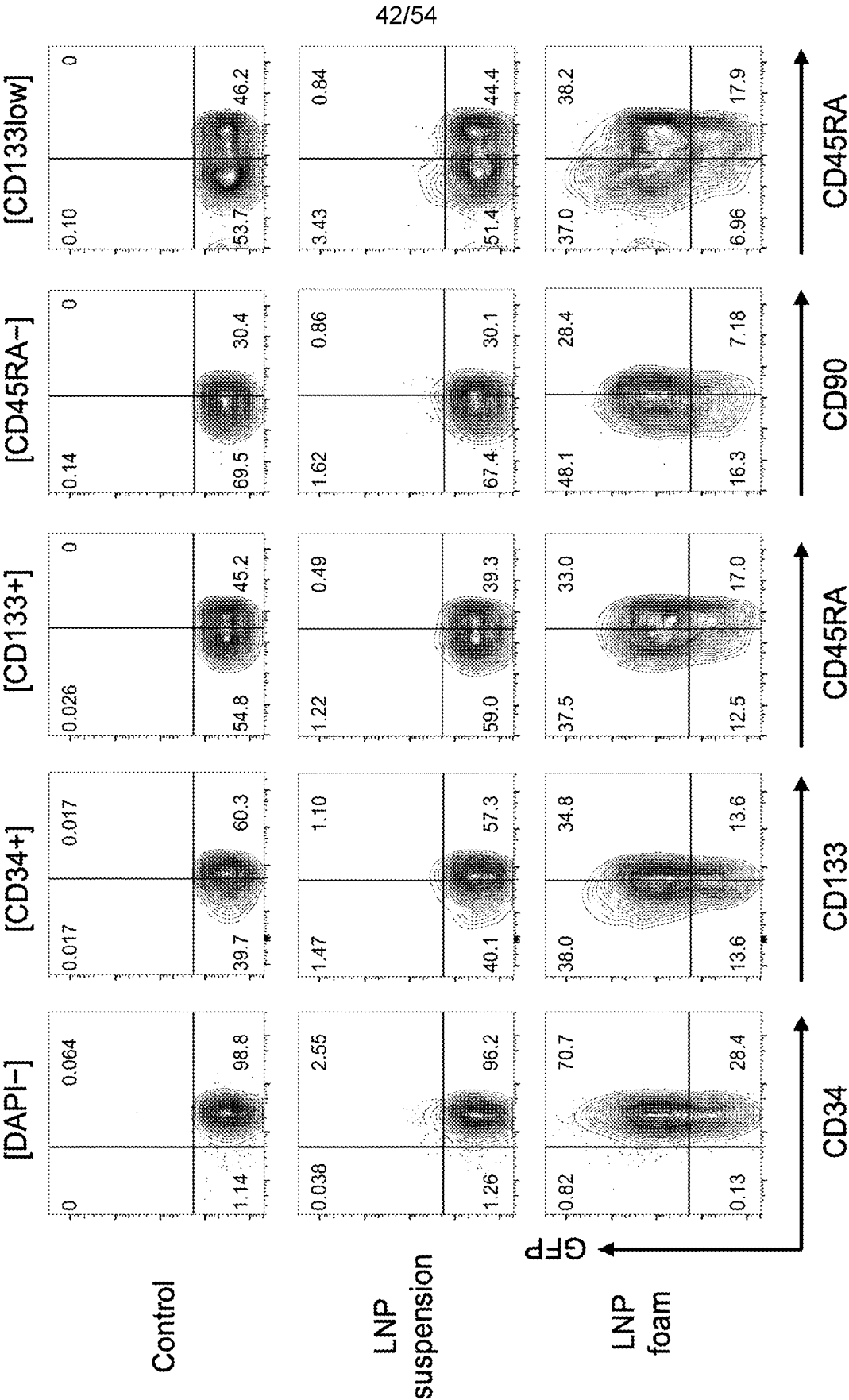


FIG. 19B



43/54

FIG. 19C

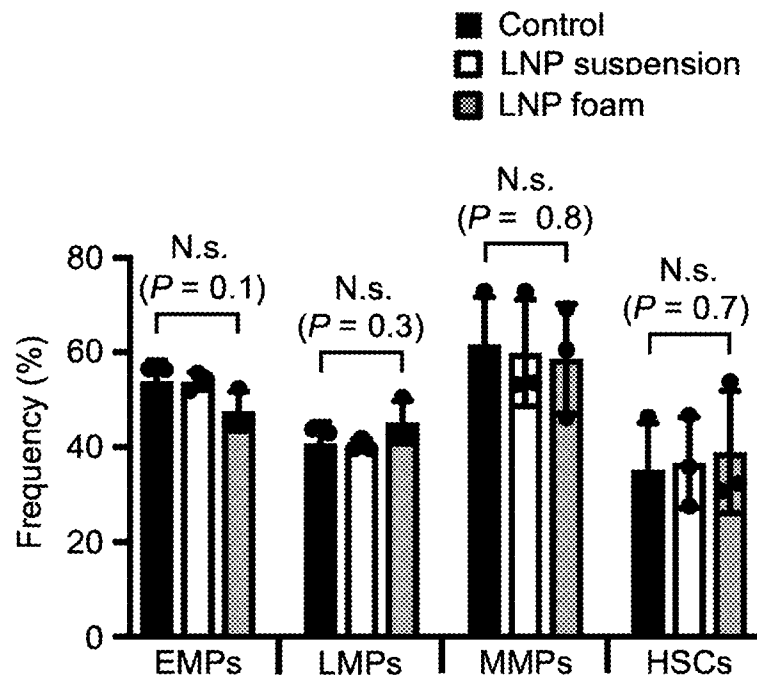
EMPs (CD34+, CD133^{low}, CD45RA⁻)LMPs (CD34+, CD133+, CD45RA⁺)MPPs (CD34+, CD133+, CD45RA⁻, CD90⁻)HSCs (CD34+, CD133+, CD45RA⁻, CD90⁺)

