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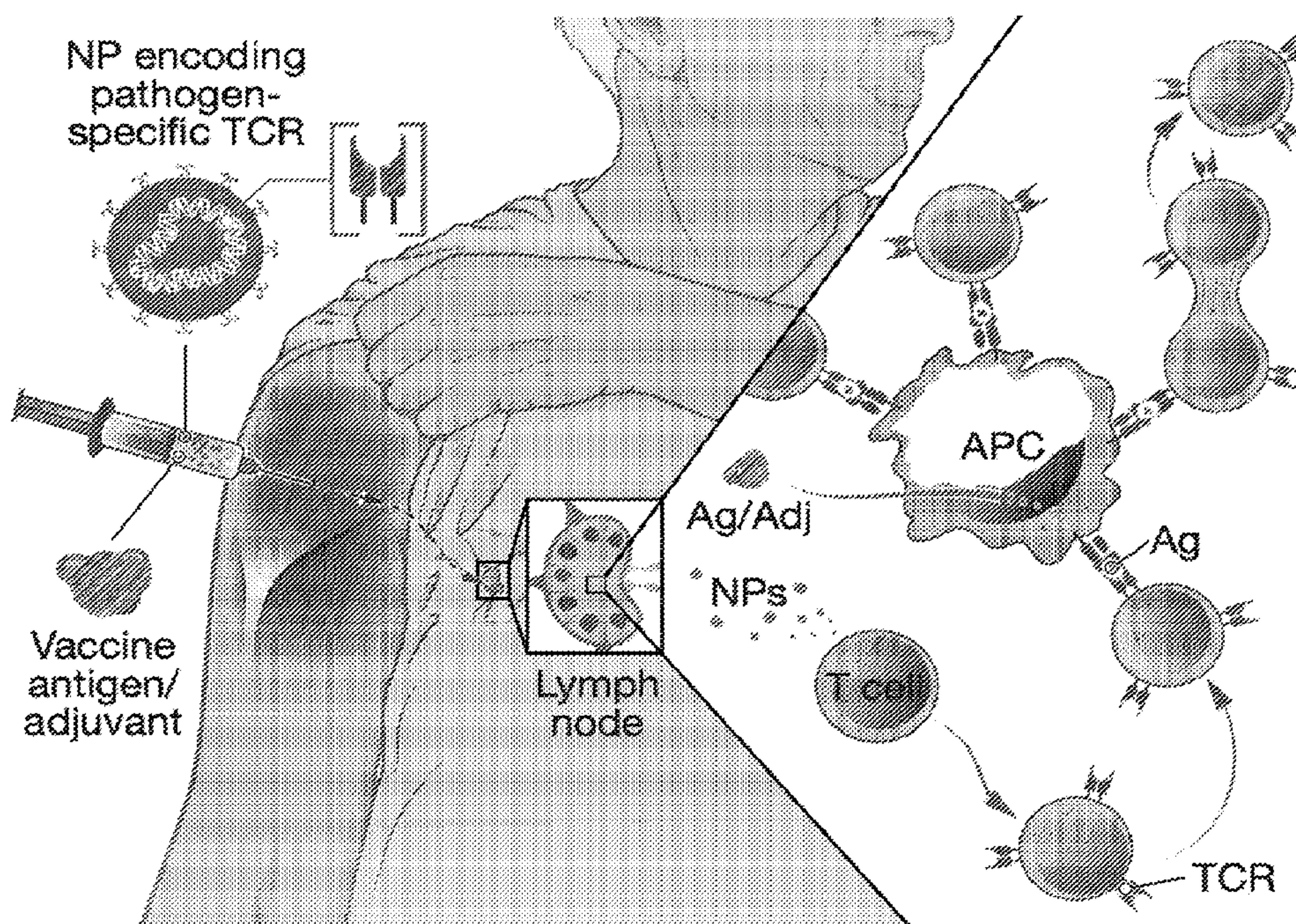
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(54) Titre : SYSTEMES ET PROCEDES POUR AMELIORER L'EFFICACITE D'UN VACCIN
(54) Title: SYSTEMS AND METHODS TO IMPROVE VACCINE EFFICACY

FIG. 1



(57) **Abrégé/Abstract:**

Systems and methods to increase the efficacy of vaccines that require or are rendered more effective with T cell mediated immunity are described. The systems and methods utilize polynucleotides that genetically modify T cells to express a T cell receptor specific for an administered vaccine antigen.

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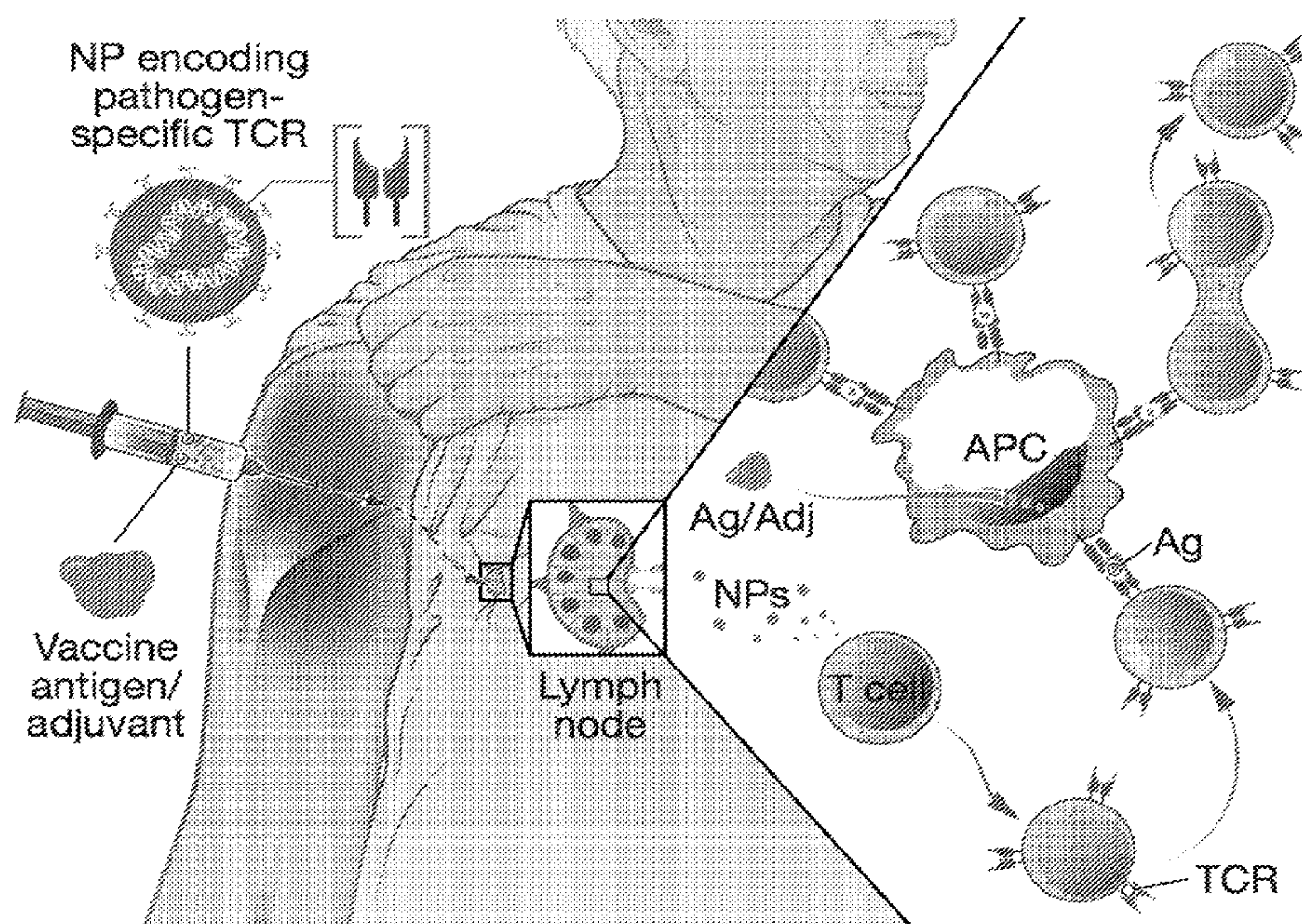
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FIG. 1



(57) Abstract: Systems and methods to increase the efficacy of vaccines that require or are rendered more effective with T cell mediated immunity are described. The systems and methods utilize polynucleotides that genetically modify T cells to express a T cell receptor specific for an administered vaccine antigen.

[Continued on next page]

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SYSTEMS AND METHODS TO IMPROVE VACCINE EFFICACY

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/442,903 filed on January 5, 2017, which is incorporated herein by reference in its entirety as if fully set forth herein.

REFERENCE TO SEQUENCE LISTING

[0002] A computer readable text file, entitled "F053-0055PCT Sequence Listing_ST25.txt" created on or about January 5, 2018, with a file size of 114 KB, contains the sequence listing for this application and is hereby incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The present disclosure provides systems and methods to increase the efficacy of vaccines that require or are rendered more effective with T cell mediated immunity. The systems and methods utilize polynucleotides that genetically modify T cells to express a T cell receptor specific for an administered vaccine antigen.

BACKGROUND OF THE DISCLOSURE

[0004] Lymphocytes are cells of the immune system involved in self/nonself recognition and acquired long-term immunity based on immunological memory. Lymphocytes can broadly be characterized as B cells or T cells. B cells are characterized by the presence of membrane-bound immunoglobulin (antibody) molecules which serve as receptors to bind soluble antigens. T cells are characterized by the presence of membrane-bound T cell receptors (TCR). TCR bind antigens only when the antigen is associated with a major histocompatibility complex (MHC) molecule (i.e., the antigen is not soluble). The specificity of T cell responses is conferred by particular TCR that bind particular antigens.

[0005] T lymphocytes include CD4⁺ T cells and CD8⁺ T cells. These types of T cells are distinguished in part by their expression of the cell surface molecules CD4 and CD8, respectively. They also, however, have different functions. CD4⁺ T cells, also referred to as helper T cells (T_H) facilitate the activities of other cell types. For example, CD4⁺ TH1 cells secrete various cytokines that activate cytotoxic T cells and macrophages to destroy cells harboring phagocytosed microorganisms. CD4⁺ TH2 cells secrete cytokines that activate B cells to produce antibodies. CD8⁺ cells are cytotoxic T lymphocytes (CTL) that can directly kill abnormal or infected cells.

[0006] Vaccines are formulations that produce an immune system response against a particular pathogen (e.g., infectious microorganism) or aberrant cell type (e.g., cancer cell) by preemptively exposing the immune system to an antigen of the pathogen or aberrant cell type. A pathogen antigen

can be an intact, but non-infectious form of a pathogen (e.g., heat-killed). Antigens can also be a protein or protein fragment of the pathogen or a protein or protein fragment preferentially expressed by the aberrant cell type. When the immune system recognizes a vaccine antigen following preemptive exposure, it can lead to long-term immune memory so that if the antigen is encountered again, the immune system can quickly and effectively mount an effective response.

[0007] When a vaccine is delivered to a subject, antigen presenting cells (APC) of the immune system take up the antigen component and present it or a fragment thereof to B cells and T cells. B cells that express receptors specific for the presented antigen will produce and secrete antibodies that circulate through the body to elicit a quick, robust immune response if the antigen is encountered again later in life. Standard vaccines are designed to function via such antibody responses created by B cells. The effectiveness of B cell immunity, however, is limited to soluble (i.e., extracellular pathogens). Pathogens that exist intracellularly (e.g., those that cause AIDS, malaria, herpes, and chlamydia) and those bound to a cell surface (e.g., cancer antigens) are not as susceptible to B cell antibodies. Further, the effectiveness of B cell immunity is enhanced when the vaccine antigen is similarly recognized by CD4+ helper T cells.

[0008] For antigens that remain cell-associated, T cell mediated immunity is required for effective immunization. Vaccines that require T cell mediated immunity often fail, however, because a host (e.g., person, research animal) does not have T cells expressing particular TCR that recognize and bind the presented vaccine antigen. People with compromised immune systems (e.g., the elderly) are especially vulnerable to this problem, because their declining production of new T cells leads to “holes” in their TCR repertoire. These issues can render vaccines ineffective and leave patients poorly protected against conditions associated with cell-associated antigens (e.g., intracellular infections and cancer).

[0009] At present, there are simply no reliable vaccines physicians can use to treat infectious diseases and cancers that require T cell mediated immunity.

SUMMARY OF THE DISCLOSURE

[0010] The current disclosure provides systems and methods to enhance the effectiveness of vaccines requiring or rendered more effective by T cell mediated immunity. The systems and methods rely on genetically modifying T cells to express a T cell receptor (TCR) that recognizes and binds a vaccine antigen that is administered to a subject. By ensuring that the subject has T cells expressing a TCR that will recognize and bind the vaccine antigen, the effectiveness of T cell mediated vaccinations is greatly expanded.

[0011] Particular embodiments include administering a polynucleotide to a subject wherein the polynucleotide encodes a TCR that binds a vaccine antigen that is administered to the subject.

[0012] In particular embodiments, the polynucleotide is administered to the subject as part of a nanoparticle (NP). The NP can include features that enhance the delivery and/or expression of the polynucleotide. For example, in particular embodiments, the NP includes a carrier molecule that condenses and protects the polynucleotide from enzymatic degradation. In particular embodiments, the NP includes a coating that shields the encapsulated polynucleotide and reduces or prevents off-target binding.

[0013] In particular embodiments, the NP includes a selective T cell targeting and delivery agent (T-DA). The T-DA allows the NP to be administered to a subject and results in selective delivery of the polynucleotide to selected T cells. Selective modification of CD4⁺ T cells to express a TCR is particularly useful to improve the efficacy of B-cell mediated vaccinations. Selective modification of CD8⁺ cytotoxic T cells to express a TCR is particularly useful to improve T cell mediated vaccinations. Both approaches provide vaccine antigen recognizing capabilities to T cells. Importantly, in embodiments incorporating a T-DA, a subject's existing T cells can be modified in vivo following, for example, intramuscular administration of the NP.

[0014] NP can also include other features to facilitate expression of polynucleotides delivered to a subject's T cells. For example, the NP can include endosomal release agents and/or nuclear targeting agents. Endosomal release agents promote escape of the delivered polynucleotide from the targeted T cell's endosome. Nuclear targeting agents direct polynucleotides towards and/or into the nucleus of the targeted cell.

[0015] Particular embodiments combine aspects of these features. For example, a NP can include (i) a polynucleotide encoding a TCR that binds a vaccine antigen that is administered to a subject; (ii) a condensing carrier molecule; (iii) a coating; (iv) a T-DA that selectively directs the NP to defined T cells (e.g., CD4⁺ or CD8⁺ T cells); (v) an endosomal release agent; and (iv) a nuclear targeting agent. This NP can be administered to the subject within a clinically relevant time window of receiving a vaccine antigen.

[0016] The systems and methods disclosed herein are particularly useful to increase the efficacy of vaccines that treat chronic conditions that require strong T cell immunity. Examples of such chronic conditions include chronic infections (e.g., acquired immune deficiency syndrome (AIDS), malaria, herpes, chlamydia, Epstein Barr virus (EBV), Pneumococcus, and Hepatitis B) and cancers.

BRIEF DESCRIPTION OF THE FIGURES

[0017] Many of the drawings submitted herein are better understood in color. Applicants consider the color versions of the drawings as part of the original submission and reserve the right to present color images of the drawings in later proceedings.

[0018] FIG.1. Schematic illustrating the overall approach to prevent vaccine failure through rational

T-cell receptor programming. Nanoparticles (NPs) are used to introduce engineered TCR genes into circulating host T cells, endowing them with antigen-recognizing capabilities, which are then selectively expanded using a peptide vaccine recognized by the transferred TCR.

[0019] FIG. 2. Schematics illustrating advantages of the disclosed systems and methods over conventional vaccines: The upper panel shows how injecting vaccine antigen/adjuvant often fails because immunized individuals have too few T cells with the appropriate receptors. The middle panel illustrates how NPs can be used to introduce engineered TCR genes into circulating T cells, endowing them with antigen-recognizing capabilities. These are then selectively expanded using a peptide vaccine recognized by the transferred TCR. The lower panel shows how programming CD4 helper T cells with vaccine-specific TCRs can boost the production of protective antibodies by generating high- affinity memory B cells.

[0020] FIGs. 3A-3C. Intramuscular injections of DNA-carrying nanoparticles (NPs) can efficiently introduce vaccine-specific TCRs into the peripheral T cell repertoire. (3A) Schematic of the T cell-targeted DNA nanoparticle used in described experiments. The NPs are prepared by mixing plasmid DNA with poly(β -amino ester) polymer, which condenses the plasmid DNA into nano-sized complexes. The particles were targeted by coupling the anti-CD8 antibody to polyglutamic acid (PGA), forming a conjugate that was electrostatically adsorbed to the particles. The inset is an electron micrograph of the NPs; scale bar, 100 nm. Also depicted are the two nanoparticle-encapsulated plasmids, which encode the OVA-specific OT-1 TCR and the hyperactive iPB7 transposase. (3B) Cytometric analysis of lymphocytes in draining lymph nodes. The percentages of cells in the bottom left quadrant and bottom right quadrant of each panel are, respectively: 82.7 and 17.3 (Vaccine only, Day 0); 75.1 and 24.8 (Vaccine only, Day 7); 85.3 and 14.7 (Vaccine only, Day 30); 85.3 and 14.7 (OVA TCR nanoparticles only, Day 0); 84.2 and 15.7 (OVA TCR nanoparticles only, Day 7); 87.5 and 12.4 (OVA TCR nanoparticles only, Day 30); 86.7 and 13.2 (Vaccine + OVA TCR nanoparticles, Day 0); 80.3 and 16.7 (Vaccine + OVA TCR nanoparticles, Day 7); 80.6 and 19.1 (Vaccine + OVA TCR nanoparticles, Day 30). (3C) Plots showing absolute numbers of NP-programmed OVA-reactive memory T cells on day 30.

[0021] FIGs. 4A, 4B. Combining T cell-targeted NPs encoding TCR₁₀₄₅ with mesothelin (MSLN) vaccines significantly prolongs survival of *Kras*^{LSL-G12D/+}; *Trp53*^{LSL-R172H/+}; *p48*^{Cre/+} (KPC) mice with established pancreatic ductal adenocarcinoma. (4A) Example tumor mass in the pancreas of a 4-month-old KPC mouse. (4B) Survival of KPC mice receiving either T cell-targeted NPs encoding the TCR₁₀₄₅, the MSLN vaccine, or both. Controls received no treatment. ms=mean survival.

[0022] FIG. 5. Representative gene sequence encoding the CD4 transmembrane domain (SEQ ID NO: 40).

[0023] FIG. 6. Representative cDNA sequence encoding a murine codon-optimized piggyBac

transposase (GenBank accession number: EF587698; SEQ ID NO: 142).

DETAILED DESCRIPTION

[0024] Lymphocytes are cells of the immune system involved in self/nonself recognition and acquired long-term immunity based on immunological memory. Lymphocytes can broadly be characterized as B cells or T cells. B cells are characterized by the presence of membrane-bound immunoglobulin (antibody) molecules which serve as receptors to bind soluble antigens. T cells are characterized by the presence of membrane-bound T cell receptors (TCR). TCR bind antigens only when the antigen is associated with a major histocompatibility complex (MHC) molecule (i.e., the antigen is not soluble). The specificity of T cell responses is conferred by particular TCR that bind particular antigens.

[0025] T lymphocytes include CD4⁺ T cells and CD8⁺ T cells. These types of T cells are distinguished in part by their expression of the cell surface molecules CD4 and CD8, respectively. They also, however, have different functions. CD4⁺ T cells, also referred to as helper T cells (T_H) facilitate the activities of other cell types. For example, CD4⁺ TH1 cells secrete various cytokines that activate cytotoxic T cells and macrophages to destroy cells harboring phagocytosed microorganisms. CD4⁺ TH2 cells secrete cytokines that activate B cells to produce antibodies. CD8⁺ cells are cytotoxic T lymphocytes (CTL) that can directly kill abnormal or infected cells.

[0026] Vaccines are formulations that produce an immune system response against a particular antigen by preemptively exposing the immune system to the antigen. A pathogen antigen can be an intact, but non-infectious form of a pathogen (e.g., heat-killed). Antigens can also be a protein or protein fragment of a pathogen or a protein or protein fragment expressed by an aberrant cell type (e.g. a cancer cell). When the immune system recognizes an antigen following preemptive exposure, it can lead to long-term immune memory so that if the antigen is encountered again, the immune system can quickly and effectively mount an effective response.

[0027] When a vaccine is delivered to a subject, antigen presenting cells (APC) of the immune system take up the antigen component and present it or a fragment thereof to B cells and T cells. B cells that express receptors specific for the presented antigen will produce and secrete antibodies that circulate through the body to elicit a quick, robust immune response if the antigen is encountered again later in life. Standard vaccines are designed to function via such antibody responses created by B cells. The effectiveness of B cell immunity, however, is limited to soluble (i.e., extracellular) pathogens. Pathogens that exist intracellularly (e.g., those that cause AIDS, malaria, herpes, and chlamydia) or remain cell-associated (e.g., cancer cell antigens) are not as susceptible to B cell antibodies. Further, the effectiveness of B cell immunity is enhanced when the vaccine antigen is similarly recognized by CD4⁺ helper T cells.

[0028] For antigens that are cell-associated (e.g., intracellular or membrane-bound), T cell mediated immunity is required for effective immunization. Vaccines that require T cell mediated immunity often fail, however, because a host (e.g., person, research animal) does not have T cells expressing particular TCR that recognize and bind the presented vaccine antigen. People with compromised immune systems (e.g., the elderly) are especially vulnerable to this problem, because their declining production of new T cells leads to “holes” in their TCR repertoire. These issues can render vaccines ineffective and leave patients poorly protected against infection by intracellular pathogens and/or cancer.

[0029] At present, there are simply no reliable vaccines physicians can use to treat infectious diseases and cancers that require T cell mediated immunity.

[0030] The current disclosure provides systems and methods to enhance the effectiveness of vaccines requiring or rendered more effective by T cell mediated immunity. The systems and methods rely on genetically modifying T cells to express a T cell receptor (TCR) that recognizes and binds a vaccine antigen that is administered to a subject. By ensuring that the subject has T cells expressing TCR that will recognize and bind the vaccine antigen, the effectiveness of T cell mediated vaccinations is greatly expanded.

[0031] Particular embodiments include administering a polynucleotide to a subject wherein the polynucleotide encodes a TCR that binds a vaccine antigen that is administered to the subject.

[0032] In particular embodiments, the polynucleotide is administered to the subject as part of a nanoparticle (NP). The NP can include features that enhance the delivery and/or expression of the polynucleotide. For example, in particular embodiments, the NP includes a carrier molecule that condenses and protects the polynucleotide from enzymatic degradation. As disclosed in more detail elsewhere herein, such carriers can include positively charged lipids and/or polymers. Particular embodiments utilize poly(β -amino ester).

[0033] In particular embodiments, the NP includes a coating that shields the encapsulated polynucleotide and reduces or prevents off-target binding. Off-target binding is reduced or prevented by reducing the surface charge of the NP to neutral or negative. As disclosed in more detail elsewhere herein, coatings can include neutral or negative polymer- and/or liposome-based coatings. Particular embodiments utilize polyglutamic acid (PGA) as a NP coating. When used, the coating need not necessarily coat the entire NP, but must be sufficient to reduce off-target binding by the NP.

[0034] In particular embodiments, the NP includes a selective T cell targeting and delivery agent (T-DA). The T-DA allows the NP to be administered to a subject and results in selective delivery of the polynucleotide to selected T cells. Selective modification of CD4⁺ T cells to express a TCR is particularly useful to improve the efficacy of B-cell mediated vaccinations. Selective modification of

CD8+ cytotoxic T cells to express a TCR is particularly useful to improve T cell mediated vaccinations. Both approaches provide vaccine antigen recognizing capabilities to T cells. Importantly, in embodiments incorporating a T-DA, a subject's existing T cells can be modified in vivo following, for example, intramuscular administration of the NP.

[0035] NP can also include other features to facilitate expression of polynucleotides delivered to a subject's T cells. For example, the NP can include endosomal release agents and/or nuclear targeting agents. Endosomal release agents promote escape of the delivered polynucleotide from the targeted T cell's endosome. Nuclear targeting agents direct polynucleotides towards and/or into the nucleus of the targeted cell.

[0036] Particular embodiments combine aspects of these features. For example, a NP can include (i) a polynucleotide encoding a TCR that binds a vaccine antigen that is administered to the subject; (ii) a positively-charged carrier; (iii) a neutral or negatively-charged coating; (iv) a T-DA that selectively directs the NP to defined T cells (e.g., CD4+ or CD8+ T cells); (v) an endosomal release agent; and (vi) a nuclear targeting agent. This NP can be administered to the subject within a clinically relevant time window of receiving a vaccine antigen.

[0037] The systems and methods disclosed herein are particularly useful to increase the efficacy of vaccines that treat chronic infections and cancers that require strong T cell immunity. Examples of such chronic infections include acquired immune deficiency syndrome (AIDS), malaria, herpes, chlamydia, Epstein Barr virus (EBV), Pneumococcus, and Hepatitis B.

[0038] FIG. 2 provides schematic representations underlying the systems and methods disclosed herein. The top 3 panels depict the poor T cell priming observed with conventional vaccine antigen administrations. The middle 3 panels depict genetic reprogramming of CD8+ T cells to recognize administered vaccine antigen to yield increased T cell priming to support T cell mediated immunity. The bottom 3 panels depict genetic reprogramming of CD4+ T cells to recognize administered vaccine antigen to yield increased T cell priming to help and support robust antibody production by B cells. Thus, particular embodiments include administering a polynucleotide to a subject wherein the polynucleotide genetically reprograms a T cell to express a TCR that binds a vaccine antigen that is administered to the subject.

[0039] Aspects of the disclosure are now described in more detail and in the following order: (I) TCRs; (II) polynucleotides (PN) encoding engineered TCRs; (III) nanoparticles (NP); (IV) T cell targeting and delivery agents (T-DA); (V) endosomal release agents (ERA); (VI) nuclear targeting agents (NTA); (VII) vaccine antigens; (VIII) vaccine adjuvants; (IX) compositions; (X) kits; and (XI) methods of use.

[0040] I. T Cell Receptors (TCRs). As indicated, TCR are molecules found on the surface of T cells that recognize and bind antigens associated with major histocompatibility complex (MHC) molecules.

[0041] Each TCR includes two disulfide-linked heterodimeric transmembrane proteins. That is, each TCR is a heterodimer. In 95% of T cells in peripheral blood, each TCR includes an alpha (α) chain and a beta (β) chain. The remaining 5% of T cells in peripheral blood, include a gamma (γ) chain and a delta (Δ) chain.

[0042] Each TCR chain includes a variable domain, which confers the antigen specificity of the T cell. These variable domains are similar to those of Ig variable (V) chains.

[0043] Other portions of the chains include several invariant domains such as a constant domain, a transmembrane domain, and a short cytoplasmic tail. Membrane-anchored C-terminal domains are analogous to Ig constant (C) domains.

[0044] To achieve functional form, TCR associate non-covalently with CD3, forming the TCR-CD3 membrane complex. CD3, the signal transduction element of the TCR, is composed of a group of invariant proteins called γ , Δ , epsilon (Σ), zeta (Z) and eta (H) chains. The γ , Δ , and Σ chains are structurally-related, each containing an Ig-like extracellular constant domain followed by a transmembrane region and a cytoplasmic domain of more than 40 amino acids. The Z and H chains have a distinctly different structure: both have a very short extracellular region of only 9 amino acids, a transmembrane region and a long cytoplasmic tail including 113 and 115 amino acids in the Z and H chains, respectively. The invariant protein chains in the CD3 complex associate to form noncovalent heterodimers of the Σ chain with a γ chain ($\Sigma\gamma$) or with a Δ chain ($\Sigma\Delta$) or of the Z and H chain (ZH), or a disulfide-linked homodimer of two Z chains (ZZ). 90% of the CD3 complex incorporate the ZZ homodimer.

[0045] The cytoplasmic regions of the CD3 chains include a motif designated the immunoreceptor tyrosine-based activation motif (ITAM). This motif is found in a number of other receptors including the Ig- α /Ig- β heterodimer of the B-cell receptor complex and Fc receptors for IgE and IgG. The ITAM sites associate with cytoplasmic tyrosine kinases and participate in signal transduction following TCR-mediated triggering. In CD3, the γ , Δ and Σ chains each contain a single copy of ITAM, whereas the Z and H chains harbor three ITAMs in their long cytoplasmic regions. Indeed, the Z and H chains have been ascribed a major role in T cell activation signal transduction pathways.

[0046] There are numerous ways to identify and select particular TCR for use within particular applications of the disclosed systems and methods. For example, the sequences of numerous TCR that bind particular antigen fragments are known and publicly available.

[0047] TCR can also be identified for use with a particular vaccine by, for example, isolating T cells that bind a particular vaccine antigen/MHC complex and sequencing the TCR chains binding the complex. As examples, antigen-specific T cells may be induced by in vitro cultivation of isolated human T cells in the presence of an antigen/MHC complex. TCR genes encoding TCR that bind the antigen/MHC complex can be readily cloned by, for example, the 5' RACE procedure using primers

corresponding to the sequences specific to the TCR α -chain gene and the TCR β -chain gene.

[0048] Various analogs of natural TCR ligands have been produced which include extracellular domains of MHC molecules bound to a specific peptide antigen. Several such analogs have been purified as detergent extracts of lymphocyte membranes or produced as recombinant proteins (see, for example, Sharma et al., PNAS. 88: 11465-69, 1991; Kozono et al., Nature 369: 151-54, 1994; Arimilli et al., J. Biol. Chem. 270: 971-77, 1995; Nag, PNAS 90: 1604-08, 1993; Nag et al., J. Biol. Chem. 271: 10413-18, 1996; Rhode et al., J. Immunol. 157: 4885-91, 1996; Fremont et al., Science 272: 1001, 1996; Sharma et al., Proc. Natl. Acad. Sci. USA 88: 11405, 1991; Nicolle et al., J. Clin. Invest. 93: 1361, 1994; Spack et al., CNS Drug Rev. 4: 225, 1998). Such analogs can be used to isolate T cells to then sequence the TCR of interest for a particular application.

[0049] In particular embodiments, it may be necessary to pair TCR chains following sequencing (i.e., to perform paired chain analysis). Various methods can be utilized to pair isolated α and β chains that bind an antigen/MHC complex such that the pairing results in a TCR that binds to an antigen/MHC complex when expressed by a genetically modified T cell. In particular embodiments post-sequencing pairing may be unnecessary or relatively simple, for example in embodiments in which the α and β chain pairing information is not lost in the procedure, such as if one were to sequence from single cells. In particular embodiments, chain pairing may be assisted in silico by computer methods. For example, specialized, publicly available immunology gene alignment software is available from IMGT, JOINSOLVER, VDJSolver, SoDA, iHMMune-align, or other similar tools for annotating VDJ gene segments.

[0050] In particular embodiments, chain pairing may be performed using VDJ antibodies. For example, one may obtain antibodies for the identified segments and use the antibodies to purify a subset of cells that express that gene segment in their (surface) receptors (e.g. using FACS, or immunomagnetic selection with microbeads). One may then sequence from this subset of cells which have been purified for the desired gene segments. If necessary, this secondary sequencing may be done more deeply (i.e. at a higher resolution) than the first round of sequencing. In this second sequence data set, there will be far fewer induced clonotypes, greatly easing the task of chain pairing. Depending on the gene segments, there may be only one induced α chain and one induced β chain for example.

[0051] In particular embodiments, chain pairing may be performed using multiwell sequencing. For example, one may isolate gene segment purified cells or unpurified cells into a microwell plate, where each microwell has a very low number of cells. One can amplify and sequence the cells in each well individually, which provides another means to pair the chains of interest by sequencing on a single cell basis, facilitating the pairing of induced α and β chains. Assays such as PairSEQ® (Adaptive Biotechnologies Corp., Seattle, WA) have also been developed.

[0052] Following selection and/or identification of a TCR of interest for a particular vaccine application, any portion of the TCR can be used and variants of the TCR can be used, so long as when expressed by a genetically modified T cell, the expressed TCR binds the intended vaccine/MHC complex and results in T cell activation.

[0053] In particular embodiments, an engineered TCR includes a single chain T cell receptor (scTCR) including V α / β and C α / β chains (e.g., V α -C α , V β -C β , V α -V β) or including V α -C α , V β -C β , V α -V β pair specific for a target of interest (e.g., peptide-MHC complex).

[0054] In particular embodiments, engineered TCR include 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 an amino acid sequence of a known or identified TCR V α , V β , C α , or C β , wherein each CDR includes zero changes or at most one, two, or three changes, from a TCR or fragment or derivative thereof that specifically binds to the target of interest.

[0055] In particular embodiments, engineered TCR include V α , V β , C α , or C β regions derived from or based on a V α , V β , C α , or C β of a known or identified TCR (e.g., a high-affinity TCR) and includes 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 V α , V β , C α , or C β of a known or identified TCR. An insertion, deletion or substitution may be anywhere in a V α , V β , C α , or C β region, including at the amino- or carboxy-terminus or both ends of these regions, provided that each CDR includes zero changes or at most one, two, or three changes and provides a target binding domain containing a modified V α , V β , C α , or C β region can still specifically bind its target with an affinity and action similar to wild type.

[0056] There are two types of MHC molecules that can be bound by TCR: MHC class I molecules and MHC class II molecules. In the context of expressed TCR and particular uses described herein, it can be useful to express MHC class I restricted or MHC class II restricted TCR. A discussion of these distinct classes of MHC molecules is therefore provided.

[0057] MHC Class I molecules include a polymorphic heavy chain (α) non-covalently associated with a monomorphic (in humans) non-MHC encoded light chain protein of 12 kDa, termed β_2 microglobulin (β_2m). The heavy α chain is a polymorphic transmembrane glycoprotein of 45 kDa including 3 extracellular domains, each including 90 amino acids (α_1 at the N-terminus, α_2 and α_3), a transmembrane region of 40 amino acids and a cytoplasmic tail of 30 amino acids. The α_1 and α_2 domains, the membrane distal domains, form the peptide-binding groove or cleft having a sufficient size to bind a peptide of 8-10 amino acids, whereas the α_3 domain is proximal to the plasma membrane. β_2m has a single immunoglobulin (Ig)-like domain, not anchored to the plasma

membrane, and interacts mainly with the α_3 chain, which also possesses a characteristic Ig fold. In humans, there are three α chain genes, called HLA-A, HLA-B and HLA-C, for each of which multiple alleles have been identified. In mice, there are three α chain genes, called H-2K, H-2D and H-2L.

[0058] MHC Class II molecules include two different polypeptide chains, a 33-kD α chain and a 28-kDa β chain, which associate by noncovalent interactions. Like class I MHC molecules, class II MHC molecules are membrane-bound glycoproteins that contain extracellular domains, a transmembrane segment and a cytoplasmic tail. Each chain in these noncovalent heterodimeric complexes includes two extracellular domains: α_1 and α_2 domains and β_1 and β_2 domains. The membrane-distal domain of a class II molecule is composed of the α_1 and β_1 domains and forms the peptide-binding groove or cleft having a sufficient size to bind a peptide, which is typically of 13-18 amino acids. The membrane-proximal domains, α_2 and β_2 , have structural similarities to Ig constant (C) domains.

[0059] The genes that encode the various polypeptide chains that associate to form MHC complexes in mammals have been studied and described in extensive detail. In humans, MHC molecules (with the exception of class I β_2m) are encoded in the HLA region of the genome, located on chromosome 6. There are three class I MHC α -chain-encoding loci, termed HLA-A, HLA-B and HLA-C. In the case of MHC class II proteins, there are three pairs of α and β chain loci, termed HLA-DR(A and B), HLA-DP(A and B), and HLA-DQ(A and B). In rats, the class I α gene is designated RT1.A, while the class II genes are termed RT1.B α and RT1.B β . More detailed description regarding the structure, function and genetics of MHC complexes can be found, for example, in Immunobiology: The Immune System in Health and Disease by Janeway and Travers, Current Biology Ltd./Garland Publishing, Inc. (1997), and in Bodmer et al. (1994) "Nomenclature for factors of the HLA system" Tissue Antigens vol. 44, pages 1-18.

[0060] During T cell development, T cells in the thymus are presented with peptide/HLA complexes and undergo selection based on this interaction. T cell selection can result in T cells that are restricted to interactions with a particular class of HLA molecule, known as HLA-restriction. For example, during selection a T cell can differentiate to become a class I restricted CD8⁺ T cell due to effective interactions between the TCR and a peptide/HLA class I complex, or can become a class II restricted CD4⁺ T cell due to effective interactions between the TCR and a peptide/HLA class II complex. The complementarity regions 1-3 (CDRs 1-3) of a TCR engage the peptide/HLA complex. Therefore, amino acid sequences of CDRs 1-3 can be determinants of whether a T cell is HLA class I or HLA class II restricted. Coreceptor expression is also an important feature of T cell class restriction. To initiate signaling for T cell activation in response to an antigen, HLA class I molecules can interact with CD4⁺ coreceptors, whereas HLA class II molecules can interact with CD8⁺ coreceptors. Therefore, a T cell engineered to express a TCR that engages a peptide/HLA class I complex can become activated if it expresses the coreceptor CD8, whereas a T cell engineered to express a TCR

that engages a peptide/HLA class II complex can become activated if it expresses the coreceptor CD4.

[0061] Thus, absent genetic engineering to alter the following, CD8+ T cells recognize MHC class I molecules while CD4+ T cells recognize MHC class II molecules. In particular embodiments then, CD8+ T cells can be genetically modified to express HLA-class I restricted TCR and CD4+ T cells can be genetically modified to express HLA-class II restricted TCR.

[0062] In particular embodiments, TCR can include: α chain:

MNSSLDFLILILMFGGTSSNSVKQTGQITVSEGASVTMNCTYTSTGYPTLFWYVEYPSKPLQLLQR
ETMENSKNFGGGNIKDKNSPIVKYSVQVSDSAVYYCLLRNHDKLIFGTGTRLQVFPNIQNPDPAVY
QLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACA
NAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRLWSS

(SEQ ID NO: 1); and β chain:

MGPGLLCWLLCCLLGAGSVETGVTQSPTHLIKTRGQQVTLRCSSQSGHNTVSWYQQALGQGPGQ
FIFQYYREEENGRGNFPPRFSGLQFPNYSSELNVNALELDDSAHYLCASSQDSYNEQFFGPGTRL
TVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQP
LKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEA
WGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSAVLMMAMVKRKDSRG (SEQ ID NO: 2).

In particular embodiments, TCR can include: α chain:

MNSSLDFLILILMFGGTSSNSVKQTGQITVSEGASVTMNCTYTSTGYPTLFWYVEYPSKPLQLLQR
ETMENSKNFGGGNIKDKNSPIVKYSVQVSDSAVYYCLLRNHDKLIFGTGTRLQVFPNIQNPDPAVY
QLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACA
NAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRLWSS

(SEQ ID NO: 1); and β chain:

MGPGLLCWLLCCLLGAGSVETGVTQSPTHLIKTRGQQVTLRCSSQSGHNTVSWYQQALGQGPGQ
FIFQYYREEENGRGNFPPRFSGLQFPNYSSELNVNALELDDSAHYLCASSLAGGYGDTQYFGPGT
RLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDP
QPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAE
AWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSAVLMMAMVKRKDSRG (SEQ ID NO:

3). In particular embodiments, TCR can include: α chain:

MKKLLAMILWLQLDRLSGELKVEQNPLFLSMQEGKNYTIYCNYSTTSDRLYWYRQDPGKSLESFL
VLLSNGAVKQEGRLMASLDTKARLSTLHITAAVHDLSATYFCAVGNYGGSQGNLIFGKGTKLSVKP
NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAW
SNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLL

MTLRLWSS (SEQ ID NO: 4); and β chain:

MGPGLLCWLLCCLLGAGSVETGVTQSPTHLIKTRGQQVTLRCSSQSGHNTVSWYQQALGQGPGQ

FIFQYYREEENGRGNFPPRFSGLQFPNYSSELNVNALELDDSAHYLCASSQDSYNEQFFGPGTRL
TVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVLSWWVNGKEVHSGVSTDPQP
LKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEA
WGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSAVLMMAMVKRKDSRG (SEQ ID NO: 2).

In particular embodiments, TCR can include: α chain:

MKKLLAMILWLQLDRLSGELKVEQNPLFLSMQEGKNYTIYCNYSTTSDRLYWYRQDPGKSLESFL
VLLSNGAVKQEGRLMASLDTKARLSTLHITAAVHDLSATYFCAVGNYGGSQGNLIFGKGTKLSVKP
NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAW
SNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLVIGFRILLKLVAGFNLL
MTLRLWSS (SEQ ID NO: 4); and β chain:

MGPGLLCWVLLCLLGAGSVETGVTQSPTHLIKTRGQQVTLRCSSQSGHNTVSWYQQALGQGPQ
FIFQYYREEENGRGNFPPRFSGLQFPNYSSELNVNALELDDSAHYLCASSLAGGYGDTQYFGPGT
RLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVLSWWVNGKEVHSGVSTDP
QPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAE
AWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSAVLMMAMVKRKDSRG (SEQ ID NO:

3). In particular embodiments, TCR can include a human α chain variable domain having a sequence of:

MNSSLDFLILILMFGGTSSNSVKQTGQITVSEGASVTMNCTYTSTGYPTLFWYVEYPSKPLQLLQR
ETMENSKNFGGGNIKDKNSPIVKYSVQVSDSAVYYCLLRNHDKLIFGTGTRLQVFPN (SEQ ID NO:
5) or

MKKLLAMILWLQLDRLSGELKVEQNPLFLSMQEGKNYTIYCNYSTTSDRLYWYRQDPGKSLESFL
VLLSNGAVKQEGRLMASLDTKARLSTLHITAAVHDLSATYFCAVGNYGGSQGNLIFGKGTKLSVKP
N (SEQ ID NO: 6). In particular embodiments, TCR can include a human β chain variable domain having a sequence of:

MGPGLLCWVLLCLLGAGSVETGVTQSPTHLIKTRGQQVTLRCSSQSGHNTVSWYQQALGQGPQ
FIFQYYREEENGRGNFPPRFSGLQFPNYSSELNVNALELDDSAHYLCASSQDSYNEQFFGPGTRL
TVLE (SEQ ID NO: 7) or

MGPGLLCWVLLCLLGAGSVETGVTQSPTHLIKTRGQQVTLRCSSQSGHNTVSWYQQALGQGPQ
FIFQYYREEENGRGNFPPRFSGLQFPNYSSELNVNALELDDSAHYLCASSLAGGYGDTQYFGPGT
RLTVLE (SEQ ID NO: 8). In particular embodiments, TCR can include a human α chain variable domain having a CDR3 sequence of: CLLRNHDKLIF (SEQ ID NO: 9) or CAVGNYGGSQGNLIF (SEQ ID NO: 10). In particular embodiments, TCR can include a human β chain variable domain having a CDR3 sequence of: CASSQDSYNEQFF (SEQ ID NO: 11) or CASSLAGGYGDTQYF (SEQ ID NO: 12). TCRs including these α and β CDR3, variable domain, and/or chain sequences bind Mesothelin (MSLN) peptide-HLA complex. In particular embodiments, TCRs including these α

and β CDR3, variable domain, and/or chain sequences bind to a SLLFLLFSL (SEQ ID NO: 13):HLA-A*201 complex or a VLPLTVAEV (SEQ ID NO: 14):HLA-A*201 complex. MSLN is a tumor antigen that is highly expressed in many human cancers, including malignant mesothelioma and pancreatic, ovarian, and lung adenocarcinomas. It is an attractive target for cancer immunotherapy because its normal expression is limited to mesothelial cells, which are dispensable. In particular embodiments, the α and β genes of human TCR specific for MSLN have been codon optimized and linked by a porcine teschovirus-1 2A element. TCR sequences specific for human MSLN are described in Stromnes, IM et al. (2015) Cancer cell 28(5): 638-652 and WO 2017/112944.

[0063] In particular embodiments, TCR can include a murine V α 4 chain having a CDR3 sequence of: LDYANKMI (SEQ ID NO: 15) and a V β 9 chain having a CDR3 sequence of: PQDTQYFF (SEQ ID NO: 16) described in Stromnes, IM et al. (2015), *supra*. The murine TCR, called TCR₁₀₄₅, was derived from T cell clones of Msln^{-/-} mice engineered to express recombinant murine Msln and specific for the Msln₄₀₆₋₄₁₄ epitope. TCR₁₀₄₅ binds Msln₄₀₆₋₄₁₄ peptide (GQKMNAQAI, SEQ ID NO: 17) with high affinity. In particular embodiments, the V α 4 and V β 9 genes of TCR₁₀₄₅ have been codon optimized and linked by a porcine teschovirus-1 2A element.

[0064] In particular embodiments, TCR can include: α chain:

MQKEVEQNSGPLSVPEGAIASLNCTYS DRGSQSFFWYRQYSGKSP ELIMFIYSNGDKEDGRFTA
QLNKASQYISLLIRDSKLSKATYLC AVR TNSGYALNFGKGT SLLVTPHIQKPDPAVYQLRDSKSSDK
SVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSA VAWSNKSDFACANAFNNSIIPED
TFFPSPESS (SEQ ID NO: 18); and β chain:

MEAGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYEEEEERQRGNFPDR
FSGHQFPNYSSELNVNALLLGDSALYLCASSDTVSYEQYFGPGTRTVTEDLKNVFPPEVAVFEPS
EAEISHTQKATLVCLATGFYPDHVELSWVWNGKEVHSGVCTDPQPLKEQPALNDSRYALSSRLRV
SATFWQDPRNHFRQCQVQFYGLSENDEWTQDRAKPV TQIVSAEAWGRAD (SEQ ID NO: 19). This
 α and β chain combination binds the HIV Gag peptide SL9 (SLYNTVATL (SEQ ID NO: 20)) and
confers anti-HIV activity to CD8⁺ T cells (see, e.g., Varela-Rohena, et al. 2008. Nature Medicine.
14(12): 1390-1395).

[0065] In particular embodiments, TCR can include: α chain:

[0066] METLLGLLILWLQLQWSSKQEVTQIPAALSVPEGENLV LNCSFTDSAIYNLQWFRQDPGK
GLTSLLLIQSSQREQ TSGRLNASLDKSSGRSTLYIAASQPGDSATYLC AVR RNDMRFGAGTRLT VK
PNIQNP (SEQ ID NO: 21); and β chain:

MGIRLLCRVAFCFLAVGLVDVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLI
YFSYDVKMKEKGDIP EGYSVSREKKERFSLILESASTNQ TSMYLCASSPGALD TDTQYFGPGTRLT
VVEDIKNVFPP (SEQ ID NO: 22). This α and β chain combination binds EBV antigen (see, e.g.,
Kobayashi, et al. 2013. Nature Medicine 19: 1542-1546).

[0067] In particular embodiments, TCR can include: α chain:

MTSIRAVFIFLWLQLDLVNGENVEQHPSTLSVQEGDSAVIKCTYSDSASNYFPWYKQELGKRPQLII
DIRSNVGEKKDQRIAVTLNKTAKHFSLHITETQPEDSAVYFCAATEDYQLIWGAGTKLIIKPDIQNPD
PAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSD
FACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNLFQNLSVIGFRILLKLVAGFNLLMTLRL
WSS (SEQ ID NO: 23); and β chain:

[0068] MSNQVLCCVVLCLGANTVDGGITQSPKYLFRKEGQNVTLSCSEQNLNHDAMYWYRQDPG
QGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASSPGALYEQYFGPG
TRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELSWWWNGKEVHSGVSTD
PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSA
EAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG (SEQ ID NO:
24). In particular embodiments, TCR can include: α chain:

MTSIRAVFIFLWLQLDLVNGENVEQHPSTLSVQEGDSAVIKCTYSDSASNYFPWYKQELGKRPQLII
DIRSNVGEKKDQRIAVTLNKTAKHFSLHITETQPEDSAVYFCAATEDYQLIWGAGTKLIIKPDIQNPD
PAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSNKSD
FACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNLFQNLSVIGFRILLKLVAGFNLLMTLRL
WSS (SEQ ID NO: 25); and β chain:

MSNQVLCCVVLCLGANTVDGGITQSPKYLFRKEGQNVTLSCSEQNLNHDAMYWYRQDPGQGLR
LIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASSPGALYEQYFGPGTRLTV
TEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELSWWWNGKEVHSGVCTDPQPLK
EQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWG
RADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG (SEQ ID NO: 26).

These α and β chain combinations bind a human Wilms tumor protein 1 (WT-1) antigen (see, e.g., US2016/0083449). WT1 is an intracellular protein that is overexpressed in a number of cancers, including acute myeloid leukemia and non-small cell lung, breast, pancreatic, ovarian, and colorectal cancers. T cells engineered with a TCR that binds a WT-1 epitope are being tested in a clinical trial for patients with high risk or relapsed acute myeloid leukemia, myelodysplastic syndrome, or chronic myelogenous leukemia, previously treated with donor stem cell transplant (Trial Number NCT01640301).

[0069] In particular embodiments, TCR can include: α chain:

MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSCYDTSESDYYLFWYKQPPSRQ
MILVIRQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCALRSSGTYYKYIFGTGTRL
KVLANIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS
AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNLFQNLSVIGFRILLKVA
GFNLLMTLRLWSS (SEQ ID NO: 27); and β chain:

MGTRLLFWWAFCLLGADHTGAGVSQSPSNKVTEKGKDVELRCDPISGHTALYWYRQSLGQGLEF
LIYFQGNAPDKSGLPSDRFSAERTGGSVSTLTIQRTQQEDSAVYLCASIRTGPFFSGNTIYFGEG
SWLTVVEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWWNGKEVHSGVSTD
PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSA
EAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO: 28).

In particular embodiments, TCR can include: α chain:

MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSCTYDTSESDYYLFWYKQPPSRQ
MILVIRQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCALRASGTYKYIFGTGTRL
KVLANIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS
AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNLFQNLSVIGFRILLKVA
GFNLLMTLRLWSS (SEQ ID NO: 29); and β chain:

MGTRLLFWWAFCLLGADHTGAGVSQSPSNKVTEKGKDVELRCDPISGHTALYWYRQSLGQGLEF
LIYFQGNAPDKSGLPSDRFSAERTGGSVSTLTIQRTQQEDSAVYLCASIRTGPFFSGNTIYFGEG
SWLTVVEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWWNGKEVHSGVSTD
PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSA
EAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO: 28).

In particular embodiments, TCR can include: α chain:

MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSCTYDTSESDYYLFWYKQPPSRQ
MILVIRQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCALRSAGTYKYIFGTGTRL
KVLANIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS
AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNLFQNLSVIGFRILLKVA
GFNLLMTLRLWSS (SEQ ID NO: 30); and β chain:

MGTRLLFWWAFCLLGADHTGAGVSQSPSNKVTEKGKDVELRCDPISGHTALYWYRQSLGQGLEF
LIYFQGNAPDKSGLPSDRFSAERTGGSVSTLTIQRTQQEDSAVYLCASIRTGPFFSGNTIYFGEG
SWLTVVEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWWNGKEVHSGVSTD
PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSA
EAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO: 28).

In particular embodiments, TCR can include: α chain:

MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSCTYDTSESDYYLFWYKQPPSRQ
MILVIRQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCALRVSGTYKYIFGTGTRL
KVLANIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS
AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNLFQNLSVIGFRILLKVA
GFNLLMTLRLWSS (SEQ ID NO: 31); and β chain:

MGTRLLFWWAFCLLGADHTGAGVSQSPSNKVTEKGKDVELRCDPISGHTALYWYRQSLGQGLEF
LIYFQGNAPDKSGLPSDRFSAERTGGSVSTLTIQRTQQEDSAVYLCASIRTGPFFSGNTIYFGEG

SWLTVVEDLNKVPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWWNGKEVHSGVSTD
PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSA
EAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO: 28).

In particular embodiments, TCR can include: α chain:

MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSTYDTSESDYYLFWYKQPPSRQ
MILVIRQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCALRSSGTYYKIFGTGTRL
KVLANIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKTVLDMKAMDSKSNGA
IAWSNQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLNFQNLSVMGLRILLKLVAGFNLL
MTLRLWSS (SEQ ID NO: 32); and β chain:

MGTRLLFWWAFCLLGADHTGAGVSQSPSNKVTEKGKDVELRCDPISGHTALYWYRQSLGQGLEF
LIYFQGNAPDKSGLPSDRFSAERTGGSVSTLTIQRTQQEDSAVYLCASIRTGPFFSGNTIYFGE
SWLTVVEDLRNVTPPKVSLFEPKAEIANKQKATLVCLARGFFPDHVELSWWWNGKEVHSGVSTD
PQAYKESNYSYCLSSRLRVSATFWHNPVNFRCQVQFHGLSEEDKWPEGSPKPVTVQNISAEAW
GRADCGITSASYHQQGVLSATILYEILLGKATLYAVLVSGLVLMAMVKRKNS (SEQ ID NO: 33).