FIG. 19D

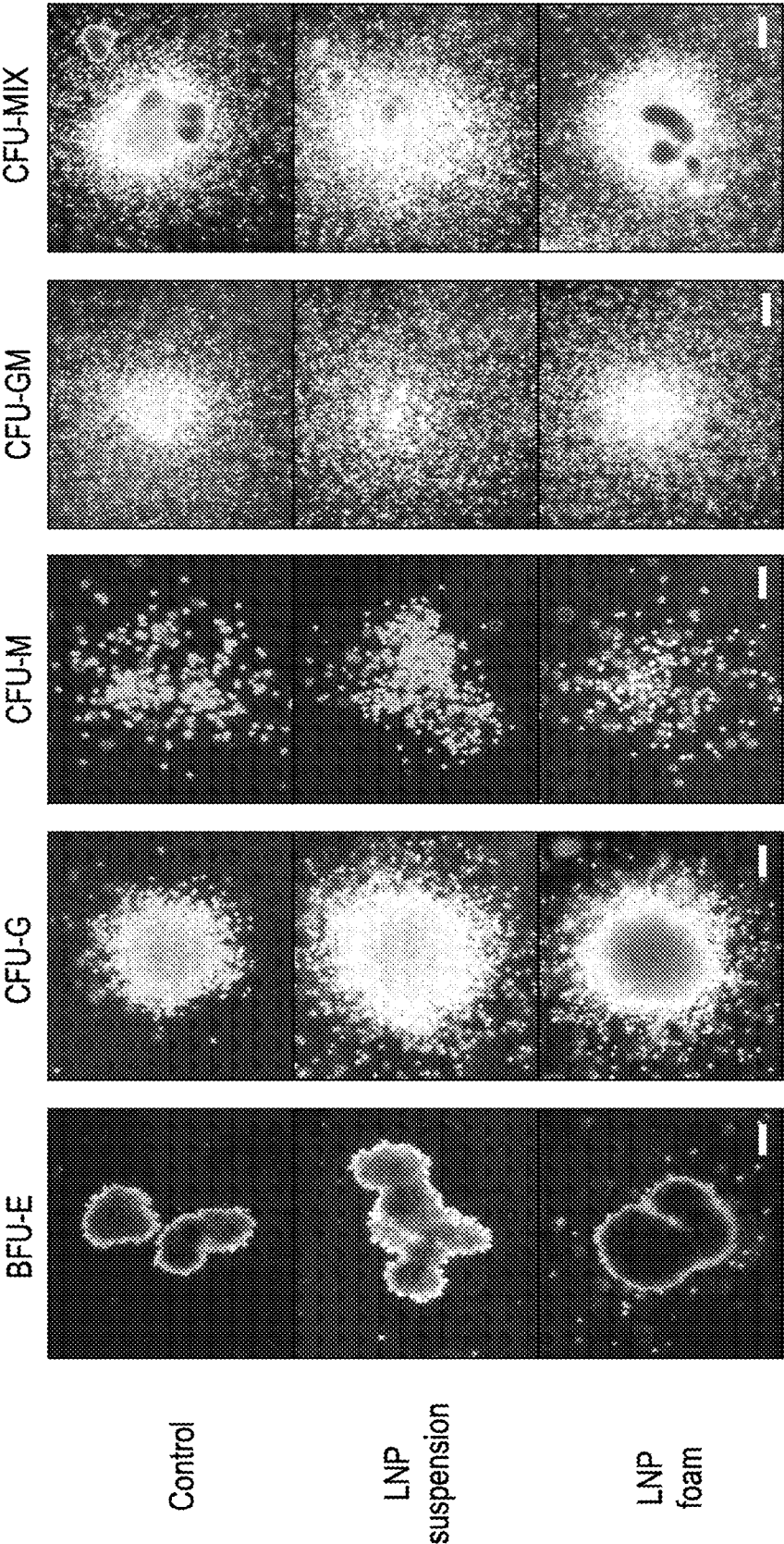


FIG. 19E

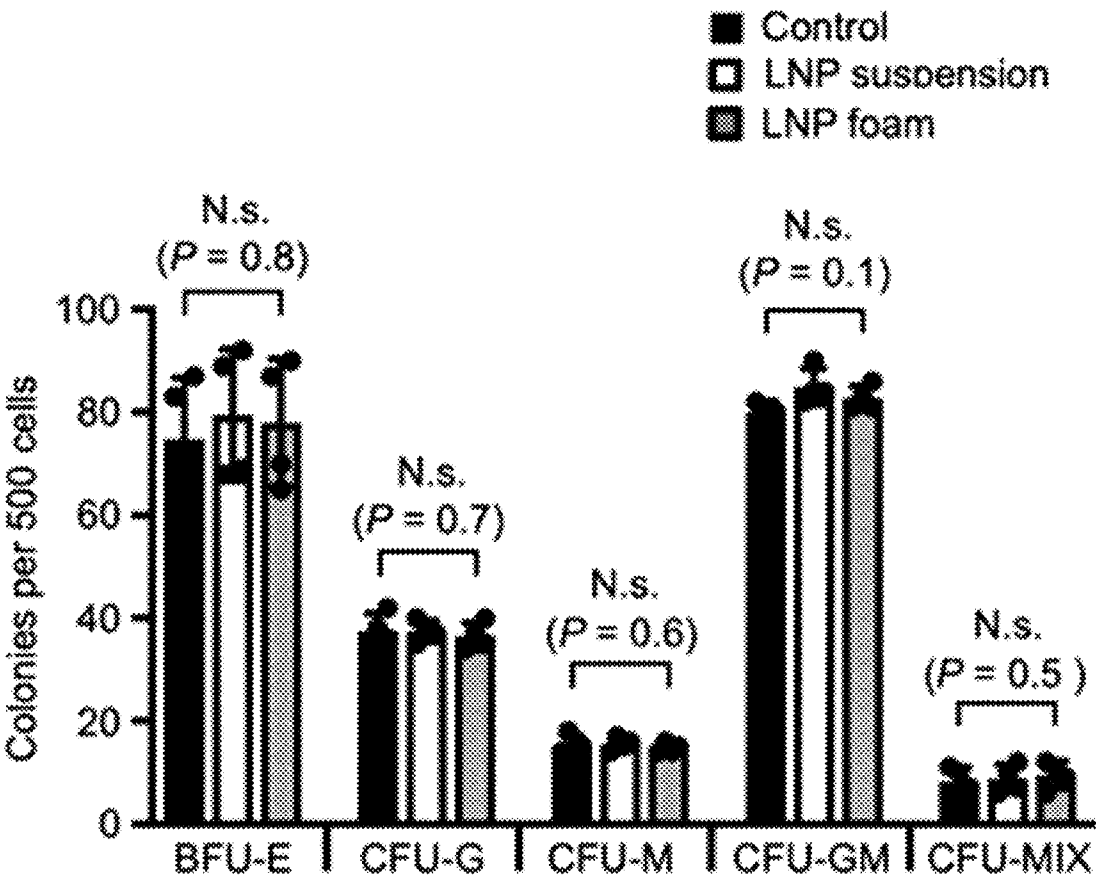