These α and β chain combinations bind MAGE A3/MAGE A6 antigens (see, e.g., US2015/0246959). MAGE A proteins are testis-specific E3 ubiquitin ligase components whose expression is upregulated in many cancers. MAGE A3 and A6 are frequently overexpressed in common solid tumors including bladder, esophageal, head and neck, lung and ovarian cancers. T cells engineered with a TCR that binds a MAGE A3/MAGE A6 antigen are being tested in a clinical trial for patients who are HLA-DPB1*04:01 positive and whose tumors are MAGE-A3 and/or MAGE-A6 positive (Trial Number NCT03139370).

[0070] In particular embodiments, TCR can include: α chain:

MQEVTVIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLTSLLLIQSSQREQTSGRLNASL
DKSSGRSTLYIAASQPGDSATYLC AVRPLYGGSYIPTFGRGTSLIVHPYIQNPDPVAVYQLRDSKSS
DKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIP
EDTFFPSPESS (SEQ ID NO: 34); and β chain:

MGVTQTPKFQVLKTGQSM TLQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQGEVPNGY
NVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNTGELFFGEGSRLTVLEDLKNVFPPEVAVFEP
EAEISHTQKATLVCLATGFYPDHVELSWWWNGKEVHSGVSTD PQPLKEQPALNDSRYALSSRLRV
SATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRAD (SEQ ID NO: 35). In

particular embodiments, TCR can include: α chain:

MQEVTVIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLTSLLLIQSSQREQTSGRLNASL
DKSSGRSTLYIAASQPGDSATYLC AVRPLYGGSYIPTFGRGTSLIVHPYIQNPDPVAVYQLRDSKSS
DKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIP
EDTFFPSPESS (SEQ ID NO: 36); and β chain:

MGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQGEVPNGY
 NVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNTGELFFGEGSRLTVLEDLKNVFPPEVAVFEPS
 EAEISHTQKATLVCLATGFYPDHVELSWVWNGKEVHSGVCTDPQPLKEQPALNDSRYALSSRLRV
 SATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRAD (SEQ ID NO: 37).

These α and β chain combinations bind the SLLMWITQC (SEQ ID NO: 38)-HLA-A*0201 complex. The SLLMWITQC (SEQ ID NO: 38) peptide is derived from a human tumor antigen NY-ESO-1 of the cancer/testis family. NY-ESO-1 is being studied as a possible target for a cancer vaccine or immunotherapy. It is highly expressed in many poor-prognosis melanomas. T cells engineered with a TCR that binds the SLLMWITQC (SEQ ID NO: 38)-HLA-A*0201 complex are being tested in a clinical trial for patients with ovarian cancer (Trial Number NCT01567891). Robbins PF *et al.* (2008) The Journal of Immunology 180(9): 6116-6131 and U.S. 8,008,438 disclose TCR α and β chain sequences that bind the SLLMWITQC (SEQ ID NO: 38)-HLA-A*0201 complex.

[0071] In particular embodiments, TCR can include an engineered TCR such as that described in WO2011039507. Such TCR include an α chain and a β chain separated by an internal self-cleaving porcine teschovirus 2A sequence and binds a human herpesvirus-5, or cytomegalovirus (CMV) antigen. One example includes the anti-CMV artificial TCR:

MEKNPLAAPLLILWFHLDCVSILNVEQSPQSLHVQEGDSTNFTCSFPSSNFYALHWYRWETAKSP
 EALFVMTLNGDEKKG (SEQ ID NO: 39).

[0072] II. Polynucleotides (PN) Encoding TCR. PN describes a nucleic acid molecule including a nucleic acid sequence encoding a TCR that binds an antigen/MHC complex such that upon introduction into a T cell, the PN causes expression of the encoded TCR. Administered PN can include a gene. The term "gene" refers to a nucleic acid sequence that encodes a TCR for use in a system or method described herein. The definition of "gene" includes various sequence polymorphisms, mutations, and/or sequence variants wherein such alterations do not significantly affect the function of the encoded TCR. The term "gene" may include not only coding sequences but also regulatory regions such as promoters, enhancers, and termination regions. The term further can include all introns and other DNA sequences spliced from the mRNA transcript, along with variants resulting from alternative splice sites. Nucleic acid sequences encoding the TCR can be DNA or RNA that direct the expression of the TCR. 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 T cell. Many gene sequences to encode TCR are available in publicly available databases and publications. Those of ordinary skill in the art can also derive such gene sequences based on

identification of a TCR of interest.

[0073] "Encoding" refers to a property of sequences of nucleotides in a PN, such as a plasmid, a gene, cDNA, or mRNA, to serve as a template for synthesis of a TCR. A PN can, e.g., encode a protein if transcription and translation of mRNA produced by a gene produces the protein in a cell or other biological system.

[0074] In particular embodiments, the PN includes a plasmid, a cDNA, or an mRNA that includes a gene for expressing a TCR. Suitable plasmids include standard plasmid vectors and minicircle plasmids that can be used to transfer a gene to a T cell. The PN (e.g., minicircle plasmids) can further include any additional sequence information to facilitate transfer of the genetic material (e.g., a sequence encoding a TCR specific for an antigen) to T cells. For example, the PN can include promoters, such as general promoters, tissue-specific promoters, cell-specific promoters, and/or promoters specific for the nucleus or cytoplasm. Promoters and plasmids (e.g., minicircle plasmids) are generally well known in the art and can be prepared using conventional techniques.

[0075] As described further herein, the PN can be used to transfect T cells. Unless otherwise specified, the terms transfect, transfected, or transfecting can be used to indicate the presence of exogenous PN or the expressed polypeptide therefrom in a T cell. A number of vectors are known to be capable of mediating transfer of PN to lymphocytes, as is known in the art.

[0076] In particular embodiments, the transfected PN can edit the antigen-specificity of T cells without affecting off-target bystander cells (i.e., provide for selective delivery as defined herein). For example, delivered genes can be expressed under the control of a T cell-specific promoter. In particular embodiments, such promoters can be included in minicircle plasmids that are a form of supercoiled DNA molecule for nonviral gene transfer, which have neither bacterial origin of replication nor antibiotic resistance marker. They are thus smaller and potentially safer than the standard plasmids currently used in gene therapy.

[0077] To sustain the expression of transferred TCR genes, for example, in rapidly dividing T cells, a scaffold/matrix attachment region can also be inserted into the PN. PN including an expression cassette linked to a S/MAR element, can autonomously replicate extra-chromosomally in dividing cells. In particular embodiments, PiggyBac or Sleeping Beauty transposase-containing plasmids can also be used to stably integrate TCR genes into the genome of transfected cells. Other options to sustain expression include homo sapiens transposon-derived Buster1 transposase-like protein gene; human endogenous retrovirus H protease/integrase-derived ORF1; homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence; homo sapiens endogenous retroviral sequence K; homo sapiens endogenous retroviral family W; homo sapiens LINE-1 type transposase domain; and homo sapiens pogo transposable element. Particular embodiments can utilize the hyperactive iPB7 transposase.

[0078] When a delivered PN is mRNA, backbone modifications can increase the mRNA's stability making resistant to premature cleavage.

[0079] In particular embodiments, self-replicating mRNA constructs can be used to ensure persistent transgene expression without the requirement of host genome integration. Self-replicating RNA can refer to RNA molecules that encode RNA replication machinery, so that upon translation, cis-encoded genes can produce new RNA copies from the original template molecule. Self-replicating RNAs can be designed using sequences derived from RNA viruses, such as alphaviruses and pestiviruses. Techniques for designing and using self-replicating RNA molecules for delivery of mRNA can be found in, for example, WO/2011/005799, WO/2009/146867, and Geall, A, et al. 2012. Proc Natl Acad Sci USA. 109(36):14604-14609.

[0080] In particular embodiments, PN include synthetic mRNA. In particular embodiments, synthetic mRNA is engineered for increased intracellular stability using 5'-capping. Multiple distinct 5'-cap structures can be used to generate the 5'-cap of a synthetic mRNA molecule. For example, the Anti-Reverse Cap Analog (ARCA) cap contains a 5'-5'-triphosphate guanine-guanine linkage where one guanine contains an N7 methyl group as well as a 3'-O-methyl group. Synthetic mRNA molecules may also be capped post-transcriptionally using enzymes responsible for generating 5'-cap structures. For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2'-O-methyltransferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-most nucleotide of an mRNA and a guanine nucleotide where the guanine contains an N7 methylation and the ultimate 5'-nucleotide contains a 2'-O-methyl generating the Cap1 structure. This results in a cap with higher translational-competency and cellular stability and reduced activation of cellular pro-inflammatory cytokines.

[0081] Synthetic mRNA or other PN may also be made cyclic. PN may be cyclized, or concatemerized, to generate a translation competent molecule to assist interactions between poly-A binding proteins and 5'-end binding proteins. The mechanism of cyclization or concatemerization may occur through at least 3 different routes: 1) chemical, 2) enzymatic, and 3) ribozyme catalyzed. The newly formed 5'-/3'-linkage may be intramolecular or intermolecular.

[0082] In the first route, the 5'-end and the 3'-end of the PN may contain chemically reactive groups that, when close together, form a new covalent linkage between the 5'-end and the 3'-end of the molecule. The 5'-end may contain an NHS-ester reactive group and the 3'-end may contain a 3'-amino-terminated nucleotide such that in an organic solvent the 3'-amino-terminated nucleotide on the 3'-end of a synthetic PN molecule will undergo a nucleophilic attack on the 5'-NHS-ester moiety forming a new 5'-/3'-amide bond.

[0083] In the second route, T4 RNA ligase may be used to enzymatically link a 5'-phosphorylated PN to the 3'-hydroxyl group of a nucleic acid forming a new phosphodiester linkage. In an example

reaction, 1 μ g of a nucleic acid molecule can be incubated at 37°C for 1 hour with 1-10 units of T4 RNA ligase (New England Biolabs, Ipswich, Mass.) according to the manufacturer's protocol. The ligation reaction may occur in the presence of a split oligonucleotide capable of base-pairing with both the 5'- and 3'-region in juxtaposition to assist the enzymatic ligation reaction.

[0084] In the third route, either the 5'- or 3'-end of a cDNA template encodes a ligase ribozyme sequence such that during in vitro transcription, the resultant nucleic acid molecule can contain an active ribozyme sequence capable of ligating the 5'-end of a nucleic acid molecule to the 3'-end of a nucleic acid molecule. The ligase ribozyme may be derived from the Group I Intron, Group II Intron, Hepatitis Delta Virus, Hairpin ribozyme or may be selected by SELEX (systematic evolution of ligands by exponential enrichment). The ribozyme ligase reaction may take 1 to 24 hours at temperatures between 0 and 37°C.

[0085] In particular embodiments, the PN encodes TCR α and β chains that specifically bind an antigen/MHC complex of interest, that is the PN encodes the TCR α and β chain variable regions. In particular embodiments, the PN can additionally encode a TCR constant domain, a transmembrane domain and/or a cytoplasmic tail. Sequences and structures of these portions of TCR are known to those of skill in the art and can be readily accessed in public databases. As one example, SEQ ID NO: 40 provides a representative gene sequence encoding the CD4 transmembrane domain (see FIG. 5). In particular embodiments, the PN can encode an invariant CD3 chain (i.e., γ , Δ , Σ , Z, H), and/or an ITAM motif (derived from, e.g., CD3-Z, FeR- γ , CD3- γ , CD3- Δ , CD3- Σ , CD5, CD22, CD79a, CD79b, and/or CD66d).

[0086] In particular embodiments, PN can include a sequence encoding a spacer region. The length of the spacer region can be customized for individual antigen/MHC complexes to optimize target recognition, binding, and T cell activation. In particular embodiments, a spacer length can be selected based upon the location of an antigen/MHC complex epitope, affinity of a TCR for the epitope, and/or the ability of the T cells expressing the TCR to proliferate in vitro and/or in vivo in response to antigen/MHC complex recognition.

[0087] Typically a spacer region is found between the α and β chains of a TCR and a transmembrane domain of the TCR. Spacer regions can provide for flexibility of the α and β chains and allows for high expression levels in genetically modified T cells. In particular embodiments, a spacer region can have at least 10 to 250 amino acids, at least 10 to 200 amino acids, at least 10 to 150 amino acids, at least 10 to 100 amino acids, at least 10 to 50 amino acids or at least 10 to 25 amino acids and including any integer between the endpoints of any of the listed ranges. In particular embodiments, a spacer region has 250 amino acids or less; 200 amino acids or less; 150 amino acids or less; 100 amino acids or less; 50 amino acids or less; 40 amino acids or less; 30 amino acids or less; 20 amino acids or less; or 10 amino acids or less.

[0088] In particular embodiments, spacer regions can be derived from a hinge region of an immunoglobulin like molecule, for example all or a portion of the hinge region from a human IgG1, human IgG2, a human IgG3, or a human IgG4. In particular embodiments, all or a portion of a hinge region can be combined with one or more domains of a constant region of an immunoglobulin. For example, a portion of a hinge region can be combined with all or a portion of a CH2 or CH3 domain or variant thereof.

[0089] In particular embodiments, introduction of PN to T cells can be carried out by any method known in the art, including transfection, electroporation, microinjection, lipofection, calcium phosphate mediated transfection, infection with a viral or bacteriophage vector containing the gene sequences, receptor-mediated endocytosis, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see e.g., Loeffler and Behr, *Meth. Enzymol*, 217, 599-618 (1993); Cohen et al., *Meth. Enzymol*, 217, 618-644 (1993); Cline, *Pharmac. Ther*, 29, 69-92 (1985)) and may be used in accordance with the present disclosure, provided that the necessary developmental and physiological functions of the T cells are not disrupted. In particular embodiments, the technique provides for the stable transfer of a gene to the T cell, so that the gene is expressible by the cell and preferably heritable and expressible by its cell progeny. In particular embodiments, the technique provides for transient expression of the gene within a cell. Methods commonly known in the art of recombinant DNA technology which can be used to genetically modify T cells are described in, for example, Ausubel et al. (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; and Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY.

[0090] III. Nanoparticles (NP). In particular embodiments, PN are administered to T cells using nanoparticles (NP). Particular NP embodiments include a positively-charged carrier. Carriers function to condense and protect PN from enzymatic degradation. Particularly useful materials to use as carriers include positively charged lipids and/or polymers, including poly(β -amino ester).

[0091] Additional examples of positively charged lipids include esters of phosphatidic acid with an aminoalcohol, such as an ester of dipalmitoyl phosphatidic acid or distearoyl phosphatidic acid with hydroxyethylenediamine. More particular examples of positively charged lipids include 3 β -[N--(N',N'-dimethylaminoethyl)carbonyl] cholesterol (DC-chol); N,N'-dimethyl-N,N'-dioctacyl ammonium bromide (DDAB); N,N'-dimethyl-N,N'-dioctacyl ammonium chloride (DDAC); 1,2-dioleoyloxypropyl-3-dimethyl-hydroxyethyl ammonium chloride (DORI); 1,2-dioleoyloxy-3-[trimethylammonio]-propane (DOTAP); N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA); dipalmitoylphosphatidylcholine (DPPC); 1,2-dioctadecyloxy-3-[trimethylammonio]-propane (DSTAP); and the cationic lipids described in e.g. Martin et al., *Current Pharmaceutical Design* 2005,

11, 375-394.

[0092] Examples of positively charged polymers that can be used as carriers within the current disclosure include polyamines; polyorganic amines (e.g., polyethyleneimine (PEI), polyethyleneimine celluloses); poly(amidoamines) (PAMAM); polyamino acids (e.g., polylysine (PLL), polyarginine); polysaccharides (e.g., cellulose, dextran, DEAE dextran, starch); spermine, spermidine, poly(vinylbenzyl trialkyl ammonium), poly(4-vinyl-N-alkyl-pyridinium), poly(acryloyl-trialkyl ammonium), and Tat proteins.

[0093] Without limiting the foregoing, particular embodiments disclosed herein can also utilize porous NP constructed from any material capable of forming a porous network. Exemplary materials include biocompatible polymers, metals, transition metals and metalloids. Exemplary biocompatible polymers include agar, agarose, alginate, alginate/calcium phosphate cement (CPC), β -galactosidase (β -GAL), (1,2,3,4,6-pentaacetyl α -D-galactose), cellulose, chitin, chitosan, collagen, elastin, gelatin, hyaluronic acid collagen, hydroxyapatite, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx), poly(lactide), poly(caprolactone) (PCL), poly(lactide-co-glycolide) (PLG), poly(lactic-co-glycolic acid) (PLGA), poly(vinyl alcohol) (PVA), silk, soy protein, and soy protein isolate, alone or in combination with any other polymer composition, in any concentration and in any ratio. Blending different polymer types in different ratios using various grades can result in characteristics that borrow from each of the contributing polymers. Various terminal group chemistries can also be adopted.

[0094] In particular embodiments, NP include a coating that shields encapsulated PN and reduces or prevents off-target binding. Off-target binding is reduced or prevented by reducing the surface charge of the NP to neutral or negative. Coatings can include neutral or negatively charged polymer- and/or liposome-based coatings. In particular embodiments, the coating is a dense surface coating of hydrophilic and/or neutrally charged hydrophilic polymer sufficient to prevent the encapsulated nucleic acids from being exposed to the environment before release into a selected cell. In particular embodiments, the coating covers at least 80% or at least 90% of the surface of the NP. In particular embodiments, the coating includes polyglutamic acid (PGA).

[0095] Examples of additional neutrally charged polymers that can be used as coatings include polyethylene glycol (PEG); poly(propylene glycol); and polyalkylene oxide copolymers, (PLURONIC®, BASF Corp., Mount Olive, NJ).

[0096] Neutrally charged polymers also include zwitterionic polymers. Zwitterionic refers to the property of overall charge neutrality while having both a positive and a negative electrical charge. Zwitterionic polymers can behave like regions of cell membranes that resist cell and protein adhesion.

[0097] Zwitterionic polymers include zwitterionic constitutional units including pendant groups (i.e.,

groups pendant from the polymer backbone) with zwitterionic groups. Exemplary zwitterionic pendant groups include carboxybetaine groups (e.g., $-Ra-N^+(Rb)(Rc)-Rd-CO_2^-$, where Ra is a linker group that covalently couples the polymer backbone to the cationic nitrogen center of the carboxybetaine groups, Rb and Rc are nitrogen substituents, and Rd is a linker group that covalently couples the cationic nitrogen center to the carboxy group of the carboxybetaine group).

[0098] Examples of negatively charged polymers include alginic acids; carboxylic acid polysaccharides; carboxymethyl cellulose; carboxymethyl cellulose-cysteine; carrageenan (e.g., Gelcarin® 209, Gelcarin® 379); chondroitin sulfate; glycosaminoglycans; mucopolysaccharides; negatively charged polysaccharides (e.g., dextran sulfate); poly(acrylic acid); poly(D-aspartic acid); poly(L-aspartic acid); poly(L-aspartic acid) sodium salt; poly(D-glutamic acid); poly(L-glutamic acid); poly(L-glutamic acid) sodium salt; poly(methacrylic acid); sodium alginate (e.g., Protanal® LF 120M, Protanal® LF 200M, Protanal® LF 200D); sodium carboxymethyl cellulose (CMC); sulfated polysaccharides (heparins, agarosectins); pectin, gelatin and hyaluronic acid.

[0099] In particular embodiments, polymers disclosed herein can include "star shaped polymers," which refer to branched polymers in which two or more polymer branches extend from a core. The core is a group of atoms having two or more functional groups from which the branches can be extended by polymerization.

[00100] In particular embodiments, the branches are zwitterionic or negatively-charged polymeric branches. For star polymers, the branch precursors can be converted to zwitterionic or negatively-charged polymers via hydrolysis, ultraviolet irradiation, or heat. The polymers also may be obtained by any polymerization method effective for polymerization of unsaturated monomers, including atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer polymerization (RAFT), photo-polymerization, ring-opening polymerization (ROP), condensation, Michael addition, branch generation/propagation reaction, or other reactions.

[00101] Liposomes are microscopic vesicles including at least one concentric lipid bilayer. Vesicle-forming lipids are selected to achieve a specified degree of fluidity or rigidity of the final complex. In particular embodiments, liposomes provide a lipid composition that is an outer layer surrounding a particle.

[0102] 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), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (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, DDAB, dioctadecyl dimethyl ammonium chloride (DODAC), 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP), DOTAP, DOTMA, DC-Chol, phosphatidic acid (PA), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylglycerol, DOPG, and dicetylphosphate. In particular embodiments, lipids used to create liposomes disclosed herein include cholesterol, hydrogenated soy phosphatidylcholine (HSPC) and, the derivatized vesicle-forming lipid PEG-DSPE.

[0103] 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).

[0104] The NP can be a variety of different shapes, including spheroidal, cuboidal, pyramidal, oblong, cylindrical, toroidal, and the like. The PN can be included in the NP in a variety of ways. For example, the PN can be encapsulated in the NP. In other aspects, the PN can be associated (e.g., covalently and/or non-covalently) with the surface or close underlying vicinity of the surface of the NP. In particular embodiments, the PN can be incorporated in the NP e.g., integrated in the material of the NP. For example, the PN can be incorporated into a polymer matrix of polymer NP. One of ordinary skill in the art will appreciate the various ways to carry the PN so as to allow delivery of the PN to cells.

[0105] The size of the NP can vary over a wide range and can be measured in different ways. For example, the NP can have a minimum dimension of 100 nm. The NP 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 NP 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 NP or coated NP. In particular embodiments, a population of NP 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 NP in a composition 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. Dimensions of the NP can be determined using, e.g., conventional techniques, such as dynamic lightscattering and/or electron microscopy.

[0106] IV. T Cell Targeting and Delivery Agents (T-DA). In particular embodiments, NP include T Cell Targeting and Delivery Agents (T-DA) to allow selective delivery of the PN to chosen cell types, either in vivo or ex vivo.

[0107] T-DA selectively bind T cells of interest. In particular embodiments, T-DA achieve selective delivery of NP to particular T cell populations through receptor-mediated endocytosis by targeting a marker expressed by the T cell type. For example, as previously indicated CD4⁺ T cells express the CD4 protein on their surface and CD8⁺ T cells express the CD8 protein on their surface.

[0108] "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 non-naïve T cells. In particular embodiments, naive T cells can be further characterized by the expression of phenotypic markers including CD62L, CCR7, CD28, CD127, and CD45RA. T-DA can bind CD62L, CCR7, CD28, CD127 and/or CD45RA to achieve selective delivery of a PN to naive T cells.

[0109] CD3 is expressed on all mature T cells. Accordingly, T-DA can bind CD3 to achieve selective delivery of a PN to all mature T cells. Activated T cells express 4-1BB (CD137). Accordingly, T-DA can bind 4-1 BB to achieve selective delivery of a PN to activated T cells. CD5 and transferrin receptor are also expressed on T cells and can be used to achieve selective delivery of a PN to T cells.

[0110] "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. T-DA can bind CD62L, CCR7, CD25, CD127, CD45RO and/or CD95 to achieve selective delivery of a polynucleotide to TCM.

[0111] "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. T-DA can bind granzyme B and/or perforin to achieve selective delivery of a PN to TEM.

[0112] Lymphocyte function-associated antigen 1 (LFA-1) is expressed by all T cells, B cells and monocytes/macrophages. Accordingly, T-DA can bind LFA-1 to achieve selective delivery of a PN to T cells, B cells and monocytes/macrophages.

[0113] "Selective delivery" means that PN are delivered and expressed by one or more selected cell populations. In particular embodiments, selective delivery is exclusive to a selected T cell population. In particular embodiments, at least 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% of administered PN are delivered and/or expressed by a T cell population. In particular embodiments, selective delivery ensures that non-selected cells do not express delivered PN. For example, when the PN encodes a TCR, selectivity can be ensured because only T cells have the Z chains required for TCR expression. Selective delivery can also be based on lack of PN uptake into unselected cells or based on the presence of a specific promoter within the PN sequence when the PN includes plasmid DNA. For example, plasmid DNA can include a T cell-specific promoter, such as the distal Ick promoter for T cells. In particular embodiments, selective delivery is observed due to the selective binding of T-DA to targeted T cells.

[0114] As indicated, T-DA can include binding domains for motifs found on T cells. T-DA can also include any selective binding mechanism allowing selective uptake into selected T cells. In particular embodiments, T-DA include binding domains for T cell receptor motifs; T cell α chains; T cell β chains; CCR7; CD3; CD4; CD8; CD28; CD45RA; CD62L; CD127; LFA-1; and combinations thereof.

[0115] In particular embodiments, binding domains include cell marker ligands, receptor ligands, antibodies, peptides, peptide aptamers, nucleic acids, nucleic acid aptamers, spiegelmers or combinations thereof. Within the context of T-DA, binding domains include any substance that binds to another substance to form a complex capable of mediating endocytosis.

[0116] "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, Fc, and single chain Fv fragments (scFvs) or any biologically effective fragments of an immunoglobulin that bind specifically to a motif expressed by a selected cell. Antibodies or antigen binding fragments include all or a portion of polyclonal antibodies, monoclonal antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, bispecific antibodies, mini bodies, and linear antibodies.

[0117] Antibodies from human origin or humanized antibodies have lowered or no immunogenicity in humans and have a lower number of non-immunogenic epitopes compared to non-human antibodies. Antibodies and their fragments will generally be selected to have a reduced level or no antigenicity in human subjects.

[0118] Antibodies that specifically bind a motif expressed by a T cell can be prepared using methods of obtaining monoclonal antibodies, methods of phage display, methods to generate human or humanized antibodies, or methods using a transgenic animal or plant engineered to produce antibodies as is known to those of ordinary skill in the art (see, for example, U.S. Pat. Nos. 6,291,161 and 6,291,158). Phage display libraries of partially or fully synthetic antibodies are available and can be screened for an antibody or fragment thereof that can bind to a T cell motif. For example, binding domains may be identified by screening a Fab phage library for Fab fragments that specifically bind to a target of interest (see Hoet et al., Nat. Biotechnol. 23:344, 2005). Phage display libraries of human antibodies are also available. Additionally, traditional strategies for hybridoma development using a target of interest as an immunogen in convenient systems (e.g., mice, HuMAb mouse®, TC mouse™, KM-mouse®, llamas, chicken, rats, hamsters, rabbits, etc.) can be used to develop binding domains. In particular embodiments, antibodies specifically bind to motifs expressed by a selected T cell and do not cross react with nonspecific components or unrelated targets. Once identified, the amino acid sequence or polynucleotide sequence coding for the antibody can be isolated and/or determined.

[0119] In particular embodiments, binding domains of selected T-DA include T cell receptor motif antibodies; T cell α chain antibodies; T cell β chain antibodies; CCR7 antibodies; CD3 antibodies; CD4 antibodies; CD8 antibodies; CD28 antibodies; CD45RA antibodies; CD62L antibodies; CD127 antibodies; and/or LFA-1 antibodies. These binding domains also can consist of scFv fragments of the foregoing antibodies.

[0120] In particular embodiments, the T-DA includes an antibody or antibody fragment that binds to CD4. An example of an antibody that binds to CD4 is TNX-355, which is described in U.S. Publication No. US20130195881. The TNX-355 anti-CD4 antibody includes a variable heavy chain including a CDRH1 sequence including GYTFTSYVIH (SEQ ID NO: 41), a CDRH2 sequence including YINPYNDGTDYDEKFKG (SEQ ID NO: 42), and a CDRH3 sequence including EKDNYATGAWFAY (SEQ ID NO: 43); and a variable light chain including a CDRL1 sequence including KSSQSLLYSTNQKNYLA (SEQ ID NO: 44), a CDRL2 sequence including WASTRES (SEQ ID NO: 45), and a CDRL3 sequence including QQYYSYRT (SEQ ID NO: 46). In particular embodiments, an antibody that binds to CD4 includes a commercially available antibody. An example of a commercially available anti-CD4 antibody is Clone GK1.5, Cat# BE0003-1, from BioXCell (West Lebanon, NH).

[0121] In particular embodiments, the T-DA includes an antibody or antibody fragment that binds to CD8. An example of an antibody that binds to CD8 is OKT8, the sequence of which is described in U.S. Publication No. US20160176969. The OKT8 anti-CD8 antibody includes a variable heavy chain including a CDRH1 sequence including FNIKDTY (SEQ ID NO: 47), a CDRH2 sequence including

DPANDN (SEQ ID NO: 48), and a CDRH3 sequence including GYGYVFDH (SEQ ID NO: 49); and a variable light chain including a CDRL1 sequence including RSISQY (SEQ ID NO: 50), a CDRL2 sequence including SGSTLQS (SEQ ID NO: 51), and a CDRL3 sequence including HNENPLT (SEQ ID NO: 52). In particular embodiments, an antibody that binds to CD8 includes a commercially available antibody. An example of a commercially available anti-CD8 antibody is Clone 2.43, Cat# BP0061, from BioXCell (West Lebanon, NH).

[0122] In particular embodiments, the T-DA includes an antibody or antibody fragment that binds to CD3. An example of an antibody that binds to CD3 is OKT3, the sequence of which is described in U.S. Patent No. 6,491,916. The OKT3 anti-CD3 antibody includes a variable heavy chain including a CDRH1 sequence including RYTMH (SEQ ID NO: 53), a CDRH2 sequence including YINPSRGYTNYNQKFKD (SEQ ID NO: 54), and a CDRH3 sequence including YYDDHYCLDY (SEQ ID NO: 55); and a variable light chain including a CDRL1 sequence including SASSSVSYMN (SEQ ID NO: 56), a CDRL2 sequence including DTSKLAS (SEQ ID NO: 57), and a CDRL3 sequence including QQWSSNPFT (SEQ ID NO: 58). In particular embodiments, an antibody that binds to CD3 includes a commercially available antibody. An example of a commercially available anti-CD3 antibody is Clone KT3, Cat# MA5-16763, from Thermo Fisher Scientific (Waltham, MA).

[0123] In particular embodiments, a binding domain VH region can be derived from or based on a VH of a known monoclonal 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 VH of a known 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.

[0124] In particular embodiments, a VL region in a binding domain is derived from or based on a VL of a known monoclonal antibody 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 VL of the known monoclonal antibody. 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.

[0125] 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 an amino acid sequence of a light chain variable region (VL) or to a heavy chain variable region (VH), or both, wherein each CDR includes zero changes or at most one, two, or three changes, from a monoclonal antibody or fragment or derivative thereof that specifically binds to target of interest.

[0126] Peptide aptamers include a peptide loop (which is specific for a target protein) attached at both ends to a protein scaffold. This double structural constraint greatly increases the binding affinity of the peptide aptamer to levels comparable to an antibody. The variable loop length is typically 8 to 20 amino acids (e.g., 8 to 12 amino acids), and the scaffold may be any protein which is stable, soluble, small, and non-toxic (e.g., thioredoxin-A, stefin A triple mutant, green fluorescent protein, eglin C, and cellular transcription factor Spl). Peptide aptamer selection can be made using different systems, such as the yeast two-hybrid system (e.g., Gal4 yeast-two-hybrid system) or the LexA interaction trap system.

[0127] Nucleic acid aptamers are single-stranded nucleic acid (DNA or RNA) ligands that function by folding into a specific globular structure that dictates binding to target proteins or other molecules with high affinity and specificity, as described by Osborne et al., *Curr. Opin. Chem. Biol.* 1:5-9, 1997; and Cerchia et al., *FEBS Letters* 528:12-16, 2002. In particular embodiments, aptamers are small (15 KD; or between 15-80 nucleotides or between 20-50 nucleotides). Aptamers are generally isolated from libraries consisting of 10^{14} - 10^{15} random oligonucleotide sequences by a procedure termed SELEX (systematic evolution of ligands by exponential enrichment; see, for example, Tuerk et al., *Science*, 249:505-510, 1990; Green et al., *Methods Enzymology* 75-86, 1991; and Gold et al., *Annu. Rev. Biochem.*, 64: 763-797, 1995). Further methods of generating aptamers are described in, for example, U.S. Pat. Nos. 6,344,318; 6,331,398; 6,110,900; 5,817,785; 5,756,291; 5,696,249; 5,670,637; 5,637,461; 5,595,877; 5,527,894; 5,496,938; 5,475,096; and 5,270,16. Spiegelmers are similar to nucleic acid aptamers except that at least one β -ribose unit is replaced by β -D-deoxyribose or a modified sugar unit selected from, for example, β -D-ribose, α -D-ribose, β -L-ribose.

[0128] Binding domains can also be selected from affibodies; affilin (Ebersbach et al., *J. Mol. Biol.* 372: 172, 2007); armadillo repeat proteins (see, e.g., Madhurantakam et al., *Protein Sci.* 21: 1015, 2012; PCT Patent Application Publication No. WO 2009/040338); atrimers; avimers; C-type lectin domains (Zelensky and Gready, *FEBS J.* 272:6179, 2005; Beavil et al., *Proc. Natl. Acad. Sci. (USA)* 89:753, 1992 and Sato et al., *Proc. Natl. Acad. Sci. (USA)* 100:7779, 2003); cytotoxic T-lymphocyte associated protein-4 (Weidle et al., *Cancer Gen. Proteo.* 10:155, 2013); designed ankyrin repeat proteins (DARPs) (Binz et al., *J. Mol. Biol.* 332:489, 2003 and Binz et al., *Nat. Biotechnol.* 22:575, 2004); fibrinogen domains (see, e.g., Weisel et al., *Science* 230:1388, 1985); fibronectin binding

domains (adnectins or monobodies) (Richards et al., J. Mol. Biol. 326:1475, 2003; Parker et al., Protein Eng. Des. Selec. 18:435, 2005 and Hackel et al. (2008) J. Mol. Biol. 381:1238-1252); fynomers; Kunitz domains (see, e.g., U.S. Pat. No. 6,423,498); leucine-rich repeat domains (Stumpp et al., J. Mol. Biol. 332:471, 2003); lipocalin domains (see, e.g., WO 2006/095164, Beste et al., Proc. Natl. Acad. Sci. (USA) 96:1898, 1999 and Schonfeld et al., Proc. Natl. Acad. Sci. (USA) 106:8198, 2009); mAb2 or Fcab™ (see, e.g., PCT Patent Application Publication Nos. WO 2007/098934; WO 2006/072620); scTCR (see, e.g., Lake et al., Int. Immunol. 11:745, 1999; Maynard et al., J. Immunol. Methods 306:51, 2005; U.S. Pat. No. 8,361,794); tetratricopeptide repeat domains (Main et al., Structure 11:497, 2003 and Cortajarena et al., ACS Chem. Biol. 3:161, 2008); V-like domains (see, e.g., U.S. Patent Application Publication No. 2007/0065431); or the like (see, e.g., Nord et al., Protein Eng. 8:601, 1995; Nord et al., Nat. Biotechnol. 15:772, 1997; Nord et al., Euro. J. Biochem. 268:4269, 2001; Binz et al., Nat. Biotechnol. 23:1257, 2005; Boersma and Pluckthun, Curr. Opin. Biotechnol. 22:849, 2011).

[0129] Other agents that can facilitate internalization by and/or transfection of T cells, such as poly(ethyleneimine)/DNA (PEI/DNA) complexes can also be used.

[0130] V. Endosomal Release Agents (ERA). Endosomal release agents (ERA) include any compound or peptide that facilitates cargo exit from the endosome of a T cell. Exemplary ERA include imidazoles, poly or oligoimidazoles, PEIs, peptides, fusogenic peptides, polycarboxylates, polycations, masked oligo or poly cations or anions, acetals, polyacetals, ketals/polyketyals, orthoesters, polymers with masked or unmasked cationic or anionic charges, amphiphilic block copolymers and dendrimers with masked or unmasked cationic or anionic charges.

[0131] Many ERA are adapted from viral elements that promote escape from the endosome and deliver polynucleotides intact into the nucleus. As one particular example, the H5WYG peptide can be used to induce the lysis of membranes at low pH. The histidine-rich peptide H5WYG is a derivative of the N-terminal sequence of the HA-2 subunit of the influenza virus hemagglutinin in which 5 of the amino acids have been replaced with histidine residues. H5WYG is able to selectively destabilize membranes at a slightly acidic pH as the histidine residues are protonated. The E1 protein from Semliki Forrest virus is also a useful ERA.

[0132] In particular embodiments, ERA include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 59). An RFGF analogue (e.g., amino acid sequence AALLPVLLAAP (SEQ ID NO: 60)) containing a hydrophobic MTS can also be used.

[0133] Additional exemplary ERA include:

Source	Sequence
Influenza virus hemagglutinin HA-2	GLFEAIAGFIENGWEG (SEQ ID NO: 61)
TAT of HIV	YGRKKRRQRRR (SEQ ID NO: 62)

N-terminal region of the S protein of duck hepatitis B	MSGTFGGILAGLIGLL (SEQ ID NO: 63)
S protein of woodchuck hepatitis B	MSPSSLLGLLAGLQVV (SEQ ID NO: 64)
Synthetic, Duguid et al. 1998	GLFEALLELLESLWELL (SEQ ID NO: 65)
Synthetic, Gupta & Kothekar, 1997	LKKLLKKLLKKLLKKL (SEQ ID NO: 66)
Derossi et al., J. Biol. Chem. 269: 10444, 1994	RQIKIWFQNRRMKWKK (SEQ ID NO: 67)
Tat fragment (48-60)	GRKKRRQRRRPPQC (SEQ ID NO: 68)
Chaloin et al., Biochem. peptide Biophys. Res. Commun., 243: 601, 1998	GALFLGWLGAAGSTMGAWSQPKKKRKV (SEQ ID NO: 69)
PVEC	LLIILRRRIRKQAHAAHSK (SEQ ID NO: 70)
Transportan	GWTLNSAGYLLKINLKALAALAKKIL (SEQ ID NO: 71)
Amphiphilic model peptide; Oehlke et al., Mol. Ther., 2: 339, 2000	KLALKLALKALKAALKLA (SEQ ID NO: 72)
Arg ₉	RRRRRRRRRR (SEQ ID NO: 73)
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES (SEQ ID NO: 74)
Cecropin P1	SWLSKTAKKLENSAKKRISGIAIAIQGGPR (SEQ ID NO: 75)
α-defensin	ACYCRIPACIAGERRYGTCTIYQGRLWAFCC (SEQ ID NO: 76)
β-defensin	DHYNCVSSGGQCLYSACPIFTKIQGTCTYRGKAKCCK (SEQ ID NO: 77)
Bactenecin	RKCRIVVIRVCR (SEQ ID NO: 78)
PR-3	RRRPRPPYLPRPRPPPPFFPRLPPRIPPGFPPRFPPRFP GKR-NH ₂ (SEQ ID NO: 79)
Indolicidin	ILPWKWPWWPWRR-NH ₂ (SEQ ID NO: 80)

[0134] VI. Nuclear Targeting Agents. Nuclear Targeting Agents (NTA) refer to sequences that enhance cellular transport to and/or entry into the nucleus of a cell. Generally, NTA are a class of short amino acid sequences from 3 to 100 amino acids in length, from 3 to 50, 4 to 30, or 4 to 20 amino acids in length.

[0135] Microtubule-associated sequence (MTAS) NTA include those that facilitate interaction with microtubules to enhance transport to the nucleus. An exemplary MTAS includes PLKTPGKKKKKGKPGKRKEQEKKKRRTR (SEQ ID NO: 81).

[0136] Nuclear localization signal (NLS) NTA include those that facilitate interaction with nuclear transport machinery. An exemplary NLS sequence includes GRYLTQETNKNVETYKEQ PLKTPGKKKKKGKP (SEQ ID NO: 82).

[0137] Particular embodiments utilize NTA derived from the human parathyroid hormone related protein (PTHrP, UniProt ID: P12272), which is a protein that includes overlapping MTAS and NLS sequences. In particular embodiments, the NTA including an overlapping MTAS and NLS sequence includes GRYLTQETNKNVETYKEQPLKTPGKKKKKGKPGKRKEQEKKKRRTR (SEQ ID NO: 83; see Narayanan, et al., Sci Rep. 2013; 3:2184).

[0138] Additional exemplary NLS sequences include (i) monopartite NLS exemplified by the SV40 large T antigen NLS (PKKKRKV) (SEQ ID NO: 84); (ii) bipartite NLS including two basic domains separated by a variable number of spacer amino acids and exemplified by the Xenopus nucleoplasmin NLS (KRXXXXXXXXXXXXKKKL) (SEQ ID NO: 85); and (iii) noncanonical sequences such as M9 of the hnRNP A1 protein, the influenza virus nucleoprotein NLS, and the yeast Ga14 protein NLS (Dingwall and Laskey, Trends Biochem Sci 16:478-481, 1991). In particular embodiments, the NLS can be a highly cationic or basic peptide. In particular embodiments, the NLS includes two or more Arg or Lys amino acid residues. In particular embodiments, the NLS can bind cytosolic proteins, such as importins and karyopherins, which recognize and transport NLS-containing sequences to the nuclear pore complex.

[0139] In particular embodiments, to direct import of delivered PN, particularly plasmid DNA, into the nucleus, PN (e.g., nanoparticle-encapsulated plasmids) can be conjugated to the SV40 T-Ag-derived NLS peptides. Exemplary SV40 T-Ag-derived NLS peptides include: PKKKRKV (SEQ ID NO: 86); PKKKRMV (SEQ ID NO: 87); PKKKRKVEDP (SEQ ID NO: 88); PKKGSKKA (SEQ ID NO: 89); PKTKRKV (SEQ ID NO: 90); CGGPKKKRKVG (SEQ ID NO: 91); PKKKIKV (SEQ ID NO: 92); CYDDEATADSQHSTPPKKKRKVEDPKDFESELLS (SEQ ID NO: 93); and CGYGPKKKRKVGG (SEQ ID NO: 94).

[0140] Additional exemplary NLS sequences include:

Source	Sequence
Polyoma large T protein	PKKARED (SEQ ID NO: 95)
Polyoma large T protein	CGYGVSRKRPRPG (SEQ ID NO: 96)
SV40 VP1 capsid polypeptide	APTKRKGS (SEQ ID NO: 97)
Polyoma virus major capsid protein VP1	APKRKSGVSKC (SEQ ID NO: 98)
SV40 VP2 capsid protein	PNKKKRK (SEQ ID NO: 99)
Polyoma virus capsid protein VP2	EEDGPQKKRRL (SEQ ID NO: 100)
Yeast histone H2B	GKKRSKA (SEQ ID NO: 101)
Adenovirus E1a	KRPRP (SEQ ID NO: 102)
Adenovirus type 2/5 E1a	CGGLSSKRPRP (SEQ ID NO: 103)
Xenopus NLS2	LKDKDAKKSKQE (SEQ ID NO: 104)
v-Rel or p59 ^{v-rel}	GNKAKRQRST (SEQ ID NO: 105)
Influenza A NS1 protein	PFLDRLRRDQK (SEQ ID NO: 106)
Human lamin A	SVTKKRKLE (SEQ ID NO: 107)
Xenopus lamin A	SASKRRRLE (SEQ ID NO: 108)
Adenovirus 5 DBP	PPKKRMRRRIE (SEQ ID NO: 109)
Rat glucocorticoid receptor	YRKCLQAGMNLEARKTKKKIKGIQQATA (SEQ ID NO: 110)
Human estrogen receptor	RKDRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAMINACIDNLWPSPLMIKRSKK (SEQ ID NO: 111)
Rabbit progesterone receptor	RKFKKFNK (SEQ ID NO: 112)
c-myb gene product	PLLKKIKQ (SEQ ID NO: 113)
N-myc gene product	PPQKKIKS (SEQ ID NO: 114)

p53	PQPKKKP (SEQ ID NO: 115)
c-erb-A gene product	SKRVAKRKL (SEQ ID NO: 116)
Yeast ribosomal protein L29	MTGSKTRKHRGSGA (SEQ ID NO: 117)
Yeast ribosomal protein L29	RHRKHP (SEQ ID NO: 118)
Yeast ribosomal protein L29	KRRKHP (SEQ ID NO: 119)
Yeast ribosomal protein L29	KYRKHP (SEQ ID NO: 120)
Yeast ribosomal protein L29	KHRRHP (SEQ ID NO: 121)
Yeast ribosomal protein L29	KHKKHP (SEQ ID NO: 122)
Yeast ribosomal protein L29	RHLKHP (SEQ ID NO: 123)
Hepatitis B core antigen	PETTVVRRRGRSPRRRTPSPRRRRSPRRRR SQS (SEQ ID NO: 124)
Viral jun	ASKSRKRKL (SEQ ID NO: 125)
Human T cell leukemia virus Tax trans- activator protein	GGLCSARLHRHALLAT (SEQ ID NO: 126)
Mouse nuclear Mx1 protein	DTREKKKFLKRLLRLDE (SEQ ID NO: 127)

[0141] Exemplary NLS are also described in Cokol et al., 2000, EMBO Reports, 1(5):411-415; Boulikas, 1993, Crit. Rev. Eukaryot. Gene Expr., 3:193-227; Collas et al., 1996, Transgenic Research, 5: 451-458; Collas and Alestrom, 1997, Biochem. Cell Biol. 75: 633-640; Collas and Alestrom, 1998, Transgenic Research, 7: 303-309; Collas and Alestrom, 1996, Mol. Reprod. Devel., 45:431-438, and U.S. Pat. Nos. 7,531,624; 7,498,177; 7,332,586; and 7,550,650.

[0142] In particular embodiments, NTA are covalently coupled to a polymer of a NP, for example, PBAE.

[0143] VII. Vaccine Antigens. Within the teachings of the current disclosure, T cells are genetically modified to express a TCR specific for a vaccine antigen that is administered to a subject. A vaccine antigen is a substance that, when introduced to the body stimulates an immune response, such as T cell activation and/or antibody production. Vaccine antigens can include natural intact pathogens, such as a killed bacterium or virus, or a live attenuated virus or can include only portions, or subunits, of a pathogen, such as a single virus or bacterium protein. Vaccine antigens can also include cancer antigens or fragments thereof.

[0144] Exemplary viral vaccine antigens can be derived from adenoviruses, arenaviruses, bunyaviruses, coronaviruses, flaviviruses, hantaviruses, hepadnaviruses, herpesviruses, papillomaviruses, paramyxoviruses, parvoviruses, picornaviruses, poxviruses, orthomyxoviruses, retroviruses, reoviruses, rhabdoviruses, rotaviruses, spongiform viruses or togaviruses. In particular embodiments, vaccine antigens include peptides expressed by viruses including CMV, EBV, flu viruses, hepatitis A, B, or C, herpes simplex, HIV, influenza, Japanese encephalitis, measles, polio, rabies, respiratory syncytial, rubella, smallpox, varicella zoster, West Nile, and/or Zika.

[0145] Examples of vaccine antigens that are derived from whole pathogens include the attenuated polio virus used for the OPV polio vaccine, and the killed polio virus used for the IPV polio vaccine.

[0146] As further particular examples, CMV vaccine antigens include envelope glycoprotein B and

CMV pp65; EBV vaccine antigens include EBV EBNA1, EBV P18, and EBV P23; hepatitis vaccine 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 vaccine antigens include immediate early proteins and glycoprotein D; human immunodeficiency virus (HIV) vaccine 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; human papillomavirus virus (HPV) viral antigens include the L1 protein; influenza vaccine antigens include hemagglutinin and neuraminidase; Japanese encephalitis vaccine antigens include proteins E, M-E, M-E-NS1, NS1, NS1-NS2A and 80% E; malaria vaccine antigens include the Plasmodium proteins circumsporozoite (CSP), glutamate dehydrogenase, lactate dehydrogenase, and fructose-bisphosphate aldolase; measles vaccine antigens include the measles virus fusion protein; rabies vaccine antigens include rabies glycoprotein and rabies nucleoprotein; respiratory syncytial vaccine antigens include the RSV fusion protein and the M2 protein; rotaviral vaccine antigens include VP7sc; rubella vaccine antigens include proteins E1 and E2; varicella zoster vaccine antigens include gpl and gpII; and zika vaccine antigens include pre-membrane, envelope (E), Domain III of the E protein, and non-structural proteins 1-5.

[0147] Additional particular exemplary viral antigen sequences include:

Source	Sequence
Nef (66-97):	VGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGL (SEQ ID NO: 128)
Nef (116-145)	HTQGYFPDWQNYTPGPGVRYPLTFGWLYKL (SEQ ID NO: 129)
Gag p17 (17-35)	EKIRLRPGGKKKYKLKHIV (SEQ ID NO: 130)
Gag p17-p24 (253-284)	NPPIPVGEIYKRWILGLNKIVRMYSPSILD (SEQ ID NO: 131)
Pol 325-355 (RT 158-188)	AIFQSSMTKILEPFRKQNPDIVIYQYMDDLY (SEQ ID NO: 132)
CSP central repeat region	NANPNANPNANPNANPNANP (SEQ ID NO: 133)
E protein Domain III	AFTFTKIPAETLHTVTEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVIT EGTENSMMLELDPPFGDSYIVIGVGE (SEQ ID NO: 134)

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

[0148] In particular embodiments, vaccine antigens are expressed by cells associated with bacterial infections. Exemplary bacteria include anthrax; gram-negative bacilli, chlamydia, diphtheria, haemophilus influenza, Helicobacter pylori, Mycobacterium tuberculosis, pertussis toxin, pneumococcus, rickettsiae, staphylococcus, streptococcus and tetanus.

[0149] As particular examples of bacterial vaccine antigens, anthrax vaccine antigens include anthrax protective antigen; gram-negative bacilli vaccine antigens include lipopolysaccharides;

haemophilus influenza vaccine antigens include capsular polysaccharides; diptheria vaccine antigens include diptheria toxin; Mycobacterium tuberculosis vaccine antigens include mycolic acid, heat shock protein 65 (HSP65), the 30 kDa major secreted protein and antigen 85A; pertussis toxin vaccine antigens include hemagglutinin, pertactin, FIM2, FIM3 and adenylate cyclase; pneumococcal vaccine antigens include pneumolysin and pneumococcal capsular polysaccharides; rickettsiae vaccine antigens include rompA; streptococcal vaccine antigens include M proteins; and tetanus vaccine antigens include tetanus toxin.

[0150] In particular embodiments, vaccine antigens are derived from multi-drug resistant "superbugs." Examples of superbugs include Enterococcus faecium, Clostridium difficile, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacteriaceae (including Escherichia coli, Klebsiella pneumoniae, Enterobacter spp.).

[0151] Vaccine antigens can also include proteins that are specifically or preferentially expressed by cancer cells in order to activate the immune system to fight cancer. Examples of cancer antigens include A33; BAGE; B-cell maturation antigen (BCMA); Bcl-2; β -catenin; CA19-9; CA125; carboxy-anhydrase-IX (CAIX); CD5; CD19; CD20; CD21; CD22; CD24; CD33; CD37; CD45; CD123; CD133; 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; L1-CAM; LRP; MAGE; MART; mesothelin; MUC; MUM-1-B; myc; NYESO-1; p53, PRAME; progesterone receptor; PSA; PSCA; PSMA; ras; ROR1; survivin; SV40 T; tenascin; TSTA tyrosinase; VEGF; and WT1.