FIG. 20

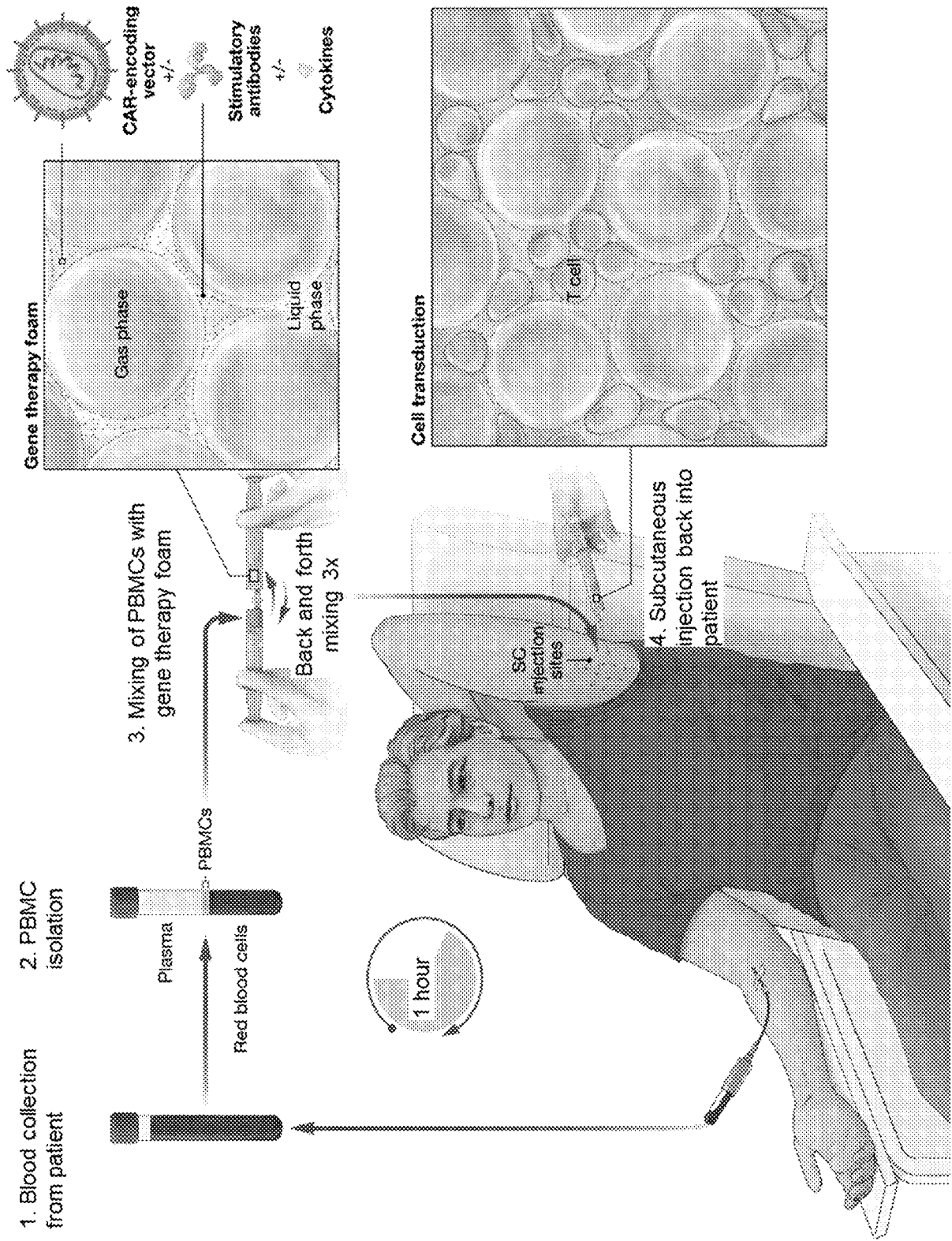


FIG. 20 (cont'd)