[0152] As more particular examples, cancer vaccine antigens can include or be derived from:

Cancer Antigen	Sequence
PSMA	MWNLLHETDSAVATARRPRWLCAGALVLAGGFFLLGFLFGWFIKSSNEATNIT PKHNMKAFLDELKAENIKKFLYNFTQIPHLAGTEQNFQLAKQIQSQWKEFGLDS VELAHYDVLLSYPNKTHPNYISIINEDGNEIFNTSLFEP PPPGYENVSDIVPPFSA FSPQGMPEGDLVYVNYARTEDFFKLERDMKINCSGKIVIARYGKVFRGNKVKN AQLAGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQQRGNILNLNGAGDPL TPGYPAN EYAYRRGIAEAVGLPSIPVHPIGYDDAQKLEKMGG SAPPDSSWRG SLKVPYNVGP GFTGNFSTQKV KMHIHSTNEVTRIYNVIGTLRGAVEPD RYVILG GHRDSWWFGGIDPQSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFG LLGSTEWAEENS RLLQERGVAYINADSSIEGNYTLRVDCTPLMYSLVHNLTKEL KSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGSGNDFEVFFQRLGIASGR ARYTKNWETNKFSGYPLYHSVYETVELVEKFYDPMFKYHLTVAQVRGGMVFE LANSIVLPFDCRDYAVVLRKYADKIYSISMKHPQEMKTYSVSFDSLFS AVKNFTE IASKFSERLQDFDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHVIYAPSS HNKYAGESFPGIYDALFDIESKVDPSKAWGEVKRQIYVAAFTVQAAAETLSEVA (SEQ ID NO: 135)
PSCA	MKAVLLALLMAGLALQPGTALLCYSCKAQVS NEDCLQVENCTQLGEQCWTARI RAVGLLTVISKGCSLNCVDDSDYYVGKKNITCCD TDLCNASGAHALQPAAAIL ALLPALGLLLWGPGQL (SEQ ID NO: 136)

Mesothelin	MALPTARPLLGSCGTPALGSLLFLLFSLGWQPSRTLGETGQEAAPLDGVLA NPPNISSLSPRQLLGFPACAEVSGLSTERVRELAVALAQKNVKLSTEQLRCLAHR LSEPPEDLDALPLDLLLFLNPDAFSGPQACTHFFSRITKANVDLLPRGAPERQR LLPAALACWGVVRGSLLSEADVRLGGLACDLPGRFVAESADEVLLPRLVSCPGP LDQDQQEAARAALQGGGPPYGPSTWSVSTMDALRGLLPVLGQPIIRSIPQGI VAAWRQRSSRDPSWRQPRTLPRFRREVEKTACPSGKKAREIDESLIFYKK WELEACVDAALLATQMDRVNAIPFTYEQLDVLKHKLDELYPQGYVESVIQHLG YLFLKMSPEDIRKWNVTSLKALLEVNKGHEMSPQVATLIDRFVKGRGQLDK DTLDTLTAFYPGYLCSLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKAR LAFQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPL TVAEVQKLLGPHVEGLKAEERHRPVRDWILRQRQDDDLTLGLGLQGGINGYL VLDLSVQEALSGTPCLLGGPVLTVLALLLASTLA (SEQ ID NO: 137)
CD19	MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWS RESPLKPFLLKLSLGLPGLGIHMRPLASWLFIFNVSSQQMGGFYLCQPGPPSEKA WQPGWTVNVEGSGELFRWNVSDLGGLGCGLNRSSEGPSSPSGKLMSPKLY VWAKDRPEIWEGEPPCVPPRDSLNSQLSQDLTMAPGSTLWLSCGVPPDSVSR GPLSWTHVHPKGPKSLLSLELKDDRPARDMWMETGLLLPRATAQDAGKYCYC HRGNLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQR ALVLRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQRW AAGLGGTAPSYGNPSSDVQADGALGSRSPPGVGPEEEEEGEGYEEDSEEDS EFYENDSNLGQDQLSQDGSQYENPEDEPLGPEDEDSFSNAESYENEDDELTQ PVARTMDFLSPHGSADWDPSREATSLGSQSYEDMRGILYAAPQLRSIRGQPGP NHEEDADSYENMDNPDGPDPAWGGGGGRMGWTR (SEQ ID NO: 138)
CD20	MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTL GAVQIMNGLFHIALGGLLMIPAGIYAPICVTWVYPLWGGIMYIISGSLLAATEKNS RKCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIYN CEPANPSEKNPSTQYCYSIQSLFLGILSVMLIAFFQELVIAGIVENEWKRTCS RPKSNIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIPIQEEEEETETN FPEPPQDQESSPIENDSSP (SEQ ID NO: 139)
ROR1	MHRPRRRGTRPPLLALLAALLAARGAAQETELSVSAELVPTSSWNISSELNK DSYLTLDPEMNNITSLGQTAELHCKVSGNPPPTIRWFKNDAPVVQEPRRLSF RSTIYGSRLRIRNLDTTDTGYFQCVATNGKEVVSSTGVLFVKFGPPPTASPGYS DEYEEDGFCQPYRGIACARFIGNRTVYMESLHMQGEIENQITAAFTMIGTSSHL SDKCSQFAIPSLCHYAFPYCDETSSVPKPRDLRDECEILENVLCQTEYIFARS NPMILMRLKLPNCEDLPQPESPEAANCIRIGIPMADPINKNHNKCYNSTGVDYRG TVSVTKSGRQCQPWNSQYPHTHTFTALRFPELNGGHSYCRNPGNQKEAPWC FTLDENFKSDLCDIPACDSKDSKEKNKMEILYILVPSVAIPLAIALFFFCVCRNN QKSSSAPVQRQPKHVRGQNVEMSMNLNAYKPKSKAKELPLSAVRFMEELGEC AFGKIYKGHLYLPGMDHAQLVAIKTLKDYNPNPQQWTEFQQEASLMAELHHPNI VCLLGAVTQEQPVCMLFEYINQGDLEHFLIMRSPHSDVGCSSDEDGTVKSSLD HGDFLHIAIQIAAGMEYLSSHFFVHKDLAARNILIGEQLHVKISDLGLSREIYSAD YYRVQSKSLLPIRWMPPEAIMYGKFSSDSDIWSFGVVLWEIFSFGGLQPYYGFS NQEVIEMVRKRQLLPCSEDCPPRMYSMLTECWNEIPSRRPRFKDIHVRLRSW EGLSSHTSSTTPSGGNATTQTTSLSASPVSNLNPNRYPNYMFPSQGITPQQGI AGFIGPPIPNQRFIPINGYPIPPGYAAFPAAHYQPTGPPRVIQHCPPPKSRSPS SASGSTSTGHVTSPLSSGSNQEANIPLLPHMSIPNHPGGMGITVFGNKSQKPY KIDSKQASLLGDANIHGHTESMISAE (SEQ ID NO: 140)
WT1	MGHHHHHHHHHHSSGHIEGRHMRRVPGVAPTLVRSASETSEKRPFMCAYPG CNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFFRSDQLKRHRHRRHTGV KPFQCKTCQRKFSRSDHLKTHTRTHTGEKPFSCRWPSQCQKKFARSDELVRHH NMHQRNMTKLQLAL (SEQ ID NO: 141)

[0153] VIII. Vaccine Adjuvants. Vaccines are often administered with vaccine adjuvants. The term “adjuvant” refers to material that enhances the immune response to an antigen and is used herein in the customary use of the term. The precise mode of action is not understood for all adjuvants, but such lack of understanding does not prevent their clinical use for a wide variety of vaccines.

[0154] Exemplary vaccine adjuvants, include any kind of Toll-like receptor ligand or combinations thereof (e.g. CpG, Cpg-28 (a TLR9 agonist), Polyribonucleosinic polyribocytidylic acid (Poly(I:C)), α -galactoceramide, MPLA, Motolimod (VTX-2337, a novel TLR8 agonist developed by VentiRx), IMO-2055 (EMD1201081), TMX-101 (imiquimod), MGN1703 (a TLR9 agonist), G100 (a stabilized emulsion of the TLR4 agonist glucopyranosyl lipid A), Entolimod (a derivative of Salmonella flagellin also known as CBLB502), Hiltonol (a TLR3 agonist), and Imiquimod), and/or inhibitors of heat-shock protein 90 (Hsp90), such as 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin).

[0155] In particular embodiments a squalene-based adjuvant can be used. Squalene is part of the group of molecules known as triterpenes, which are all hydrocarbons with 30 carbon molecules. Squalene can be derived from certain plant sources, such as rice bran, wheat germ, amaranth seeds, and olives, as well as from animal sources, such as shark liver oil. In particular embodiments, the squalene-based adjuvant is MF59® (Novartis, Basel, Switzerland). An example of a squalene-based adjuvant that is similar to MF59® but is designed for preclinical research use is Addavax™ (InvivoGen, San Diego, CA). MF59 has been FDA approved for use in an influenza vaccine, and studies indicate that it is safe for use during pregnancy (Tsai T, et al. Vaccine. 2010. 17:28(7):1877-80; Heikkinen T, et al. Am J Obstet Gynecol. 2012. 207(3):177). In particular embodiments, squalene based adjuvants can include 0.1% -20% (v/v) squalene oil. In particular embodiments, squalene based adjuvants can include 5%(v/v) squalene oil.

[0156] In particular embodiments the adjuvant alum can be used. Alum refers to a family of salts that contain two sulfate groups, a monovalent cation, and a trivalent metal, such as aluminum or chromium. Alum is an FDA approved adjuvant. In particular embodiments, vaccines can include alum in the amounts of 1-1000ug/dose or 0.1mg-10mg/dose. In particular embodiments, the adjuvant Vaxfectin® (Vical, Inc., San Diego, CA) can be used. Vaxfectin® is a cationic lipid based adjuvant.

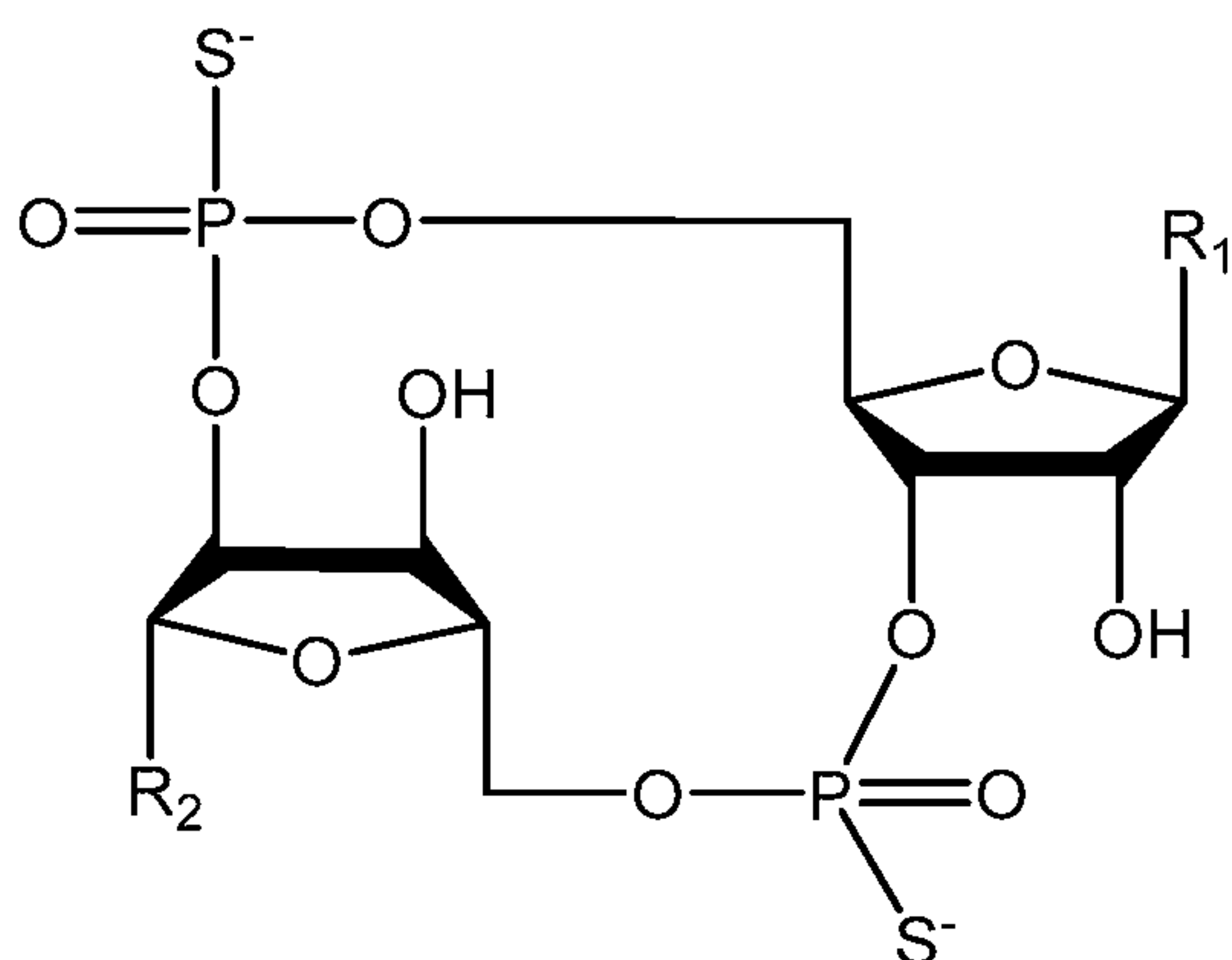
[0157] In particular embodiments, one or more STING agonists are used as a vaccine adjuvant. “STING” is an abbreviation of “stimulator of interferon genes”, which is also known as “endoplasmic reticulum interferon stimulator (ERIS)”, “mediator of IRF3 activation (MITA)”, “MPYS” or “transmembrane protein 173 (TM173)”. STING is a transmembrane receptor protein and is encoded by the gene TMEM173 in human. Activation of STING leads to production of Type I interferons (e.g. IFN- α and IFN- β), via the IRF3 (interferon regulatory factor 3) pathway; and to production of pro-inflammatory cytokines (e.g. TNF- α and IL-1 β), via the NF- κ B pathway and/or the NLRP3 inflammasome.

[0158] Human and murine STING are naturally activated two ways: via binding of exogenous (3',3') cyclic dinucleotides (c-diGMP, c-diAMP and c-GAMP) that are released by invading bacteria or archaea; and via binding of (2',3')cyclic guanosine monophosphate-adenosine monophosphate ((2',3')c-GAMP), an endogenous cyclic dinucleotide that is produced by the enzyme cyclic GMP-AMP synthase (cGAS; also known as C6orf150 or MB21D1) in the presence of exogenous double-stranded DNA (e.g. that released by invading bacteria, viruses or protozoa).

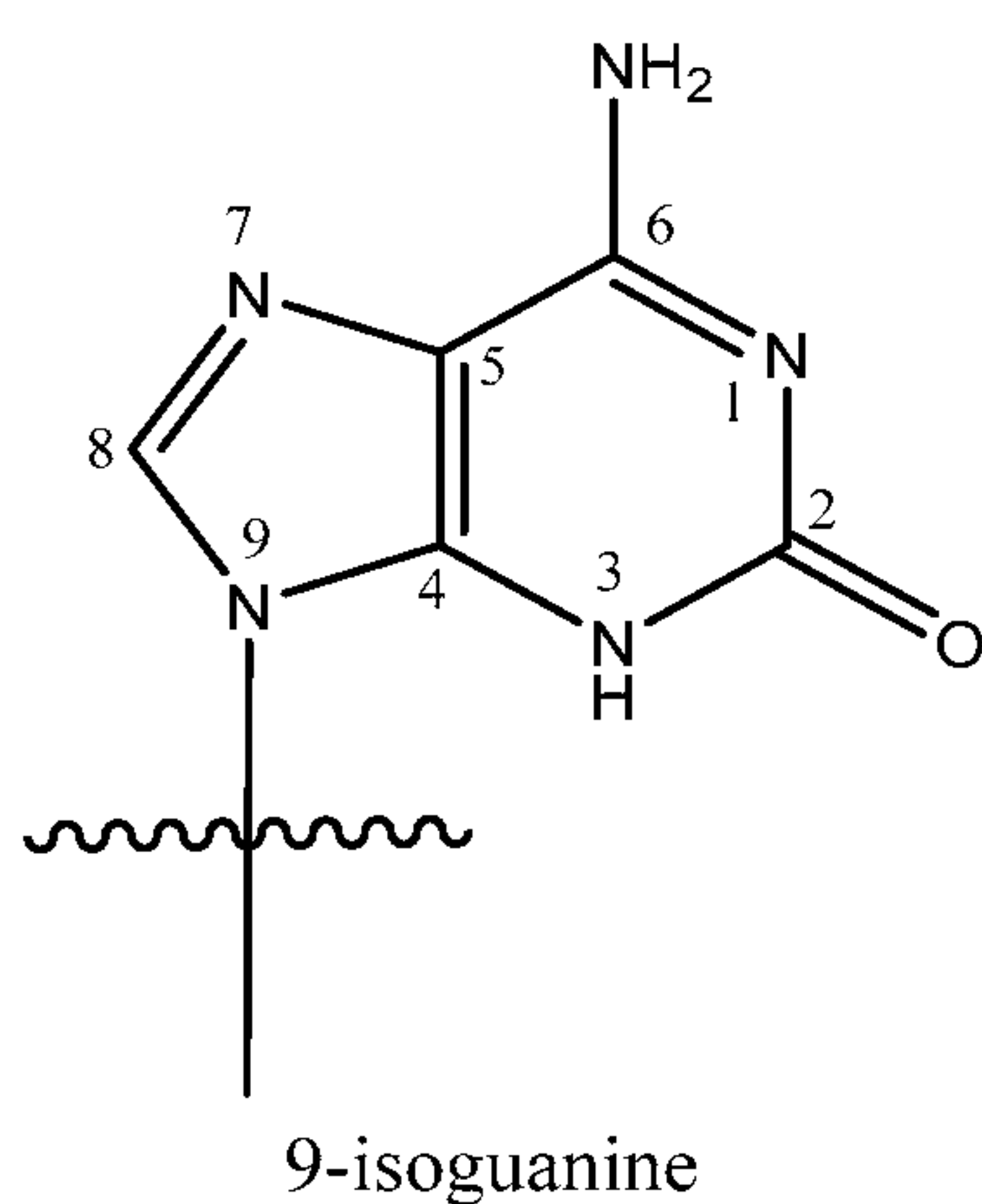
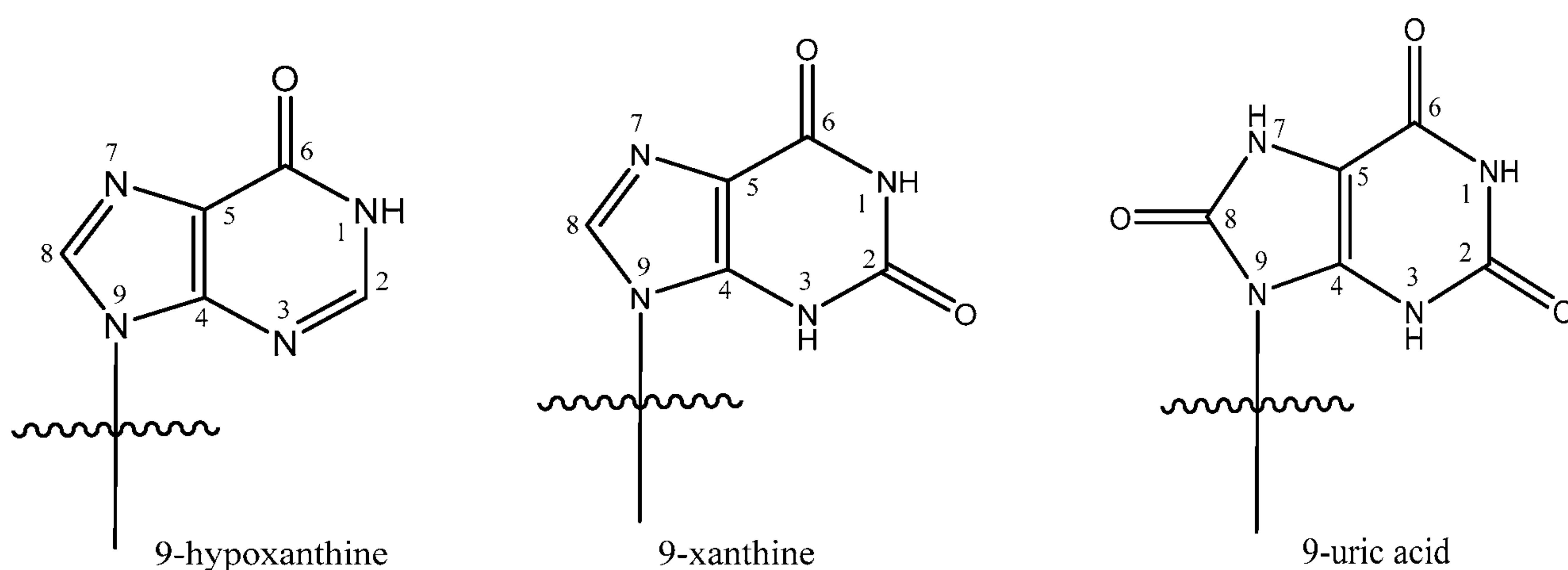
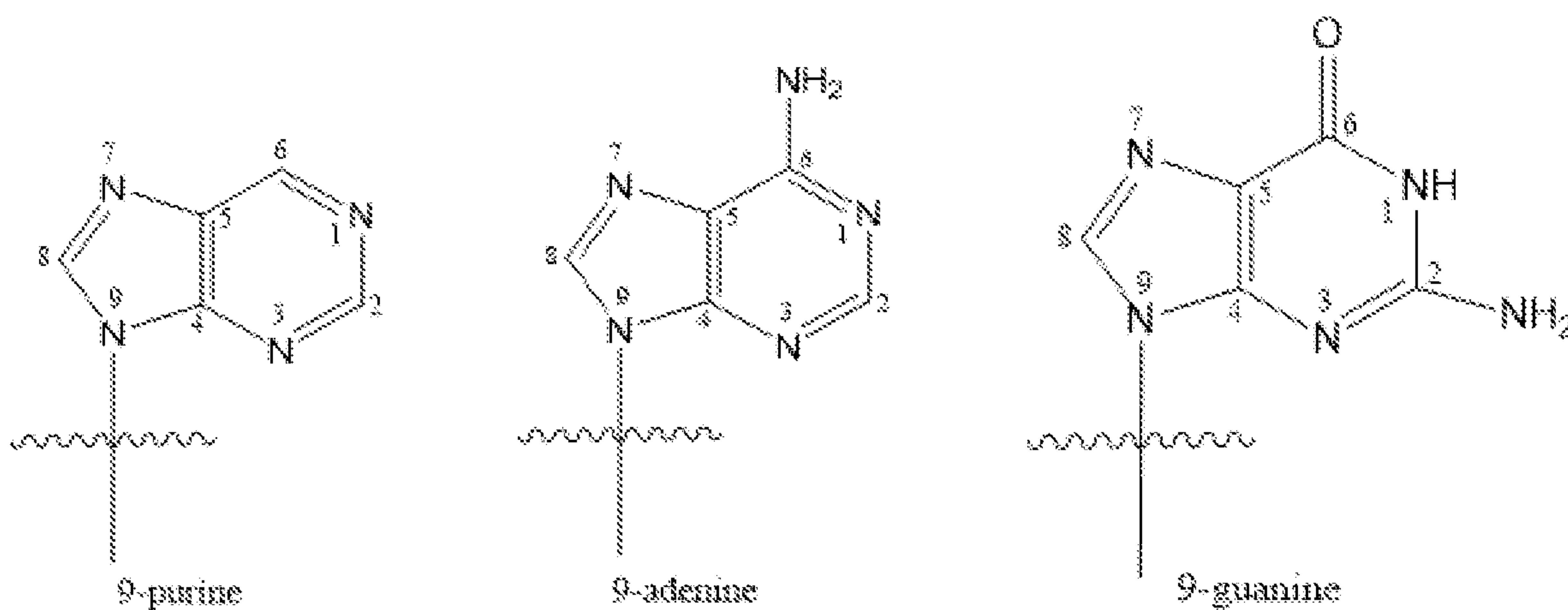
[0159] The term "STING agonist" refers to a substance that activates the STING receptor in vitro or in vivo. A compound can be deemed a STING agonist if: (i) induces Type I interferons in vitro in human or animal cells that contain STING; and (ii) does not induce Type I interferons in vitro in human or animal cells that do not contain STING or does not contain functioning STING. A typical test to ascertain whether a ligand is a STING agonist is to incubate the ligand in a wild-type human or animal cell line and in the corresponding cell line in which the STING coding gene has been genetically inactivated by a few bases or a longer deletion (e.g. a homozygous STING knockout cell line). An agonist of STING will induce Type I interferon in the wild-type cells but will not induce Type I interferon in the cells in which STING is inactivated.

[0160] In particular embodiments, STING agonists include cyclic molecules with one or two phosphodiester linkages, and/or one or two phosphorothioate diester linkages, between two nucleotides. This includes (3',5')-(3',5') nucleotide linkages (abbreviated as (3',3')); (3',5')-(2',5') nucleotide linkages (abbreviated as (3',2')); (2',5')-(3',5') nucleotide linkages (abbreviated as (2',3')); and (2',5')-(2',5') nucleotide linkages (abbreviated as (2',2')). "Nucleotide" refers to any nucleoside linked to a phosphate group at the 5', 3' or 2' position of the sugar moiety.

[0161] In particular embodiments, STING agonists include compounds of the formula:

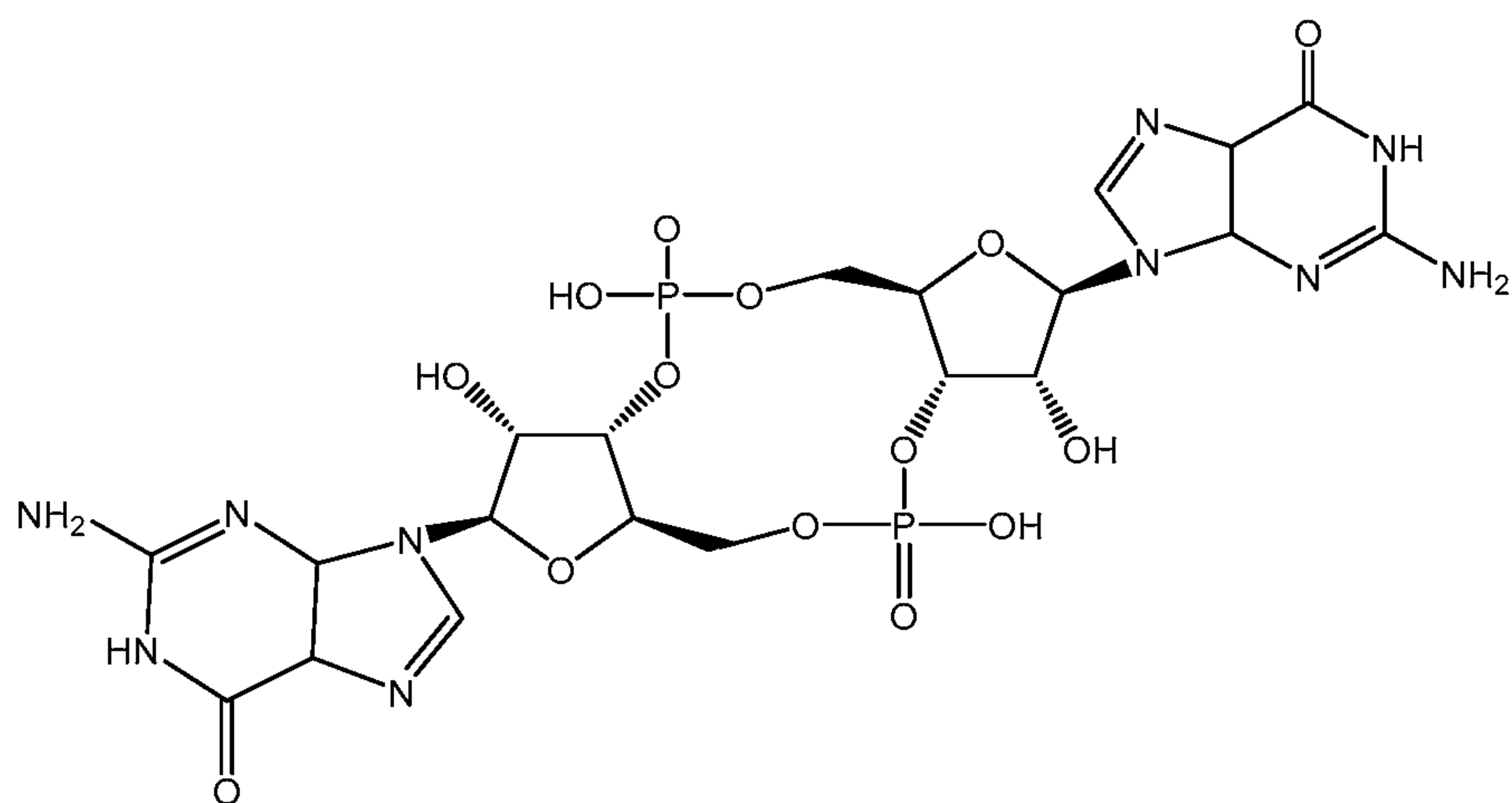


[0162] In particular embodiments, R1 and R2 may be independently 9-purine, 9-adenine, 9-guanine, 9-hypoxanthine, 9-xanthine, 9-uric acid, or 9-isoguanine, as shown below:

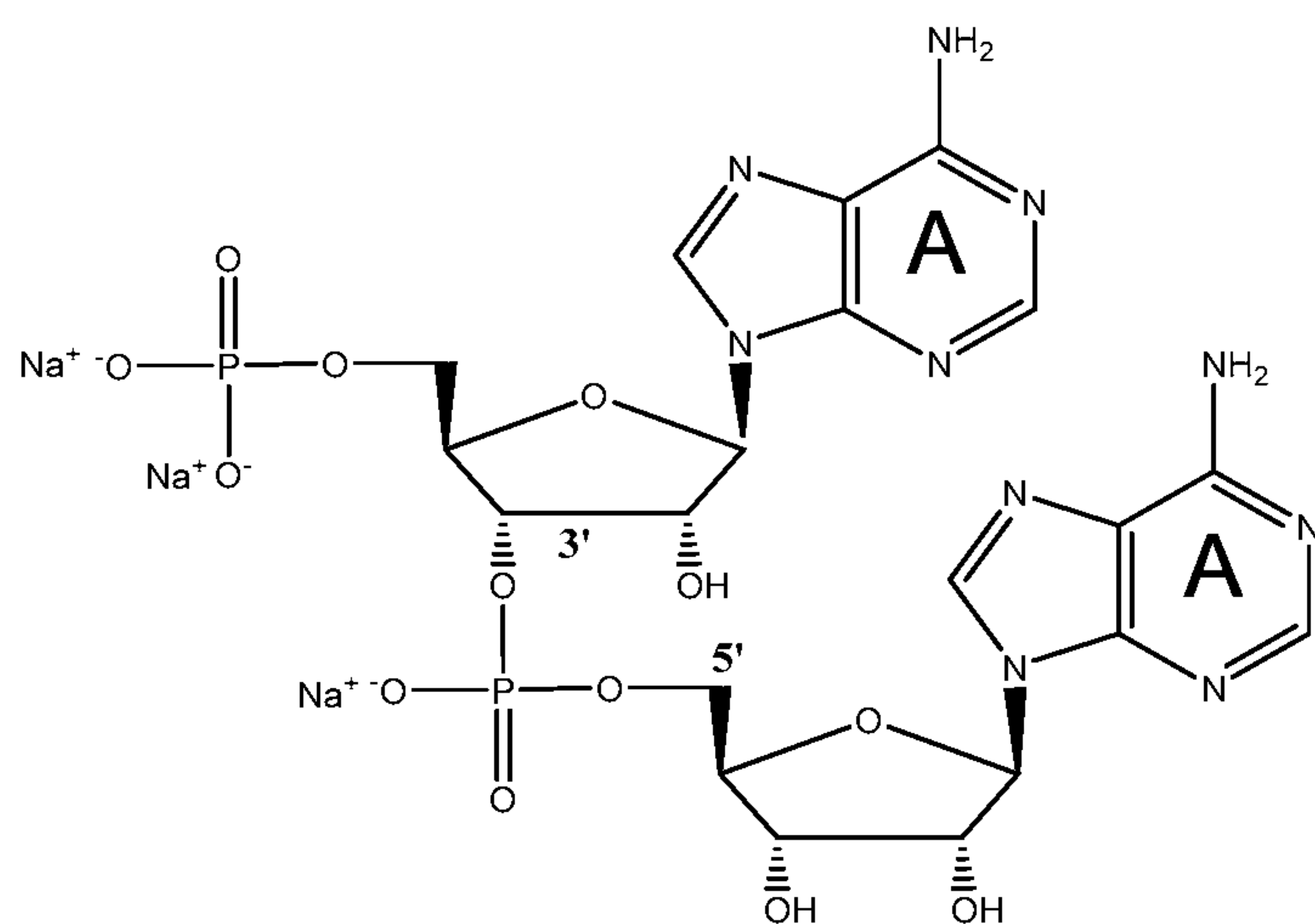


[0163] In particular embodiments, the STING agonist can include dithio-(RP, RP)-[cyclic[A(2',5')pA(3',5')p]] (also known as 2'-5', 3'-5' mixed phosphodiester linkage (ML) RR-S2 c-di-AMP or ML RR-S2 CDA), ML RR-S2-c-di-GMP (ML-CDG), ML RR-S2 cGAMP, or any mixtures thereof.

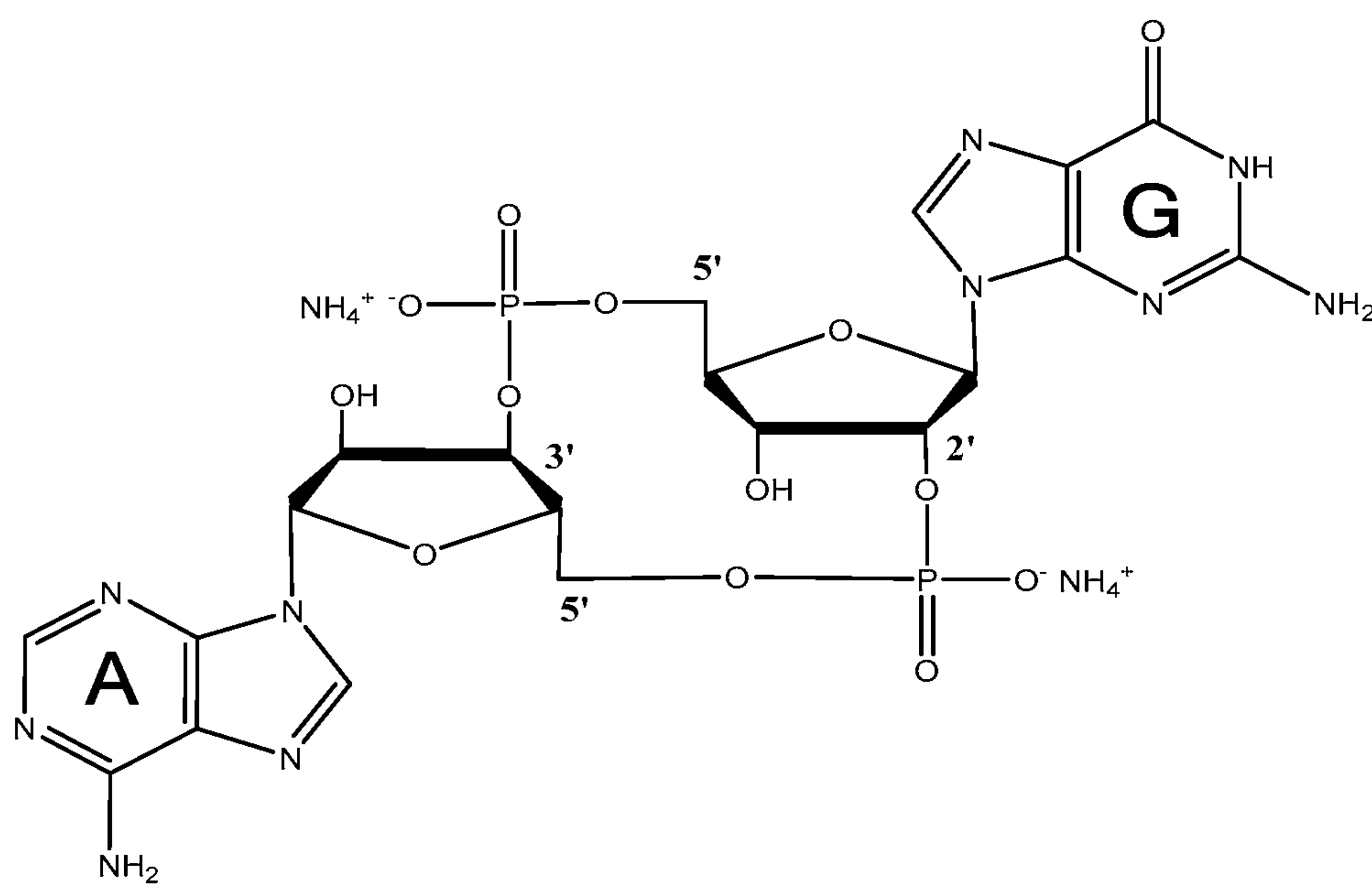
[0164] The structure of c-diGMP includes:



[0165] The structure of c-diAMP includes:



[0166] The structure of c-GAMP includes:



[0167] Additional particular examples of STING agonists include c-AIMP; (3',2')c-AIMP; (2',2')c-AIMP; (2',3')c-AIMP; c-AIMP(S); c-(dAMP-dIMP); c-(dAMP-2'FdIMP); c-(2'FdAMP-2'FdIMP); (2',3')c-(AMP-2'FdIMP); c-[2'FdAMP(S)-2'FdIMP(S)]; c-[2'FdAMP(S)-2'FdIMP(S)](POM)₂; and DMXAA. Additional examples of STING agonists are described in WO2016/145102.

[0168] Other immune stimulants can also be used as vaccine adjuvants. Additional exemplary small molecule immune stimulants include TGF- β inhibitors, SHP-inhibitors, STAT-3 inhibitors, and/or STAT-5 inhibitors. Exemplary siRNA capable of down-regulating immune-suppressive signals or oncogenic pathways (such as kras) can be used whereas any plasmid DNA (such as minicircle DNA) encoding immune-stimulatory proteins can also be used.

[0169] Exemplary cytokines include IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, TNF α , IFN- α , IFN- β , IFN- γ , or GM-CSF. In particular embodiments, the immune stimulant may be a cytokine and or a combination of cytokines, such as IL-2, IL-12 or IL-15 in combination with IFN- α , IFN- β or IFN- γ , or GM-CSF, or any effective combination thereof, or any other effective combination of cytokines. The above-identified cytokines stimulate T_H1 responses, but cytokines that stimulate T_H2 responses may also be used, such as IL-4, IL-10, IL-11, or any effective combination thereof. Also, combinations of cytokines that stimulate T_H1 responses along with cytokines that stimulate T_H2 responses may be used.

[0170] Immune stimulants derived from the molecules noted in the preceding paragraphs can also be used. For example, RLI is an IL-15-IL-15 receptor- α fusion protein that exhibits 50-fold greater potency than IL-15 alone. IL-15 particularly impacts anti-tumor immune response at multiple points. It can differentiate monocytes into stimulatory antigen presenting cells; promote the effector functions and proliferation of tumor-reactive T cells; and recruit and activate NK cells.

[0171] IX. Compositions. The polynucleotides, NP, vaccine antigens, and/or vaccine adjuvants disclosed herein (individually, collectively, or in grouped combinations referred to as "active ingredients") can be provided as part of compositions formulated for administration to subjects.

[0172] In particular embodiments, the active ingredients are provided as part of a composition that can include, for example, at least 0.1% w/v or w/w of active ingredient(s); at least 1% w/v or w/w of active ingredient(s); at least 10% w/v or w/w of active ingredient(s); at least 20% w/v or w/w of active ingredient(s); at least 30% w/v or w/w of active ingredient(s); at least 40% w/v or w/w of active ingredient(s); at least 50% w/v or w/w of active ingredient(s); at least 60% w/v or w/w of active ingredient(s); at least 70% w/v or w/w of active ingredient(s); at least 80% w/v or w/w of active ingredient(s); at least 90% w/v or w/w of active ingredient(s); at least 95% w/v or w/w of active ingredient(s); or at least 99% w/v or w/w of active ingredient(s).

[0173] If cells are genetically modified ex vivo, compositions can include greater than 10² cells, greater than 10³ cells, greater than 10⁴ cells, greater than 10⁵ cells, greater than 10⁶ 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} . In particular embodiments, compositions can be calibrated to provide 1 million – 20 million genetically modified cells per kilogram when administered to a subject.

[0174] The compositions disclosed herein can be formulated for administration by, for example, injection, inhalation, infusion, perfusion, lavage or ingestion. The compositions can further be formulated for, for example, intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, oral and/or subcutaneous administration and more particularly by intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, oral and/or subcutaneous injection.

[0175] For injection, compositions can be formulated as aqueous solutions, such as in buffers including Hanks' solution, Ringer's solution, or physiological saline. The aqueous solutions can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the formulation can be in lyophilized and/or powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0176] For oral administration, the compositions can be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. For oral solid formulations such as, for example, powders, capsules and tablets, suitable excipients include binders (gum tragacanth, acacia, cornstarch, gelatin), fillers such as sugars, e.g. lactose, sucrose, mannitol and sorbitol; dicalcium phosphate, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate; cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP); granulating agents; and binding agents. If desired, disintegrating agents can be added, such as corn starch, potato starch, alginic acid, cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. If desired, solid dosage forms can be sugar-coated or enteric-coated using standard techniques. Flavoring agents, such as peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc. can also be used.

[0177] For administration by inhalation, compositions can be formulated as aerosol sprays from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the therapeutic and a suitable powder base such as lactose or starch.

[0178] Any composition formulation disclosed herein can advantageously include any other pharmaceutically acceptable carriers which include those that do not produce significantly adverse, allergic or other untoward reactions that outweigh the benefit of administration, whether for research, prophylactic and/or therapeutic treatments. Exemplary pharmaceutically acceptable carriers and formulations are disclosed in Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990. Moreover, formulations can be prepared to meet sterility, pyrogenicity, general safety and purity standards as required by United States FDA Office of Biological Standards and/or other relevant foreign regulatory agencies.

[0179] Exemplary generally used pharmaceutically acceptable carriers include any and all bulking agents or fillers, solvents or co-solvents, dispersion media, coatings, surfactants, antioxidants (e.g., ascorbic acid, methionine, vitamin E), preservatives, isotonic agents, absorption delaying agents, salts, stabilizers, buffering agents, chelating agents (e.g., EDTA), gels, binders, disintegration agents, and/or lubricants.

[0180] Exemplary buffering agents include citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers and/or trimethylamine salts.

[0181] 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.

[0182] Exemplary isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol or mannitol.

[0183] Exemplary stabilizers include organic sugars, polyhydric sugar alcohols, polyethylene glycol; sulfur-containing reducing agents, amino acids, low molecular weight polypeptides, proteins, immunoglobulins, hydrophilic polymers or polysaccharides.

[0184] Compositions can also be formulated as depot preparations. Depot preparations can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as sparingly soluble salts.

[0185] Additionally, compositions can be formulated as sustained-release systems utilizing semipermeable matrices of solid polymers containing at least one active ingredient. Various sustained-release materials have been established and are well known by those of ordinary skill in the art. Sustained-release systems may, depending on their chemical nature, release active ingredients following administration for a few weeks up to over 100 days.

[0186] X. Kits. Combinations of active ingredients can also be provided as kits. Kits can include

containers including one or more or more PN, NP, vaccine antigens, and/or vaccine adjuvants described herein formulated individually, or in various combinations. Generally, the kit will include PN, NP, vaccine antigens, and/or vaccine adjuvants specific to enhance vaccine efficacy against a particular infectious agent or cancer antigen, such as those described elsewhere herein.

[0187] Kits can also include a notice in the form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use, or sale for human administration. The notice may state that the provided active ingredients can be administered to a subject. The kits can include further instructions for using the kit, for example, instructions regarding preparation of PN, NP, vaccine antigens, and/or vaccine adjuvants for administration; proper disposal of related waste; and the like. The instructions can be in the form of printed instructions provided within the kit or the instructions can be printed on a portion of the kit itself. Instructions may be in the form of a sheet, pamphlet, brochure, CD-Rom, or computer-readable device, or can provide directions to instructions at a remote location, such as a website. In particular embodiments, kits can also include some or all of the necessary medical supplies needed to use the kit effectively, such as syringes, ampules, tubing, facemask, an injection cap, sponges, sterile adhesive strips, Chloraprep, gloves, and the like. Variations in contents of any of the kits described herein can be made. The instructions of the kit will direct use of the active ingredients to effectuate a new clinical use described herein.

[0188] XI. Methods of Use. Once formed, the compositions find use in a number of applications in subjects. Subjects include human subjects, veterinary animals (dogs, cats, reptiles, birds, etc. and also including animals found within zoos), livestock (horses, cattle, goats, pigs, chickens, etc.), and research animals (monkeys, rats, mice, fish, etc.). "Subjects in need thereof" include those in need of treatment, such as, those with a condition (e.g., an infection, cancer), as well as those prone to have or develop a condition (e.g., an infection, cancer), or those in whom a condition is to be prevented, such as those in a high risk group for exposure to a pathogen or cancer recurrence.

[0189] The skilled artisan will appreciate that the immune system produces an innate immune response and an adaptive immune response following a vaccination. An innate immune response generally can be characterized as not being substantially antigen specific and/or not generating immune memory. An adaptive immune response can be characterized as being substantially antigen specific, maturing over time (e.g., increasing affinity and/or avidity for antigen), and can produce immunologic memory. Even though these and other functional distinctions between innate and adaptive immunity can be discerned, the skilled artisan will appreciate that the innate and adaptive immune systems can be integrated and therefore can act in concert.

[0190] In particular embodiments, an adaptive immune response can be a "primary immune response" which refers to an immune response occurring on the first exposure of a "naive" subject

to a vaccine antigen. For example, in the case of a primary antibody response, after a lag or latent period of from 3 to 14 days depending on, for example, the composition, dose, and subject, antibodies to the vaccine antigen can be produced. Generally, IgM production lasts for several days followed by IgG production and the IgM response can decrease. Antibody production can terminate after several weeks but memory cells can be produced. The primary immune response also triggers CD4+ and CD8+ T cell activation and proliferation. In particular embodiments, an adaptive immune response can be a "secondary immune response", "anamnestic response," or "booster response" which refer to the immune response occurring on a second and subsequent exposure of a subject to a vaccine antigen disclosed herein. Generally, in a secondary immune response, memory cells respond to the vaccine antigen and therefore the secondary immune response can differ from a primary immune response qualitatively and/or quantitatively. For example, in comparison to a primary immune response, the lag period of a secondary immune response can be shorter, the peak response can be higher, higher affinity antibodies and TCRs can be produced, and/or the response can persist for a greater period of time. In particular embodiments, "immune responses" can be measured by expansion, persistence, and/or activity of memory T cells (e.g., TCM and/or TEM).

[0191] In particular embodiments, improving the efficacy of a vaccination results in at least one of the following after administration of a therapeutically effective amount of a composition disclosed herein within a clinically relevant time window: increased activation and/or proliferation of CD4+ and/or CD8+ T cells, increased production and retention of memory T cells (e.g., TCM and/or TEM), a shortened lag time before a secondary immune response, a higher peak response during a secondary immune response, and/or a greater persistence of a secondary immune response.

[0192] In particular embodiments, improving the efficacy of a vaccination results in at least one of the following after administration of a therapeutically effective amount of a composition disclosed herein within a clinically relevant time window: an improved prophylactic treatment and/or an improved therapeutic treatment.

[0193] Prophylactic treatments prevent or reduce the occurrence or severity of, or slow down or lessen the development of a potential disorder or disease. Prophylactic vaccine treatments increase the immunity of a subject against an infectious pathogen or type of cancer. Therefore, in particular embodiments, a vaccine may be administered prophylactically, for example to a subject that is immunologically naive (e.g., no prior exposure or experience with an infectious pathogen or cancer).

[0194] The compositions can be administered prophylactically in subjects who are at risk of developing a condition (e.g., an infection caused by HIV, malaria, herpes, chlamydia, EBV, Pneumococcus, and/or Hepatitis or cancer), or who have been exposed an agent leading to such an infection, to prevent, reduce, or delay the development of the infection or associated disease. For example, the compositions can be administered to a subject likely to have been exposed to HIV,

malaria, herpes, chlamydia, EBV, Pneumococcus, and/or Hepatitis or to a subject who is at high risk for exposure to HIV, malaria, herpes, chlamydia, EBV, Pneumococcus, and/or Hepatitis or cancer recurrence.

[0195] Therapeutic treatments include reducing, eliminating, or slowing down the progression of an existing disorder or disease. In particular embodiments, a vaccine may be administered therapeutically to a subject who has been exposed to an infectious pathogen or cancer. Thus, a vaccine can be used to ameliorate a symptom associated with an infectious pathogen such as a reduced T cell count in the context of HIV infection and AIDS.

[0196] In particular embodiments, improving the efficacy of a vaccination provides an improved anti-infection effect. An anti-infection effect can reduce the number of cells that become infected, increase the time before cells become infected, prevent a higher level of infection, decrease the number of infected cells, decrease the volume of infected tissue, increase life expectancy, induce sensitivity of infected cells to immune clearance, reduce infection-associated pain, and/or prevent, reduce, delay, or eliminate a symptom associated with the treated infection.

[0197] In particular embodiments, improving the efficacy of a vaccination provides an improved anti-cancer effect. An anti-cancer effect can include a decrease in the occurrence of cancer cells, a decrease in the number of cancer cells, a decrease in the occurrence of metastases, a decrease in the number of metastases, a decrease in tumor volume, an increase in life expectancy, induced sensitivity of cancer cells to immune clearance, inhibited cancer cell proliferation, inhibited tumor growth, prolonged subject life, reduced cancer-associated pain, and/or reduced or delayed relapse or re-occurrence of cancer following treatment.

[0198] The actual dose of active ingredients administered to a particular subject can be determined by a physician, veterinarian, or researcher taking into account parameters such as physical and physiological factors including target, body weight, presence and/or severity of infection or cancer, stage of infection or cancer, previous or concurrent therapeutic interventions, idiopathy of the subject, and route of administration.

[0199] For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from in vitro assays and/or animal model studies.

[0200] Exemplary doses of compositions include 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240 or 250 $\mu\text{g/kg}$ body mass or mg/kg body mass although higher and/or lower doses can be used. The number of doses that can be administered as a function of time can be from 1, 2, 3, 4 or 5 doses over 1, 2, 3, 4, 5 or 6 weeks but can be increased or decreased depending at least in part on the immune status of a subject.

[0201] When genetically modified cells are administered as part of a composition, exemplary

therapeutically effective amounts to administer can include 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} cells. In particular embodiments, therapeutically effective amounts include 1 million – 20 million cells per kilogram.

[0202] In particular embodiments, a composition can be administered initially, and thereafter maintained by further administration. For example, a composition can be administered by intramuscular injection. The subject's levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition, may be used. In the instance of a vaccine and NP composition, the composition may be administered as a single dose, or the composition may incorporate set booster doses. For example, booster doses may include variants of vaccine antigens and TCR to provide protection against multiple clades of infectious agents.

[0203] In particular embodiments, active ingredients for administration in one or more compositions can be (i) a PN and/or a PN within a NP, (ii) a vaccine antigen and (iii) a vaccine adjuvant. In particular embodiments, when included in combinations, the substituents in the combination can be provided in exemplary ratios such as: 1:1:1; 1:2:1; 1:3:1; 1:4:1; 1:5:1; 1:10:1; 1:2:2; 1:2:3; 1:3:4; 1:4:2; 1:5:3; 9:10:20; 5:2:1; 5:3:11; 5:4:1; 5:5:1; 5:100:1; 5:20:2; 5:2:3; 5:14:200; 5:10:20; or additional beneficial ratios depending on the number and identity of substituents in a combination to reach an intended effect. The substituents in a combination can be provided within the same composition or within different compositions, as will be understood by one of ordinary skill in the art.

[0204] Therapeutically effective amounts can be achieved by administering single or multiple doses during the course of a treatment regimen (e.g., QID, TID, BID, daily, every other day, every 3 days, every 4 days, every 5 days, every 6 days, weekly, every 2 weeks, every 3 weeks, monthly, every 2 months, every 3 months, every 4 months, every 5 months, every 6 months, every 7 months, every 8 months, every 9 months, every 10 months, every 11 months, or yearly).

[0205] In particular embodiments, PN (in any of the various disclosed forms (e.g., naked or within NP) are administered within 1 month of vaccine antigen, within 3 weeks of vaccine antigen, within 2 weeks of vaccine antigen, within 1 week of vaccine antigen, within 7 days of vaccine antigen, within 6 days of vaccine antigen, within 5 days of vaccine antigen, within 4 days of vaccine antigen, within 3 days of vaccine antigen, within 2 days of vaccine antigen, within 24 hours of vaccine antigen, within 22 hours of vaccine antigen, within 20 hours of vaccine antigen, within 18 hours of vaccine antigen, within 16 hours of vaccine antigen, within 14 hours of vaccine antigen, within 12 hours of vaccine antigen, within 10 hours of vaccine antigen, within 8 hours of vaccine antigen, within 6 hours of vaccine antigen, within 4 hours of vaccine antigen, within 2 hours of vaccine antigen, or within 1 hours of vaccine antigen. "Within" includes before or after vaccine administration, and each of these

times can provide a clinically relevant time window.

[0206] In particular embodiments, enhanced vaccine efficacy decreases a subject's development of a condition. Conditions can be evaluated through clinical endpoints such as blood tests, evaluation of biopsy samples, and symptoms of conditions as fever, chills, rash, joint pain, nausea, vomiting, red eyes, cancer recurrence, etc..

[0207] Unless otherwise indicated, the practice of the present disclosure can employ conventional techniques of immunology, molecular biology, microbiology, cell biology and recombinant DNA. These methods are described in the following publications. See, e.g., Sambrook, et al. *Molecular Cloning: A Laboratory Manual*, 2nd Edition (1989); F. M. Ausubel, et al. eds., *Current Protocols in Molecular Biology*, (1987); the series *Methods IN Enzymology* (Academic Press, Inc.); M. MacPherson, et al., *PCR: A Practical Approach*, IRL Press at Oxford University Press (1991); MacPherson et al., eds. *PCR 2: Practical Approach*, (1995); Harlow and Lane, eds. *Antibodies, A Laboratory Manual*, (1988); and R. I. Freshney, ed. *Animal Cell Culture* (1987).