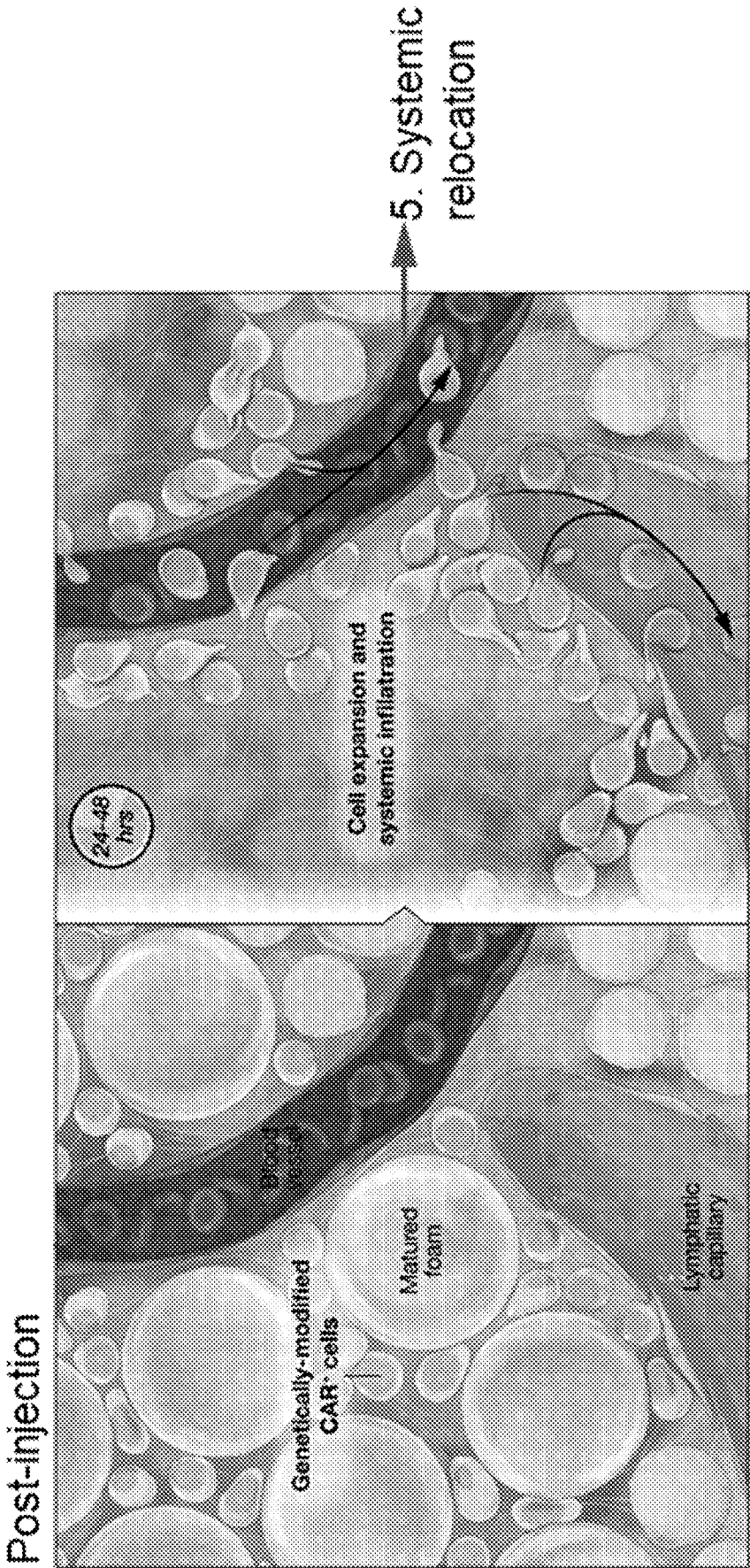
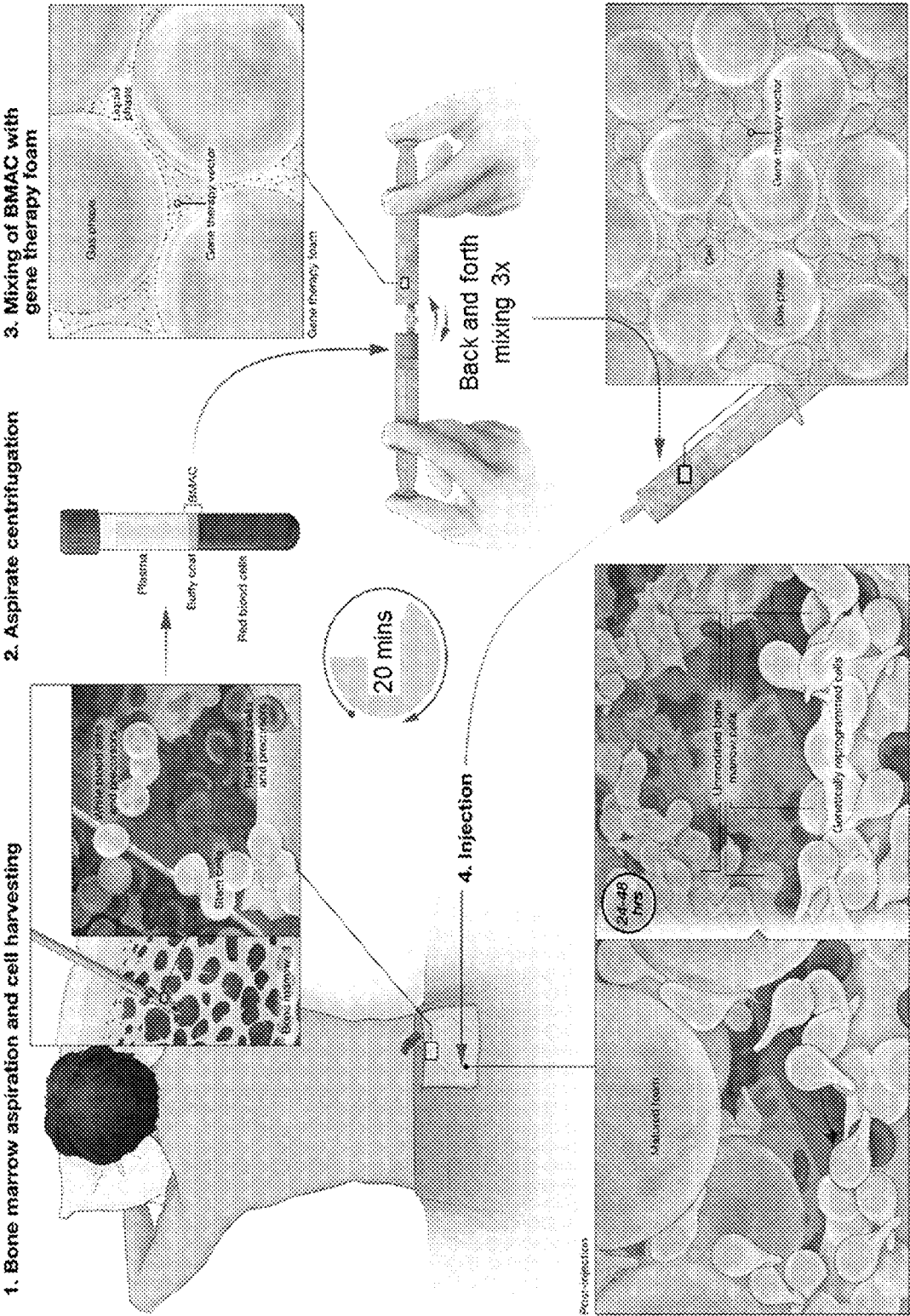


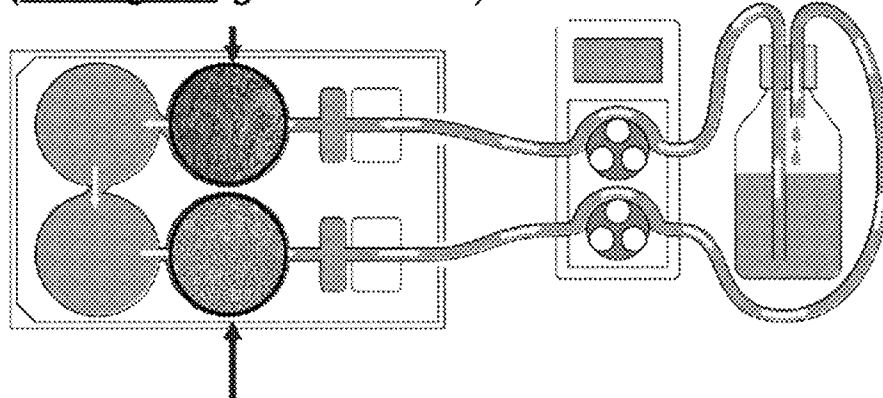
FIG. 21



49/54

FIG. 22

Place 3D HSC culture in here
(on target – gene transfer)



Place 3D culture of HEK 293
cells in here (off target- gene
transfer/mimicking tissue
outside the bone marrow