[0208] Sequence information provided by public database can be used to identify additional gene and protein sequences that can be used with the systems and methods disclosed herein.

[0209] Variants of the sequences disclosed and referenced herein are also included. Variants of proteins can include those having one or more conservative amino acid substitutions. As used herein, a "conservative substitution" involves a substitution found in one of the following conservative substitutions groups: Group 1: Alanine (Ala), Glycine (Gly), Serine (Ser), Threonine (Thr); Group 2: Aspartic acid (Asp), Glutamic acid (Glu); Group 3: Asparagine (Asn), Glutamine (Gln); Group 4: Arginine (Arg), Lysine (Lys), Histidine (His); Group 5: Isoleucine (Ile), Leucine (Leu), Methionine (Met), Valine (Val); and Group 6: Phenylalanine (Phe), Tyrosine (Tyr), Tryptophan (Trp).

[0210] Additionally, amino acids can be grouped into conservative substitution groups by similar function or chemical structure or composition (e.g., acidic, basic, aliphatic, aromatic, sulfur-containing). For example, an aliphatic grouping may include, for purposes of substitution, Gly, Ala, Val, Leu, and Ile. Other groups containing amino acids that are considered conservative substitutions for one another include: sulfur-containing: Met and Cysteine (Cys); acidic: Asp, Glu, Asn, and Gln; small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, and Gly; polar, negatively charged residues and their amides: Asp, Asn, Glu, and Gln; polar, positively charged residues: His, Arg, and Lys; large aliphatic, nonpolar residues: Met, Leu, Ile, Val, and Cys; and large aromatic residues: Phe, Tyr, and Trp. Additional information is found in Creighton (1984) *Proteins*, W.H. Freeman and Company.

[0211] As indicated elsewhere, variants of gene sequences can include codon optimized variants, sequence polymorphisms, splice variants, and/or mutations that do not affect the function of an encoded product to a statistically-significant degree.

[0212] Variants of the protein, nucleic acid, and gene sequences disclosed herein also include sequences with at least 70% sequence identity, 80% sequence identity, 85% sequence identity, 90% sequence identity, 95% sequence identity, 96% sequence identity, 97% sequence identity, 98% sequence identity, or 99% sequence identity to the protein, nucleic acid, or gene sequences disclosed herein.

[0213] "% sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between protein, nucleic acid, or gene sequences as determined by the match between strings of such sequences. "Identity" (often referred to as "similarity") can be readily calculated by known methods, including those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1994); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (Von Heijne, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Oxford University Press, NY (1992). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR, Inc., Madison, Wisconsin). Multiple alignment of the sequences can also be performed using the Clustal method of alignment (Higgins and Sharp CABIOS, 5, 151-153 (1989) with default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Relevant programs also include the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin); BLASTP, BLASTN, BLASTX (Altschul, et al., J. Mol. Biol. 215:403-410 (1990); DNASTAR (DNASTAR, Inc., Madison, Wisconsin); and the FASTA program incorporating the Smith-Waterman algorithm (Pearson, Comput. Methods Genome Res., [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, N.Y.). Within the context of this disclosure it will be understood that where sequence analysis software is used for analysis, the results of the analysis are based on the "default values" of the program referenced. As used herein "default values" will mean any set of values or parameters, which originally load with the software when first initialized.

[0214] The Exemplary Embodiments and Examples below are included to demonstrate particular embodiments of the disclosure. Those of ordinary skill in the art should recognize in light of the present disclosure that many changes can be made to the particular embodiments disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the disclosure. Exemplary Embodiments.

1. A method of vaccinating a subject including:
administering a therapeutically effective amount of a polynucleotide encoding a T cell receptor (TCR) to a subject wherein the encoded TCR specifically binds a vaccine antigen administered to the subject within a clinically relevant time window of the administering, thereby vaccinating the subject.
2. A method of embodiment 1 wherein the administering improves the efficacy of the vaccination as compared to administration of the vaccine antigen alone.
3. A method of embodiment 1 or 2 wherein the subject is in need of improved vaccine efficacy due to age or immune status.
4. A method of embodiment 3 wherein the immune status includes a low T cell count.
5. A method of any of embodiments 1-4 wherein the vaccinating provides a treatment for AIDS, malaria, herpes, chlamydia, Epstein-Barr virus, Pneumococcus, or Hepatitis B.
6. A method of any of embodiments 1-5 wherein the TCR is Class I restricted.
7. A method of any of embodiments 1-5 wherein the TCR is Class II restricted.
8. A method of embodiment 6 wherein the TCR is Class I restricted and the improved vaccine efficacy is due to CD8⁺ T helper cell activity that improves a T cell cytotoxic response.
9. A method of embodiment 7 wherein the TCR is Class II restricted and the improved vaccine efficacy is due to CD4⁺ T helper cell activity that improves a B cell antibody response.
10. A method of any of embodiments 1-9 wherein the TCR includes the variable regions of an α chain and a β chain.
11. A method of any of embodiments 1-10 wherein the TCR includes the constant regions of an α chain and a β chain.
12. A method of any of embodiments 1-11 wherein the TCR includes a transmembrane domain and a cytoplasmic tail.
13. A method of any of embodiments 1-12 wherein the TCR includes an α chain selected from SEQ ID NOs: 1, 4, 18, 21, 23, 25, 27, 29-32, 34, and 36.
14. A method of any of embodiments 1-13 wherein the TCR includes an β chain selected from SEQ ID NOs: 2, 3, 19, 22, 24, 26, 28, 33, 35, and 37.
15. A method of any of embodiments 1-12 wherein the TCR includes sequences selected from SEQ ID NOs: 5-12, 15, 16, and 39.
16. A method of any of embodiments 1-15 wherein the vaccine antigen includes a viral antigen.
17. A method of embodiment 16 wherein the viral antigen is derived from an adenovirus, arenavirus, bunyavirus, coronavirus, flavivirus, hantavirus, hepadnavirus, herpesvirus, papillomavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, orthomyxovirus, retrovirus, reovirus, rhabdovirus, rotavirus, spongiform virus or togavirus.
18. A method of embodiment 16 or 17 wherein the viral antigen includes a peptide expressed by

cytomegalovirus, cold virus, Epstein-Barr virus, flu virus, hepatitis A, B, or C virus, herpes simplex virus, human immunodeficiency virus, influenza virus, Japanese encephalitis virus, measles virus, polio virus, rabies virus, respiratory syncytial virus, rubella virus, smallpox virus, varicella zoster virus, West Nile virus, or Zika virus.

19. A method of any of embodiments 16-18 wherein the viral antigen includes a cytomegaloviral antigen selected from envelope glycoprotein B and/or CMV pp65; an Epstein-Barr antigen selected from EBV EBNA1, EBV P18, and/or EBV P23; a hepatitis vaccine antigen selected from the S, M, and/or L proteins or the pre-S antigen of hepatitis B virus; a herpes simplex vaccine antigen selected from glycoprotein D; a human immunodeficiency virus (HIV) vaccine antigen selected from 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/or HIV reverse transcriptase; a human papillomavirus virus (HPV) viral antigen selected from the L1 protein; an influenza vaccine antigen selected from hemagglutinin and neuraminidase; a Japanese encephalitis vaccine antigen selected from proteins E, M-E, M-E-NS1, NS1, or NS1-NS2A; a malaria vaccine antigen selected from circumsporozoite (CSP), glutamate dehydrogenase, lactate dehydrogenase, or fructose-bisphosphate aldolase; a measles vaccine antigen selected from measles virus fusion protein; a rabies vaccine antigen selected from rabies glycoprotein or rabies nucleoprotein; a respiratory syncytial vaccine antigen selected from RSV fusion protein or M2 protein; a rotaviral vaccine antigen selected from VP7sc; a rubella vaccine antigen selected from protein E1 or E2; a varicella zoster vaccine antigen selected from gpl or gpII; or a zika vaccine antigen selected from pre-membrane, envelope (E), Domain III of the E protein, or non-structural proteins 1, 2, 3, 4, or 5.

20. A method of any of embodiments 16-18 wherein the viral antigen is selected from Nef (66-97), Nef (116-145), Gag p17 (17-35), Gag p17-p24 (253-284), Pol 325-355 (RT 158-188), CSP central repeat region, or E protein Domain III.

21. A method of any of embodiments 16-20 wherein the viral antigen includes any one of SEQ ID NOs: 128-134.

22. A method of any of embodiments 1-15 wherein the vaccine antigen includes a cancer antigen.

23. A method of embodiment 22 wherein the cancer antigen 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; ROR1; survivin; tenascin; TSTA tyrosinase; VEGF; or WT1.

24. A method of embodiment 22 or 23 wherein the cancer antigen includes PSMA, PSCA, mesothelin, CD19, CD20, ROR1, or WT1.

25. A method of any of embodiments 22-24 wherein the cancer antigen includes any one of SEQ ID NOs: 135-141.
26. A method of any of embodiments 1-25 further including administering a vaccine adjuvant.
27. A method of embodiment 26 wherein the vaccine adjuvant includes (i) a Toll-like receptor ligand selected from CpG, Cpg-28, Poly(I:C), α -galactoceramide, MPLA, VTX-2337, EMD1201081, imiquimod, MGN1703, G100, CBLB502, Hiltonol, and Imiquimod, and/or (ii) 17-dimethylaminoethylamino-17-demethoxygeldanamycin).
28. A method of embodiment 26 wherein the vaccine adjuvant includes a STING agonist.
29. A method of embodiment 28 wherein the STING agonist includes c-diGMP, c-diAMP, c-GAMP, c-AIMP, (3',2')c-AIMP, (2',2')c-AIMP, (2',3')c-AIMP, c-AIMP(S), c-(dAMP-dIMP), c-(dAMP-2'FdIMP), c-(2'FdAMP-2'FdIMP), (2',3')c-(AMP-2'FdIMP), c-[2'FdAMP(S)-2'FdIMP(S)], c-[2'FdAMP(S)-2'FdIMP(S)](POM)², and/or DMXAA.
30. A method of any of embodiments 1-29 wherein the polynucleotide includes a plasmid, a minicircle plasmid, or a self-replicating mRNA molecule.
31. A method of any of embodiments 1-30 wherein the administering includes via intramuscular injection.
32. A method of any of embodiments 1-31 wherein the polynucleotide is within a nanoparticle.
33. A method of embodiment 32 wherein the nanoparticle includes liposomes, polymeric particles, metallic particles, polymeric micelles, polyethyleneimine (PEI)/DNA complexes, or a combination thereof.
34. A method of embodiment 32 or 33 wherein the nanoparticle includes a poly(β -amino ester) polymer.
35. A method of any of embodiments 32-34 wherein the nanoparticle includes a lipid coating.
36. A method of embodiment 35 wherein the lipid coating includes a liposome, a lipid bilayer, or a polymeric micelle.
37. A method of any of embodiments 32-36 wherein the nanoparticle includes poly(β -amino ester) with a PGA coating.
38. A method of any of embodiments 32-37 wherein the nanoparticle includes a T cell targeting and delivery agent (T-DA).
39. A method of embodiment 38 wherein the T-DA includes a binding domain that selectively binds to T cells *in vivo*.
40. A method of embodiment 38 wherein the T-DA includes a binding domain that selectively binds a T cell receptor motif; a T cell α chain; a T cell β chain; CCR7; CD3; CD4; CD8; CD28; CD45RA; CD62L; CD127; or LFA-1.
41. A method of embodiment 40 wherein the T-DA binding domain selectively binds CD4.

42. A method of embodiment 41 wherein the T-DA binding domain includes any one of SEQ ID NOs: 41-46.
43. A method of embodiment 40 wherein the T-DA binding domain selectively binds CD8.
44. A method of embodiment 43 wherein the T-DA binding domain includes any one of SEQ ID NOs: 47-52.
45. A method of embodiment 40 wherein the T-DA binding domain selectively binds CD3.
46. A method of embodiment 45 wherein the T-DA binding domain includes any one of SEQ ID NOs: 53-58.
47. A method of any of embodiments 38-40 wherein the T-DA includes a binding domain that selectively binds CD4+ or CD8+ T cells in vivo and ex vivo.
48. A method of any of embodiments 39-47 wherein the T-DA binding domain includes a T cell receptor motif antibody; a T cell α chain antibody; a T cell β chain antibody; a CCR7 antibody; a CD3 antibody; a CD4 antibody; a CD8 antibody; a CD28 antibody; a CD45RA antibody; a CD62L antibody; a CD127 antibody; a LFA-1 antibody; or an effective fragment of the foregoing antibodies.
49. A method of any of embodiments 32-48 wherein the nanoparticle includes an endosomal release agent (ERA).
50. A method of embodiment 49 wherein the ERA includes any one of SEQ ID NOs: 40, and 59-80, or combinations thereof.
51. A method of any of embodiments 32-50 wherein the nanoparticle includes a nuclear targeting agent (NTA).
52. A method of embodiment 51 wherein the NTA includes any one of SEQ ID NOs: 81-127, or combinations thereof.
53. A method of any of embodiments 32-52 wherein the nanoparticle includes an iPB7 transposase, a S/MAR element, a PiggyBac transposase-containing plasmid, a Sleeping Beauty transposase-containing plasmid; a homo sapiens transposon-derived Buster1 transposase-like protein gene; a human endogenous retrovirus H protease/integrase-derived ORF1; a homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence; a homo sapiens endogenous retroviral sequence K; a homo sapiens endogenous retroviral family W sequence; a homo sapiens LINE-1 type transposase domain; or a homo sapiens pogo transposable element.
54. A method of embodiment 53 wherein the iBP7 transposase includes SEQ ID NO: 142.
55. A method of any of embodiments 1-54 wherein the administering results in expression of the polynucleotide selectively by T cells within 10 days; within 9 days; within 8 days; within 7 days; within 6 days; within 5 days; within 4 days; or within 3 days of administration.
56. A kit including a vaccine antigen and a polynucleotide (PN) encoding a T cell receptor (TCR) that binds the vaccine antigen when expressed by a T cell.

57. A kit of embodiment 56 wherein the TCR is Class I restricted.
58. A kit of embodiment 56 wherein the TCR is Class II restricted.
59. A kit of any of embodiments 56-58 wherein the TCR includes the variable regions of an α chain and a β chain.
60. A kit of any of embodiments 56-59 wherein the TCR includes the constant regions of an α chain and a β chain.
61. A kit of any of embodiments 56-60 wherein the TCR includes a transmembrane domain and a cytoplasmic tail.
62. A kit of any of embodiments 56-61 wherein the TCR includes an α chain including SEQ ID NOs: 1, 4, 18, 21, 23, 25, 27, 29-32, 34, and 36.
63. A kit of any of embodiments 56-62 wherein the TCR includes an β chain including SEQ ID NOs: 2, 3, 19, 22, 24, 26, 28, 33, 35, and 37.
64. A kit of any of embodiments 56-61 wherein the TCR includes SEQ ID NOs: 5-12, 15, 16, and 39.
65. A kit of any of embodiments 56-64 wherein the vaccine antigen includes a viral antigen.
66. A kit of embodiment 65 wherein the viral antigen is derived from an adenovirus, arenavirus, bunyavirus, coronavirus, flavivirus, hantavirus, hepadnavirus, herpesvirus, papillomavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, orthomyxovirus, retrovirus, reovirus, rhabdovirus, rotavirus, spongiform virus or togavirus.
67. A kit of embodiment 65 wherein the viral antigen includes a peptide expressed by cytomegalovirus, cold virus, Epstein-Barr virus, flu virus, hepatitis A, B, or C virus, herpes simplex virus, human immunodeficiency virus, influenza virus, Japanese encephalitis virus, measles virus, polio virus, rabies virus, respiratory syncytial virus, rubella virus, smallpox virus, varicella zoster virus, West Nile virus, or Zika virus.
68. A kit of any of embodiments 65-67 wherein the viral antigen includes a cytomegaloviral antigen selected from envelope glycoprotein B and/or CMV pp65; an Epstein-Barr antigen selected from EBV EBNA1, EBV P18, and/or EBV P23; a hepatitis vaccine antigen selected from the S, M, and/or L proteins or the pre-S antigen of hepatitis B virus; a herpes simplex vaccine antigen selected from glycoprotein D; a human immunodeficiency virus (HIV) vaccine antigen selected from 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/or HIV reverse transcriptase; a human papillomavirus virus (HPV) viral antigen selected from the L1 protein; an influenza vaccine antigen selected from hemagglutinin and neuraminidase; a Japanese encephalitis vaccine antigen selected from proteins E, M-E, M-E-NS1, NS1, or NS1-NS2A; a malaria vaccine antigen selected from circumsporozoite (CSP), glutamate dehydrogenase, lactate dehydrogenase, or fructose-bisphosphate aldolase; a measles vaccine antigen selected from measles virus fusion protein; a rabies vaccine antigen selected from rabies

glycoprotein or rabies nucleoprotein; a respiratory syncytial vaccine antigen selected from RSV fusion protein or M2 protein; a rotaviral vaccine antigen selected from VP7sc; a rubella vaccine antigen selected from protein E1 or E2; a varicella zoster vaccine antigen selected from gpl or gpII; or a zika vaccine antigen selected from pre-membrane, envelope (E), Domain III of the E protein, or non-structural proteins 1, 2, 3, 4, or 5.

69. A kit of any of embodiments 65-68 wherein the viral antigen includes Nef (66-97), Nef (116-145), Gag p17 (17-35), Gag p17-p24 (253-284), Pol 325-355 (RT 158-188), CSP central repeat region, or E protein Domain III.

70. A kit of any of embodiments 65-69 wherein the viral antigen includes any of SEQ ID NOs: 128-134.

71. A kit of any of embodiments 56-70 wherein the vaccine antigen includes a cancer antigen.

72. A kit of embodiment 71 wherein the cancer antigen 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; ROR1; survivin; tenascin; TSTA tyrosinase; VEGF; or WT1.

73. A kit of embodiment 71 or 72 wherein the cancer antigen includes PSMA, PSCA, mesothelin, CD19, CD20, ROR1, or WT1.

74. A kit of embodiment 73 wherein the cancer antigen includes any one of SEQ ID NOs: 135-141.

75. A kit of any of embodiments 56-74 further including administering a vaccine adjuvant.

76. A kit of embodiment 75 wherein the vaccine adjuvant includes a STING agonist.

77. A kit of any of embodiments 56-76 wherein the polynucleotide includes a plasmid, a minicircle plasmid, or a self-replicating mRNA molecule.

78. A kit of any of embodiments 56-77 wherein the polynucleotide is within a nanoparticle.

79. A kit of embodiment 78 wherein the nanoparticle includes liposomes, polymeric particles, metallic particles, polymeric micelles, polyethyleneimine (PEI)/DNA complexes, or a combination thereof.

80. A kit of embodiment 78 wherein the nanoparticle includes a poly(β -amino ester) polymer.

81. A kit of any of embodiments 78-80 wherein the nanoparticle includes a lipid coating.

82. A kit of embodiment 81 wherein the lipid coating includes a liposome, a lipid bilayer, or a polymeric micelle.

83. A kit of any of embodiments 78-82 wherein the nanoparticle includes a poly(β -amino ester) polymer with a PGA coating.

84. A kit of any of embodiments 78-83 wherein the nanoparticle includes a T cell targeting and delivery agent (T-DA).

85. A kit of embodiment 84 wherein the T-DA includes a binding domain that selectively binds a T cell receptor motif; a T cell α chain; a T cell β chain; CCR7; CD3; CD4; CD8; CD28; CD45RA; CD62L; CD127; or LFA-1.
86. A kit of embodiment 85 wherein the T-DA binding domain selectively binds CD4.
87. A kit of embodiment 86 wherein the T-DA binding domain includes any one of SEQ ID NOs: 41-46.
88. A kit of embodiment 85 wherein the T-DA binding domain selectively binds CD8.
89. A kit of embodiment 88 wherein the T-DA binding domain includes any one of SEQ ID NOs: 47-52.
90. A kit of embodiment 85 wherein the T-DA binding domain selectively binds CD3.
91. A kit of embodiment 90 wherein the T-DA binding domain includes any one of SEQ ID NOs: 53-58.
92. A kit of embodiment 84 or 85 wherein the T-DA includes a binding domain that selectively binds CD4⁺ or CD8⁺ T cells in vivo and ex vivo.
93. A kit of any of embodiments 85-92 wherein the T-DA binding domain includes a T cell receptor motif antibody; a T cell α chain antibody; a T cell β chain antibody; a CCR7 antibody; a CD3 antibody; a CD4 antibody; a CD8 antibody; a CD28 antibody; a CD45RA antibody; a CD62L antibody; a CD127 antibody; a LFA-1 antibody; or an effective fragment of the foregoing antibodies.
94. A kit of any of embodiments 56-93 wherein the nanoparticle includes an endosomal release agent (ERA).
95. A kit of embodiment 94 wherein the ERA includes any one of SEQ ID NOs: 40, and 59-80, or combinations thereof.
96. A kit of any of embodiments 56-95 wherein the nanoparticle includes a nuclear targeting agent (NTA).
97. A kit of embodiment 96 wherein the NTA includes any one of SEQ ID NOs: 81-127, or combinations thereof.
98. A kit of any of embodiments 56-97 wherein the nanoparticle includes an iPB7 transposase, a S/MAR element, a PiggyBac transposase-containing plasmid, a Sleeping Beauty transposase-containing plasmid; a homo sapiens transposon-derived Buster1 transposase-like protein gene; a human endogenous retrovirus H protease/integrase-derived ORF1; a homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence; a homo sapiens endogenous retroviral sequence K; a homo sapiens endogenous retroviral family W sequence; a homo sapiens LINE-1 type transposase domain; or a homo sapiens pogo transposable element.
99. A kit of embodiment 98 wherein the iBP7 transposase includes SEQ ID NO: 142.
100. Use of a method or kit of any of embodiments 1-99 to provide vaccine antigen recognizing

capabilities to the T cells of a subject.

101. Use of a method or kit of any of embodiments 1-99 to render a subject's immune system responsive to a vaccine antigen.

102. Use of a method or kit of any of embodiments 1-99 to increase a subject's immune system response to a vaccine antigen.

[0215] Examples. The impact of many vaccines can be enhanced if they are co-delivered with agents that program T cells to produce TCRs that react with the vaccine antigens. This premise was tested by loading CD8-targeted nanoparticles (NP) with plasmids encoding the ovalbumin (OVA)-specific OT-I TCR (FIG. 3A). The design of these DNA-carrying NPs was based on versions developed to program tumor recognition abilities into circulating lymphocytes—in those studies, it was demonstrated that when the NPs are outfitted with lymphocyte-targeting ligands, chimeric antigen receptor genes are transfected into host T cells. This NP platform was adapted to program host T cells so they express vaccine-specific TCRs. P14 TCR-transgenic mice (containing only CD8 T cells specific for Lymphocytic Choriomeningitis Virus) were intramuscularly injected with a single dose of 10^{11} T cell-targeted NPs delivering genes that encode the OVA-specific OT-1 TCR, along with a myc-tag and the hyperactive iPB7 transposase. These NPs were either injected alone or in combination with an OVA peptide vaccine. As controls, mice were immunized with the OVA vaccine only, or left untreated. On days 7 and 30 after the immunizations, draining lymph nodes were isolated so the percentages of NP-programmed (OVA-tetramer+) T cells could be quantified by flow cytometry. It was found that intramuscularly injected NPs effectively deliver engineered TCR genes into host T cells so that they recognize the vaccine antigen (FIG. 3B). Following their rapid vaccine-induced expansion, the NP-programmed T cells differentiate into long-lived memory T cells (FIGs. 3B,3C).

[0216] The *Kras*^{LSL-G12D/+}; *Trp53*^{LSL-R172H/+}; *p48*^{Cre/+} (KPC) mouse model was utilized to test the NP vaccine strategy in a clinically relevant in vivo test system. The KPC model expresses mutant Kras and p53 at endogenous gene loci known to drive pancreatic tumorigenesis. This model recapitulates the cardinal features of human pancreatic ductal adenocarcinoma (PDA), including molecular progression, histopathology, and clinical syndrome (FIG. 4A). KPC mice carrying a defined tumor burden (2-5 mm diameter, as determined by high-resolution ultrasound) were intramuscularly injected with a single dose of 10^{13} T cell-targeted NPs delivering genes that encode the tumor antigen mesothelin (MSLN)-specific receptor TCR₁₀₄₅ (Stromnes, IM et al. (2015) *supra*), along with a myc-tag and the hyperactive iPB7 transposase (to ensure efficient integration of the vector into chromosomes via a “cut and paste” mechanism). In particular embodiments, the hyperactive iPB7 transposase is a murine codon-optimized piggyBac transposase cDNA (GenBank accession number: EF587698, Cadiñanos, J and Bradley, A (2007) *Nucleic Acids Res* 35: e87, see FIG. 6, SEQ ID NO: 142). These NPs were either injected alone or in combination with an MSLN vaccine

(5×10^8 pfu of an attenuated recombinant adenovirus expressing murine MSLN). As controls, mice were immunized with the MSLN vaccine only, were not treated. Only animals treated with a combination of both the TCR₁₀₄₅ NPs and the MSLN vaccine exhibited tumor regression; they also had an average 27-day improvement in survival (FIG. 4B).

[0217] 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. As used herein, the transition term “comprise” or “comprises” means includes, 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. As used herein, a material effect would cause a statistically-significant reduction in the ability to increase a subject’s immune system response to a vaccine antigen within 7 days of vaccine administration.

[0218] 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.

[0219] 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.

[0220] 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.

[0221] 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.

[0222] Particular 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.

[0223] Furthermore, numerous references have been made to patents and printed publications throughout this specification. Each of the above-cited references and printed publications are individually incorporated herein by reference in their entirety.

[0224] 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.

[0225] 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.