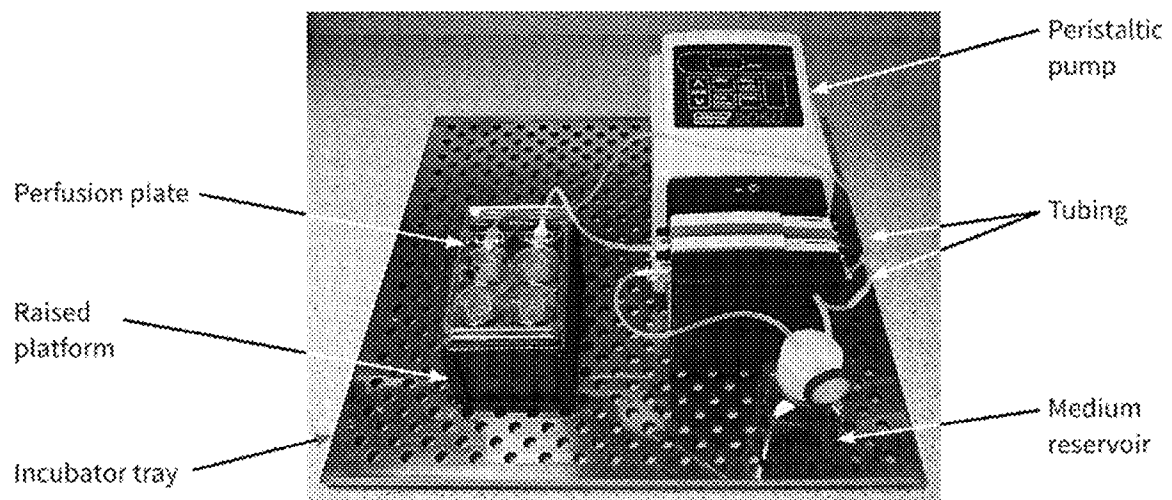
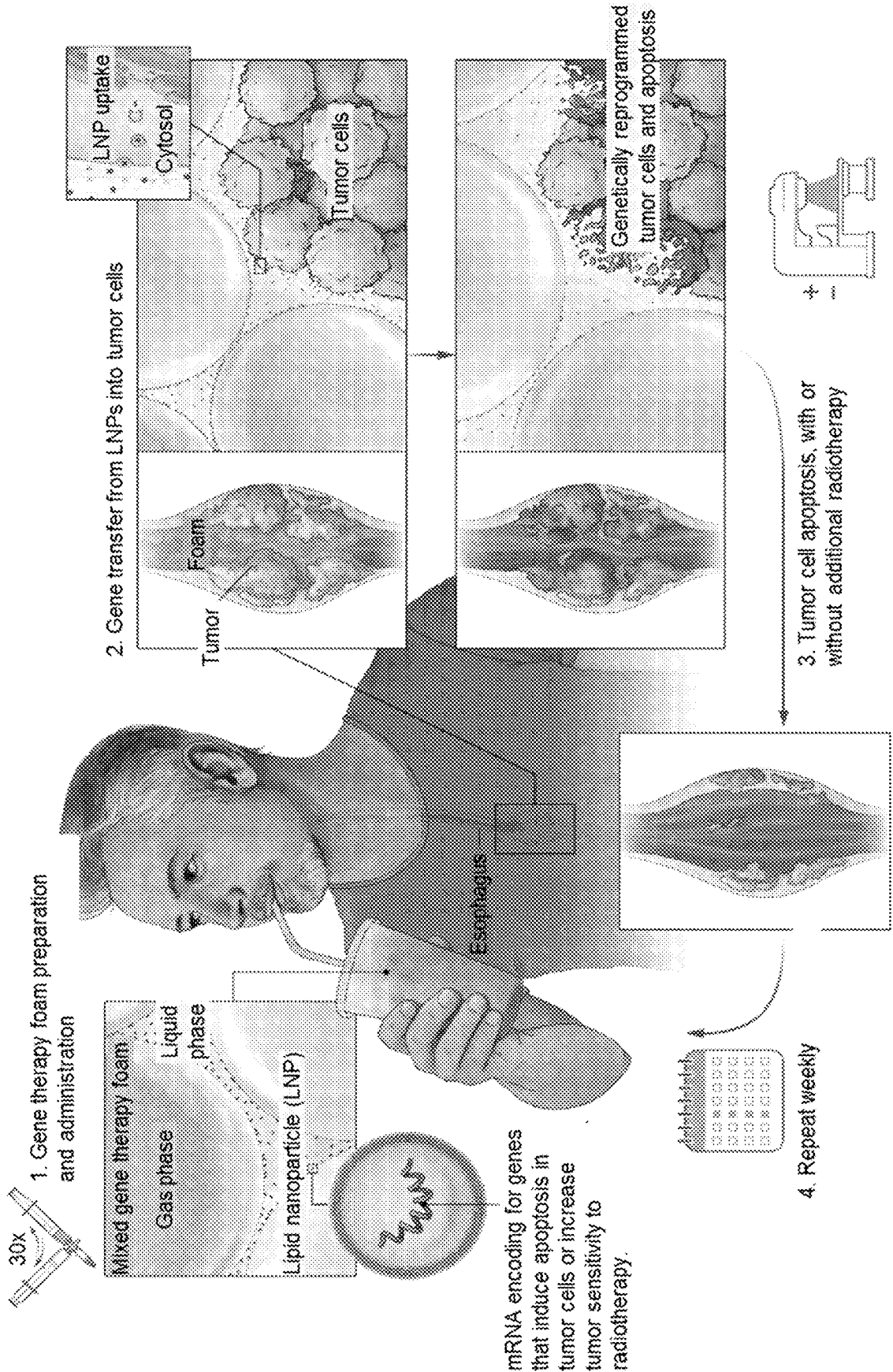
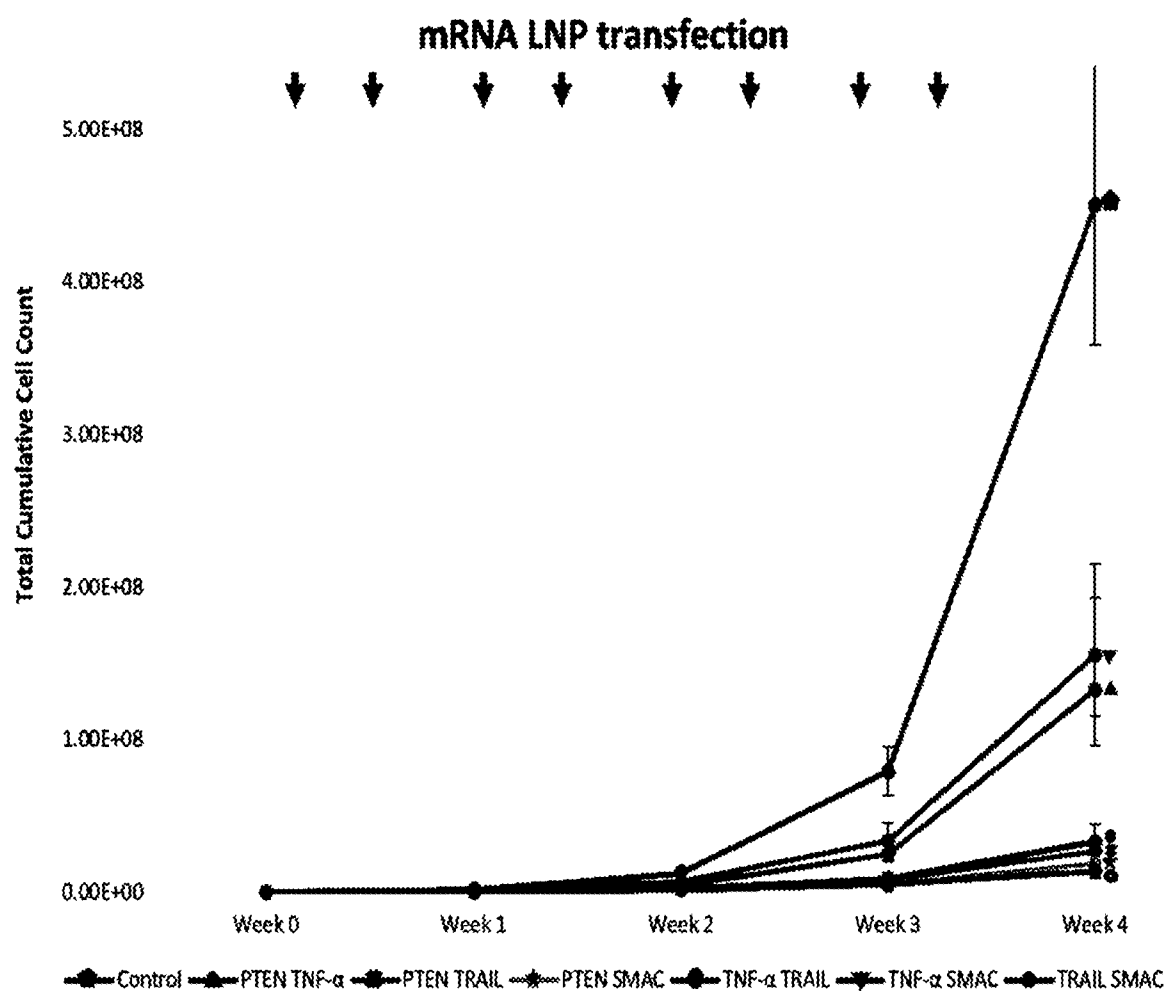


FIG. 23



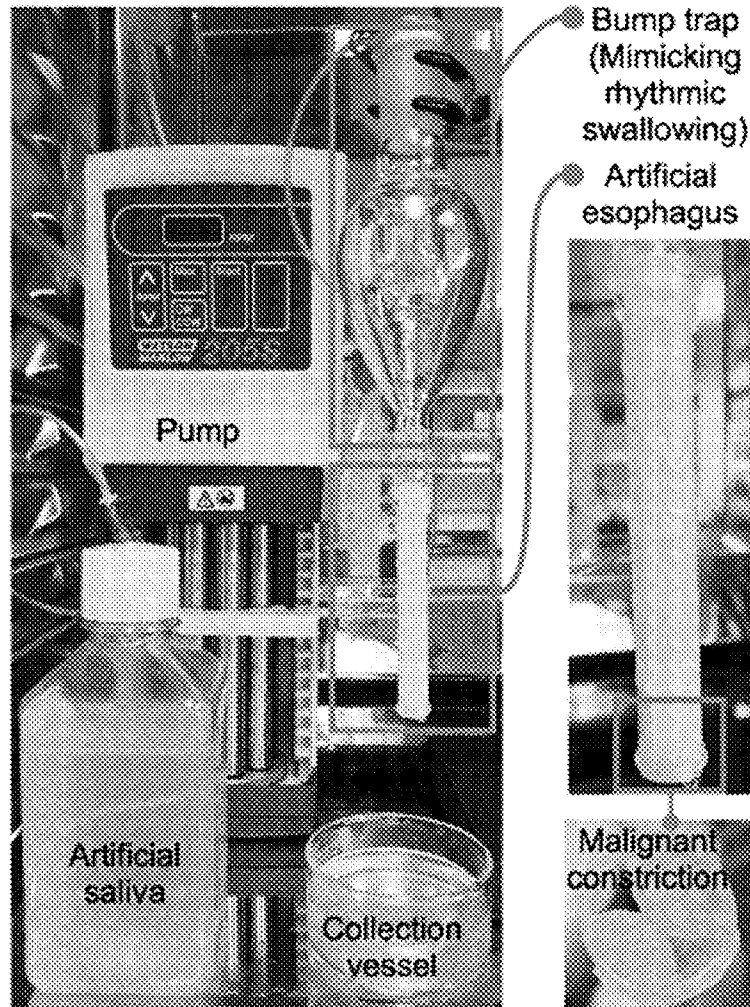
51/54

FIG. 24



52/54

FIG. 25



53/54

FIG. 26

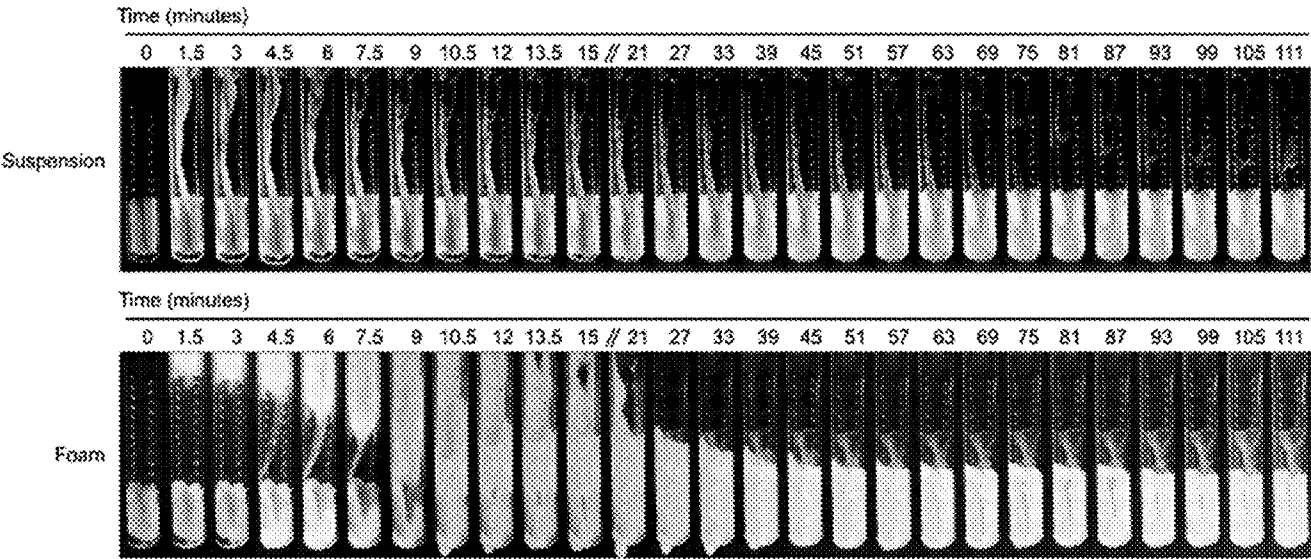
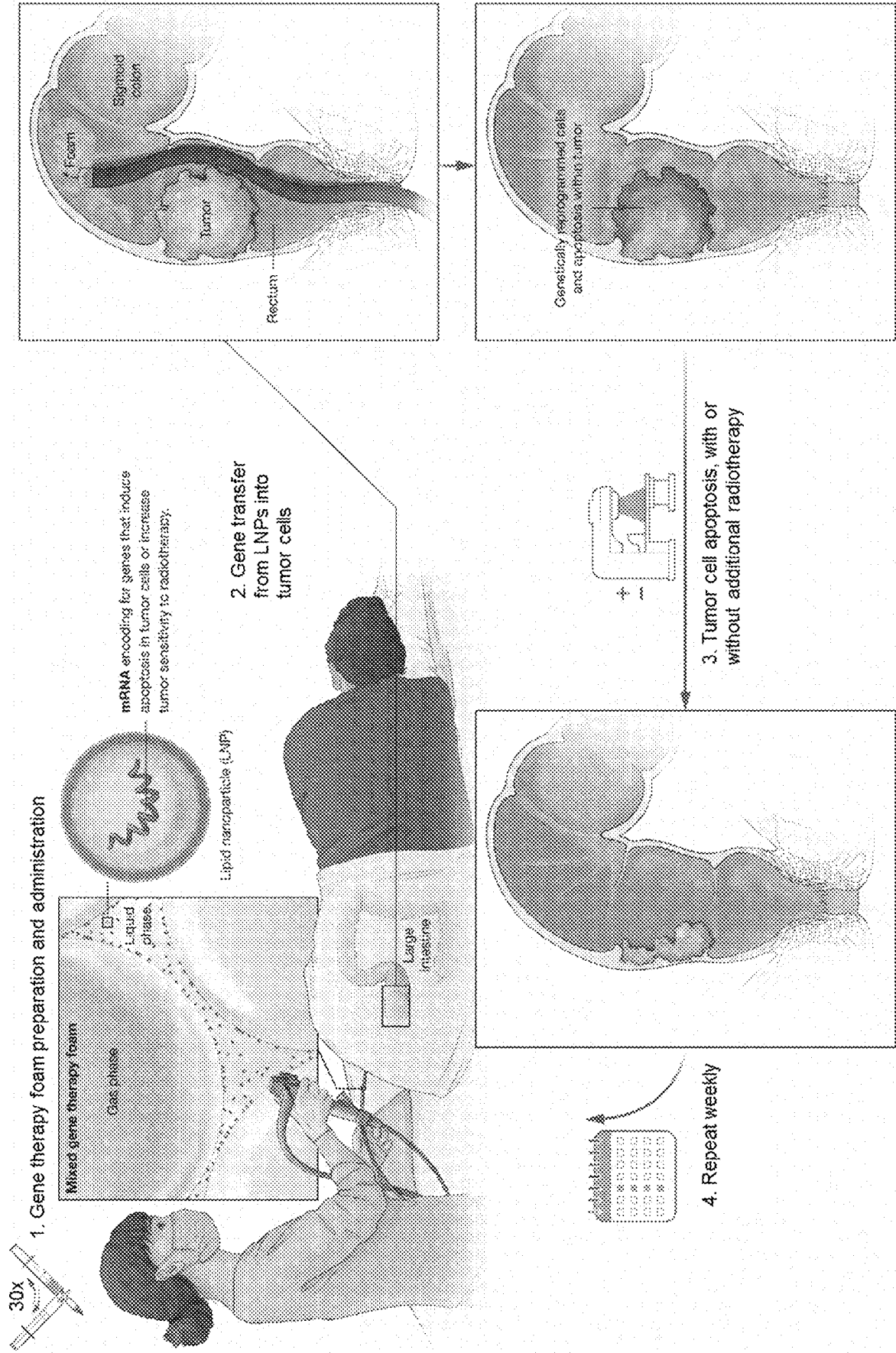


FIG. 27



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/056261

A. CLASSIFICATION OF SUBJECT MATTER IPC: A61K 9/51 (2025.01); <i>A61K 9/12</i> (2025.01); <i>A61K 31/7088</i> (2025.01) CPC: A61K 9/5192; A61K 9/5161 ; <i>A61K 9/122</i> ; <i>A61K 31/7088</i> 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) See Search History Document Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched See Search History Document Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) See Search History Document		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2022/162396 A1 (OXFORD UNIVERSITY INNOVATION LIMITED) 04 August 2022 (04.08.2022) abstract, pg. 2, ln 10-14, pg. 3, ln 16, pg. 12, ln 21, pg. 27, ln 14-19	1-4, 9-11, 15, 19-35, 37-40, 44, 68-82, 148-151, 156-158, 162, 166-188, 190-193, 197, 224-234, 239, 241-242, 246-248, 251, 255-260, 263, 265-268, 272, 277-279
☒ Further documents are listed in the continuation of Box C. ☐ 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 “D” document cited by the applicant in the international application “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 27 February 2025 (27.02.2025)		Date of mailing of the international search report 03 April 2025 (03.04.2025)
Name and mailing address of the ISA/US COMMISSIONER FOR PATENTS MAIL STOP PCT, ATTN: ISA/US P.O. Box 1450 Alexandria, VA 22313-1450 UNITED STATES OF AMERICA		Authorized officer KARI RODRIQUEZ
Facsimile No. 571-273-8300		Telephone No. PCT Help Desk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/056261

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2002/0159952 A1 (Unger) 31 October 2002 (31.10.2002) abstract, para [0013], [0150], [0172], [0213], [0216]	1-4, 9-11, 15, 19-35, 37-40, 44, 68-82, 148- 151, 156-158, 162, 166-188, 190-193, 197, 224-234, 239, 241-242, 246-248, 251, 255-260, 263, 265-268, 272, 277-279
A	WO 2017/151862 A1 (UNIVERSITY OF PITTSBURGH - OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) 08 September 2017 (08.09.2017) entire document	1-4, 9-11, 15, 19-35, 37-40, 44, 68-82, 148- 151, 156-158, 162, 166-188, 190-193, 197, 224-234, 239, 241-242, 246-248, 251, 255-260, 263, 265-268, 272, 277-279
A	WO 2023/144330 A1 (CUREVAC SE et al.) 03 August 2023 (03.08.2023) entire document	1-4, 9-11, 15, 19-35, 37-40, 44, 68-82, 148- 151, 156-158, 162, 166-188, 190-193, 197, 224-234, 239, 241-242, 246-248, 251, 255-260, 263, 265-268, 272, 277-279
A	Chen et al. "Efficient gene delivery to pancreatic islets with ultrasonic microbubble destruction technology" PNAS. 30 May 2006, vol 103, pg. 8469-8474 pg. 8469, right col, para 1, pg. 8473, left col, para 3	1-4, 9-11, 15, 19-35, 37-40, 44, 68-82, 148- 151, 156-158, 162, 166-188, 190-193, 197, 224-234, 239, 241-242, 246-248, 251, 255-260, 263, 265-268, 272, 277-279

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: Claims 1-89 and 148-309, directed to a gas-bubble based degrading liquid foam comprising a nucleic acid. The foam of claim 1 will be searched to the extent that it encompasses the first species of claim 1, represented by a gas-bubble, which is the first gas of claim 15, air; based degrading liquid foam which is the first foam of claim 9, a GRAS material, which is the first material of claim 10, cellulose, which is the first cellulose of claim 11, methylcellulose; comprising a nucleic acid, which is the first nucleic acid of claim 2, a nucleic acid, which is the first nucleic acid of claim 44, wherein the nucleic acid encodes IRF5; within a vector; which is the first vector of claim 3, a lipid nanoparticle, which is the first nanoparticle of claim 4, a lipid nanoparticle comprising 2-(Di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethan-1-ol (wherein optional groups are omitted). It is believed that claims 1-4, 9-11, 15, 19-35, 37-40, 44, 68-82, 148-151, 156-158, 162, 166-188, 190-193, 197, 224-234, 239, 241-242, 246-248, 251, 255-260, 263, 265-268, 272 and 277-279 read on this first named invention, and thus these claims will be searched without fee.

Group II: Claims 90-95 and 143-147, directed to a use of a foam to treat a condition in a subject / a method of treating a condition.

Group III: Claims 96-104, directed to a method of forming a gas-bubble based degrading liquid foam.

Group IV: Claims 105-142, directed to a method of delivering a nucleic to a cell / method of modifying cells.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-4, 9-11, 15, 19-35, 37-40, 44, 68-82, 148-151, 156-158, 162, 166-188, 190-193, 197, 224-234, 239, 241-242, 246-248, 251, 255-260, 263, 265-268, 272, 277-279**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.