[0226] 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 following 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 (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

CLAIMS

What is claimed is:

1. A method of improving the efficacy of a vaccination in a subject comprising
Identifying a subject who has or will receive a vaccine antigen; and
Administering a therapeutically effective amount of a nanoparticle (NP) to the subject within a clinically relevant time window of vaccine antigen receipt wherein the NP comprises (i) a polynucleotide (PN) encoding a T cell receptor (TCR) that specifically binds the vaccine antigen; and (ii) a T cell targeting and delivery agent (T-DA)
Thereby improving the efficacy of the vaccination as compared to administration of the vaccine antigen alone.
2. The method of claim 1 wherein the vaccine antigen is mesothelin.
3. The method of claim 1 wherein the subject is in need of improved vaccine efficacy due to age or immune status.
4. The method of claim 3 wherein the immune status is a low T cell count.
5. The method of claim 1 wherein the vaccinating provides a treatment for AIDS, malaria, herpes, chlamydia, Epstein-Barr virus, Pneumococcus, or Hepatitis B.
6. The method of claim 1 wherein the vaccinating provides a treatment for cancer.
7. The method of claim 1 wherein the TCR is Class I restricted.
8. The method of claim 1 wherein the TCR is Class II restricted.
9. The method of claim 1 wherein the TCR is Class I restricted and the improved vaccine efficacy is due to CD8+ T helper cell activity that improves a T cell cytotoxic response.
10. The method of claim 1 wherein the TCR is Class II restricted and the improved vaccine efficacy is due to CD4+ T helper cell activity that improves an antibody response.
11. The method of claim 1 wherein the TCR comprises an α chain selected from SEQ ID NOs: 1, 4, 18, 21, 23, 25, 27, 29-32, 34, and 36.
12. The method of claim 1 wherein the TCR comprises an β chain selected from SEQ ID NOs: 2, 3, 19, 22, 24, 26, 28, 33, 35, and 37.
13. The method of claim 1 wherein the TCR comprises a sequence selected from SEQ ID NOs: 5-12, 15, 16, and 39.
14. The method of claim 1 wherein the vaccine antigen comprises a viral antigen.
15. The method of claim 14 wherein the viral antigen is derived from an adenovirus, arenavirus, bunyavirus, coronavirus, flavivirus, hantavirus, hepadnavirus, herpesvirus, papillomavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, orthomyxovirus, retrovirus, reovirus, rhabdovirus, rotavirus, spongiform virus or togavirus.

16. The method of claim 14 wherein the viral antigen is a peptide expressed by cytomegalovirus, cold virus, Epstein-Barr virus, flu virus, hepatitis A, B, or C virus, herpes simplex virus, human immunodeficiency virus, influenza virus, Japanese encephalitis virus, measles virus, polio virus, rabies virus, respiratory syncytial virus, rubella virus, smallpox virus, varicella zoster virus, West Nile virus, or Zika virus.
17. The method of claim 14 wherein the viral antigen is
 - a cytomegaloviral antigen selected from envelope glycoprotein B and/or CMV pp65;
 - an Epstein-Barr antigen selected from EBV EBNA1, EBV P18, and/or EBV P23;
 - a hepatitis vaccine antigen selected from the S, M, and/or L proteins or the pre-S antigen of hepatitis B virus;
 - a herpes simplex vaccine antigen selected from glycoprotein D;
 - a human immunodeficiency virus (HIV) vaccine antigen selected from 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/or HIV reverse transcriptase;
 - a human papillomavirus virus (HPV) viral antigen selected from the L1 protein;
 - an influenza vaccine antigen selected from hemagglutinin and neuraminidase;
 - a Japanese encephalitis vaccine antigen selected from proteins E, M-E, M-E-NS1, NS1, or NS1-NS2A;
 - a malaria vaccine antigen selected from circumsporozoite (CSP), glutamate dehydrogenase, lactate dehydrogenase, or fructose-bisphosphate aldolase;
 - a measles vaccine antigen selected from measles virus fusion protein;
 - a rabies vaccine antigen selected from rabies glycoprotein or rabies nucleoprotein;
 - a respiratory syncytial vaccine antigen selected from RSV fusion protein or M2 protein;
 - a rotaviral vaccine antigen selected from VP7sc;
 - a rubella vaccine antigen selected from protein E1 or E2;
 - a varicella zoster vaccine antigen selected from gpl or gpII; or
 - a zika vaccine antigen selected from pre-membrane, envelope (E), Domain III of the E protein, or non-structural proteins 1, 2, 3, 4, or 5.
18. The method of claim 14 wherein the viral antigen is selected from Nef (66-97), Nef (116-145), Gag p17 (17-35), Gag p17-p24 (253-284), Pol 325-355 (RT 158-188), CSP central repeat region, or E protein Domain III.
19. The method of claim 14 wherein the viral antigen is selected from SEQ ID NOs: 128-134.
20. The method of claim 1 wherein the vaccine antigen comprises a cancer antigen.
21. The method of claim 20 wherein the cancer antigen comprises PSMA, PSCA, mesothelin, CD19, CD20, ROR1, or WT1 or a fragment of PSMA, PSCA, mesothelin, CD19, CD20, ROR1, or WT1.

22. The method of claim 20 wherein the cancer antigen is selected from SEQ ID NOs. 135-141.
23. The method of claim 1 further comprising administering the vaccine antigen.
24. The method of claim 1 further comprising administering a vaccine adjuvant within the clinically relevant time window.
25. The method of claim 24 wherein the vaccine adjuvant is selected from CpG, Cpg-28, Poly(I:C), α -galactoceramide, MPLA, VTX-2337, EMD1201081) imiquimod, MGN1703, G100, CBLB502, Hiltonol, and Imiquimod, and/or (ii) 17-dimethylaminoethylamino-17-demethoxygeldanamycin).
26. The method of claim 24 wherein the vaccine adjuvant is a STING agonist.
27. The method of claim 26 wherein the STING agonist is selected from c-diGMP, c-diAMP, c-GAMP, c-AIMP, (3',2')c-AIMP, (2',2')c-AIMP, (2',3')c-AIMP, c-AIMP(S), c-(dAMP-dIMP), c-(dAMP-2'FdIMP), c-(2'FdAMP-2'FdIMP), (2',3')c-(AMP-2'FdIMP), c-[2'FdAMP(S)-2'FdIMP(S)], c-[2'FdAMP(S)-2'FdIMP(S)](POM)², and/or DMXAA
28. The method of claim 1 wherein the polynucleotide is a plasmid, a minicircle plasmid, or self-replicating mRNA.
29. The method of claim 1 wherein the administering is via intramuscular injection.
30. The method of claim 1 wherein the nanoparticle comprises a poly(β -amino ester) polymer.
31. The method of claim 1 wherein the nanoparticle comprises a lipid coating.
32. The method of claim 31 wherein the lipid coating is a liposome, a lipid bilayer, or a polymeric micelle.
33. The method of claim 1 wherein the nanoparticle comprises a poly(β -amino ester) polymer and a PGA coating.
34. The method of claim 1 wherein the T-DA is covalently coupled to the poly(β -amino ester) polymer.
35. The method of claim 1 wherein the T-DA comprises a binding domain that selectively binds to T cells in vivo.
36. The method of claim 1 wherein the T-DA comprises a binding domain that selectively binds CD4+ or CD8+ T cells in vivo and ex vivo.
37. The method of claim 1 wherein the T-DA comprises a binding domain comprising a sequence selected from SEQ ID NOs: 41-58.
38. The method of claim 1 wherein the nanoparticle comprises an endosomal release agent (ERA).
39. The method of claim 38 wherein the ERA is selected from any one of SEQ ID NOs: 40, and 59-80, or combinations thereof.
40. The method of claim 1 wherein the nanoparticle comprises a nuclear targeting agent (NTA).
41. The method of claim 40 wherein the NTA is selected from any one of SEQ ID NOs: 81-127, or combinations thereof.

42. The method of claim 1 wherein the nanoparticle comprises a iPB7 transposase, a S/MAR element, a PiggyBac transposase-containing plasmid, a Sleeping Beauty transposase-containing plasmid; a homo sapiens transposon-derived Buster1 transposase-like protein gene; a human endogenous retrovirus H protease/integrase-derived ORF1; a homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence; a homo sapiens endogenous retroviral sequence K; a homo sapiens endogenous retroviral family W sequence; a homo sapiens LINE-1 type transposase domain; or a homo sapiens pogo transposable element.
43. The method of claim 42 wherein the iPB7 transposase comprises SEQ ID NO: 142.
44. The method of claim 1 wherein the administering results in expression of the polynucleotide selectively by T cells within 10 days; within 9 days; within 8 days; within 7 days; within 6 days; within 5 days; within 4 days; or within 3 days of administration.
45. A kit comprising a vaccine antigen and a polynucleotide (PN) encoding a T cell receptor (TCR) that binds the vaccine antigen when expressed by a T cell.
46. The kit of claim 45 wherein the polynucleotide is within a nanoparticle.
47. The kit of claim 45 wherein the encoded TCR is Class I restricted.
48. The kit of claim 45 wherein the encoded TCR is Class II restricted
49. The kit of claim 45 wherein the encoded TCR comprises an α chain selected from SEQ ID NOs: 1, 4, 18, 21, 23, 25, 27, 29-32, 34, and 36.
50. The kit of claim 45 wherein the encoded TCR comprises an β chain selected from SEQ ID NOs: 2, 3, 19, 22, 24, 26, 28, 33, 35, and 37.
51. The kit of claim 45 wherein the encoded TCR comprises a sequence selected from SEQ ID NOs: 5-12, 15, 16, and 39.
52. The kit of claim 45 wherein the vaccine antigen comprises a viral antigen.
53. The kit of claim 52 wherein the viral antigen is derived from an adenovirus, arenavirus, bunyavirus, coronavirus, flavivirus, hantavirus, hepadnavirus, herpesvirus, papillomavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, orthomyxovirus, retrovirus, reovirus, rhabdovirus, rotavirus, spongiform virus or togavirus.
54. The kit of claim 52 wherein the viral antigen is a peptide expressed by cytomegalovirus, cold virus, Epstein-Barr virus, flu virus, hepatitis A, B, or C virus, herpes simplex virus, human immunodeficiency virus, influenza virus, Japanese encephalitis virus, measles virus, polio virus, rabies virus, respiratory syncytial virus, rubella virus, smallpox virus, varicella zoster virus, West Nile virus, or Zika virus.
55. The kit of claim 52 wherein the viral antigen is
a cytomegaloviral antigen selected from envelope glycoprotein B and/or CMV pp65;
an Epstein-Barr antigen selected from EBV EBNA1, EBV P18, and/or EBV P23;

a hepatitis vaccine antigen selected from the S, M, and/or L proteins or the pre-S antigen of hepatitis B virus;

a herpes simplex vaccine antigen selected from glycoprotein D;

a human immunodeficiency virus (HIV) vaccine antigen selected from 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/or HIV reverse transcriptase;

a human papillomavirus virus (HPV) viral antigen selected from the L1 protein;

an influenza vaccine antigen selected from hemagglutinin and neuraminidase;

a Japanese encephalitis vaccine antigen selected from proteins E, M-E, M-E-NS1, NS1, or NS1-NS2A;

a malaria vaccine antigen selected from circumsporozoite (CSP), glutamate dehydrogenase, lactate dehydrogenase, or fructose-bisphosphate aldolase;

a measles vaccine antigen selected from measles virus fusion protein;

a rabies vaccine antigen selected from rabies glycoprotein or rabies nucleoprotein;

a respiratory syncytial vaccine antigen selected from RSV fusion protein or M2 protein;

a rotaviral vaccine antigen selected from VP7sc;

a rubella vaccine antigen selected from protein E1 or E2;

a varicella zoster vaccine antigen selected from gpl or gpII; or

a zika vaccine antigen selected from pre-membrane, envelope (E), Domain III of the E protein, or non-structural proteins 1, 2, 3, 4, or 5.

56. The kit of claim 52 wherein the viral antigen is selected from Nef (66-97), Nef (116-145), Gag p17 (17-35), Gag p17-p24 (253-284), Pol 325-355 (RT 158-188), CSP central repeat region, or E protein Domain III.
57. The kit of claim 52 wherein the viral antigen comprises one of SEQ ID NOs: 128-134.
58. The kit of claim 45 wherein the vaccine antigen is a cancer antigen.
59. The kit of claim 58 wherein the cancer antigen comprises PSMA, PSCA, mesothelin, CD19, CD20, ROR1, or WT1.
60. The kit of claim 58 wherein the cancer antigen comprises one of SEQ ID NOs: 135-141.
61. The kit of claim 45 further comprising a vaccine adjuvant.
62. The kit of claim 61 wherein the vaccine adjuvant is selected from CpG, Cpg-28, Poly(I:C), α -galactoceramide, MPLA, VTX-2337, EMD1201081) imiquimod, MGN1703, G100, CBLB502, Hiltonol, and Imiquimod, and/or (ii) 17-dimethylaminoethylamino-17-demethoxygeldanamycin).
63. The kit of claim 61 wherein the vaccine adjuvant comprises a STING agonist.
64. The kit of claim 63 wherein the STING agonist is selected from c-diGMP, c-diAMP, c-GAMP, c-AIMP, (3',2')c-AIMP, (2',2')c-AIMP, (2',3')c-AIMP, c-AIMP(S), c-(dAMP-dIMP), c-(dAMP-

2'FdIMP), c-(2'FdAMP-2'FdIMP), (2',3')c-(AMP-2'FdIMP), c-[2'FdAMP(S)-2'FdIMP(S)], c-[2'FdAMP(S)-2'FdIMP(S)](POM)², and/or DMXAA

65. The kit of claim 45 wherein the polynucleotide comprises a plasmid, a minicircle plasmid, or self-replicating mRNA.
66. The kit of claim 65 further comprising a syringe for intramuscular injection.
67. The kit of claim 46 wherein the nanoparticle comprises a poly(β -amino ester) polymer.
68. The kit of claim 46 wherein the nanoparticle comprises a lipid coating.
69. The kit of claim 68 wherein the lipid coating is a liposome, a lipid bilayer, or a polymeric micelle.
70. The kit of claim 46 wherein the nanoparticle comprises a poly(β -amino ester) polymer and a PGA coating.
71. The kit of claim 45 further comprising a T-DA.
72. The kit of claim 46 wherein the nanoparticle comprises a polymer and a T-DA covalently coupled to the polymer.
73. The kit of claim 71 or 72 wherein the T-DA comprises a binding domain that selectively binds to T cells in vivo.
74. The kit of claim 71 or 72 wherein the T-DA comprises a binding domain that selectively binds CD4⁺ or CD8⁺ T cells in vivo and ex vivo.
75. The kit of claim 46 wherein the nanoparticle comprises an endosomal release agent (ERA).
76. The kit of claim 75 wherein the ERA is selected from any one of SEQ ID NOs: 40, and 59-80, or combinations thereof.
77. The kit of claim 46 wherein the nanoparticle comprises a nuclear targeting agent (NTA).
78. The kit of claim 77 wherein the NTA is selected from any one of SEQ ID NOs: 81-127, or combinations thereof.
79. The kit of claim 46 wherein the nanoparticle comprises an iPB7 transposase, a S/MAR element, a PiggyBac transposase-containing plasmid, a Sleeping Beauty transposase-containing plasmid; a homo sapiens transposon-derived Buster1 transposase-like protein gene; a human endogenous retrovirus H protease/integrase-derived ORF1; a homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence; a homo sapiens endogenous retroviral sequence K; a homo sapiens endogenous retroviral family W sequence; a homo sapiens LINE-1 type transposase domain; or a homo sapiens pogo transposable element.
80. The kit of claim 79 wherein the iPB7 transposase comprises SEQ ID NO: 142.

FIG. 1

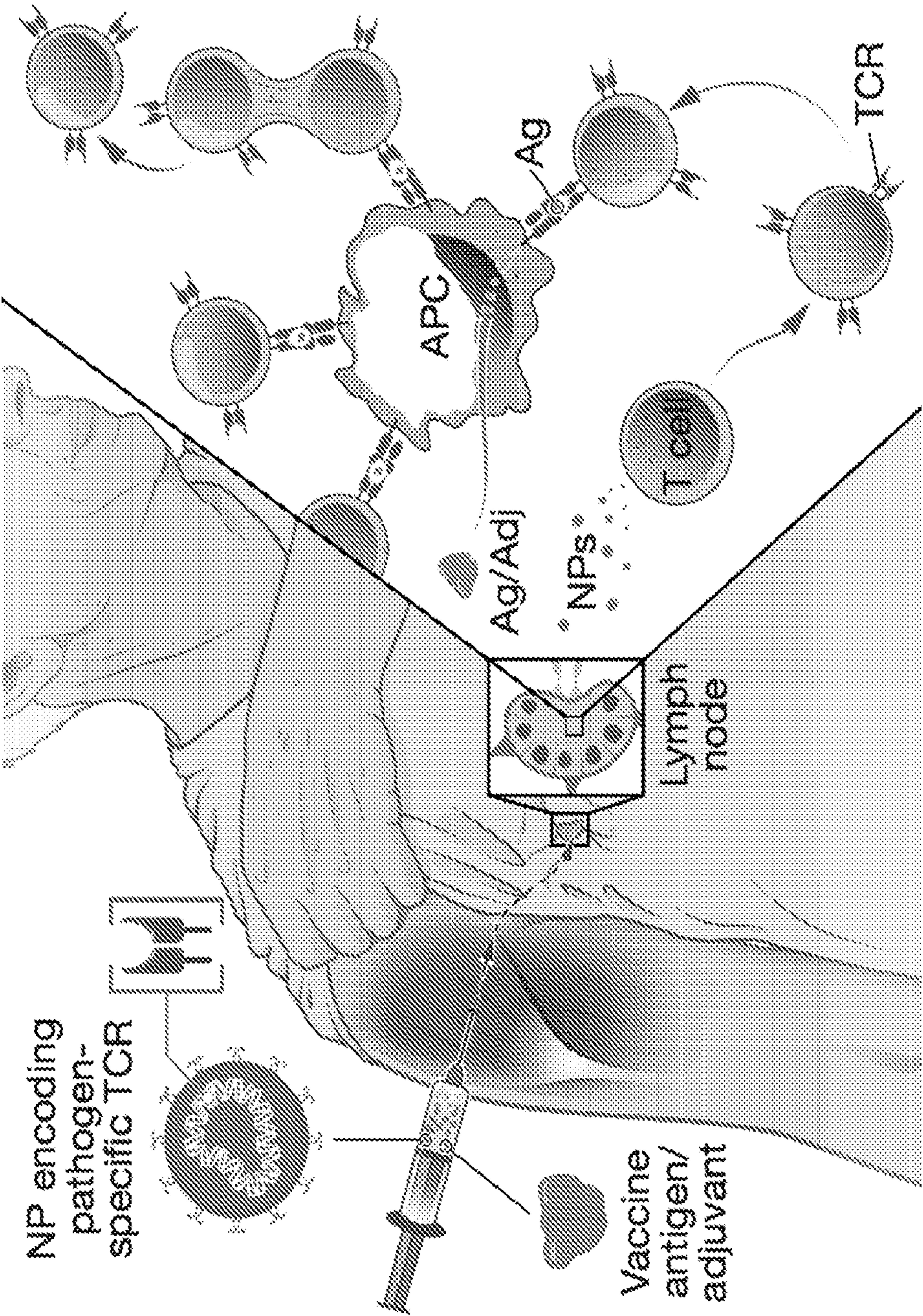


FIG. 2

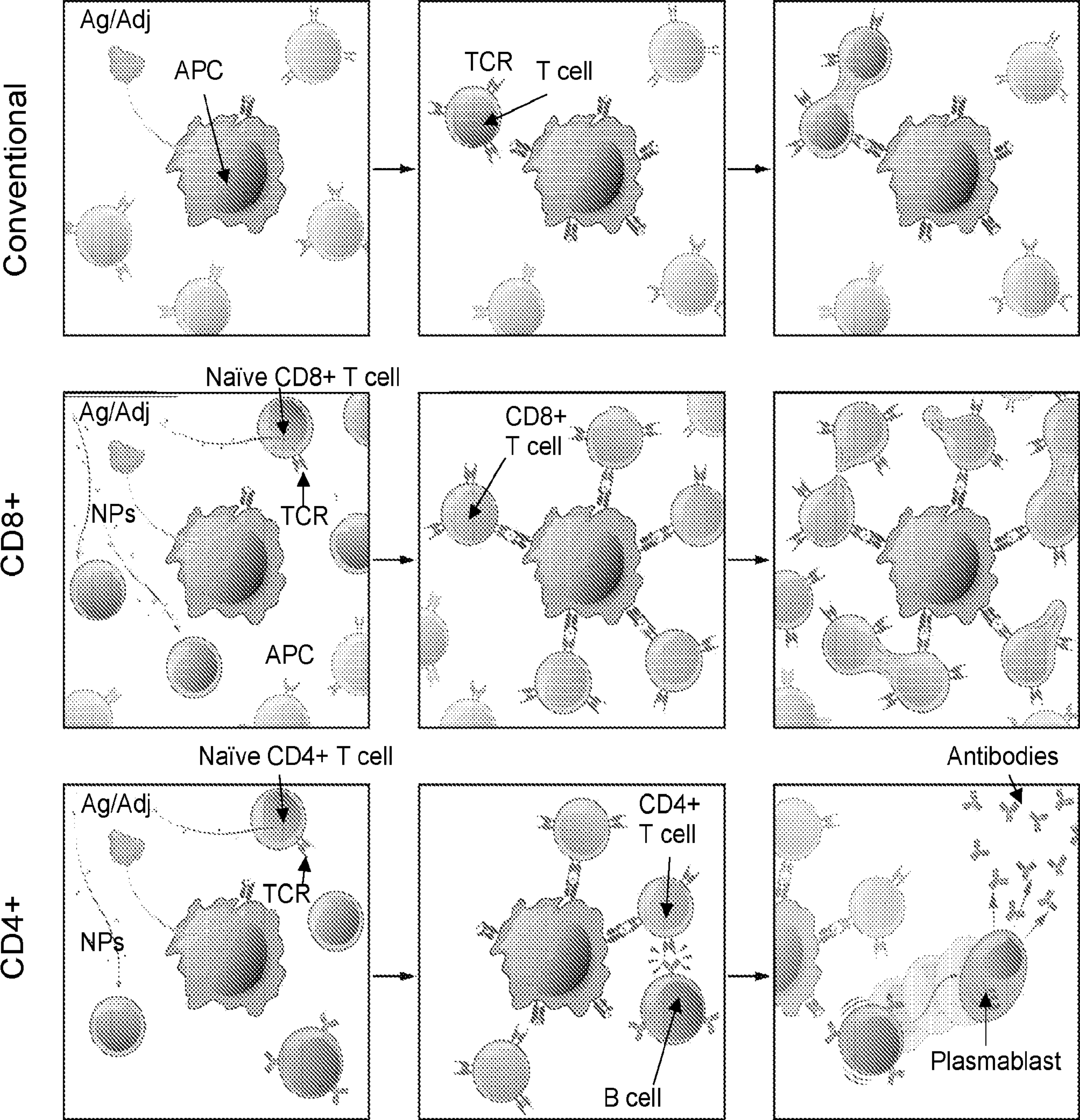


FIG. 3A

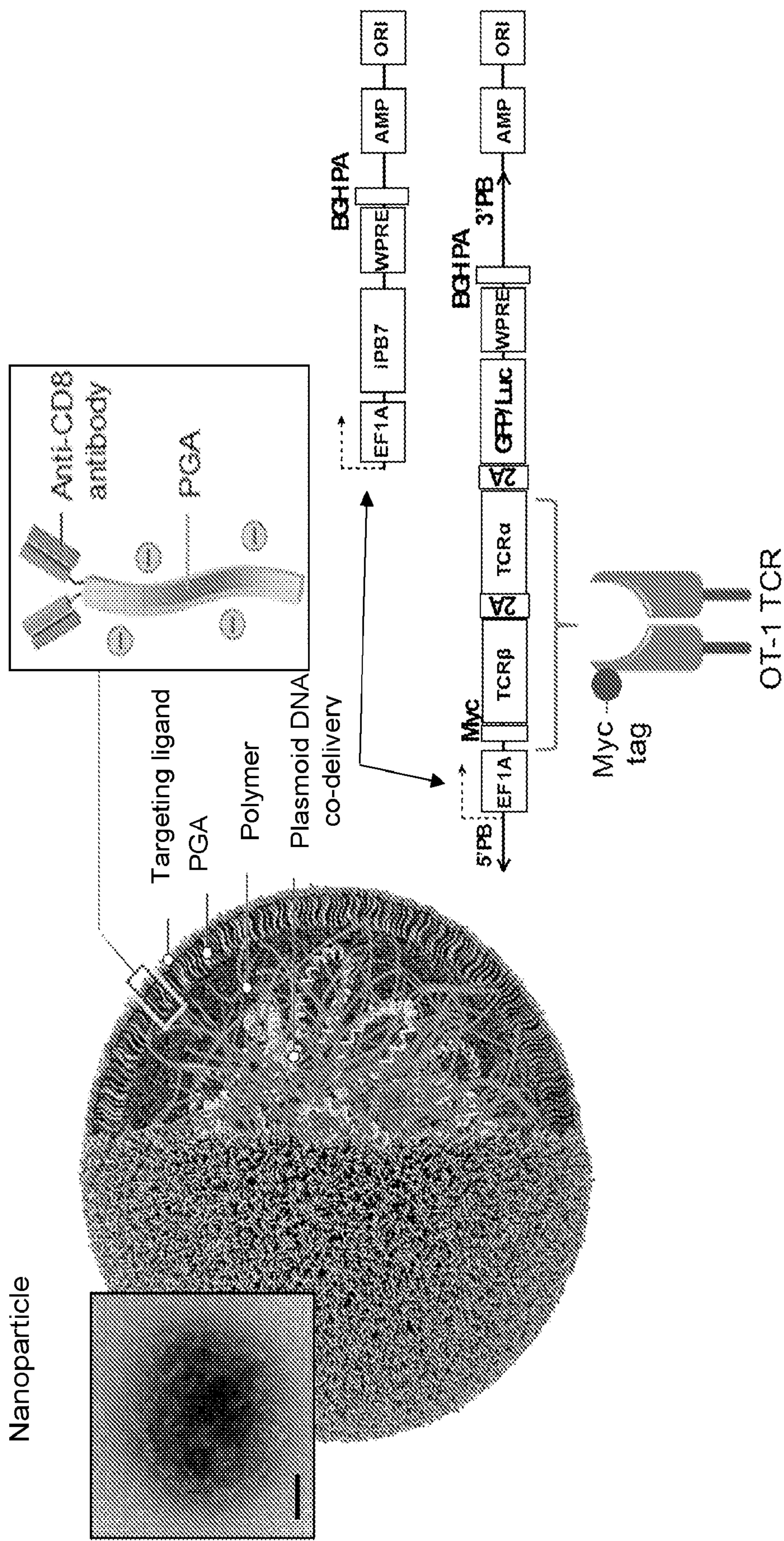


FIG. 3B

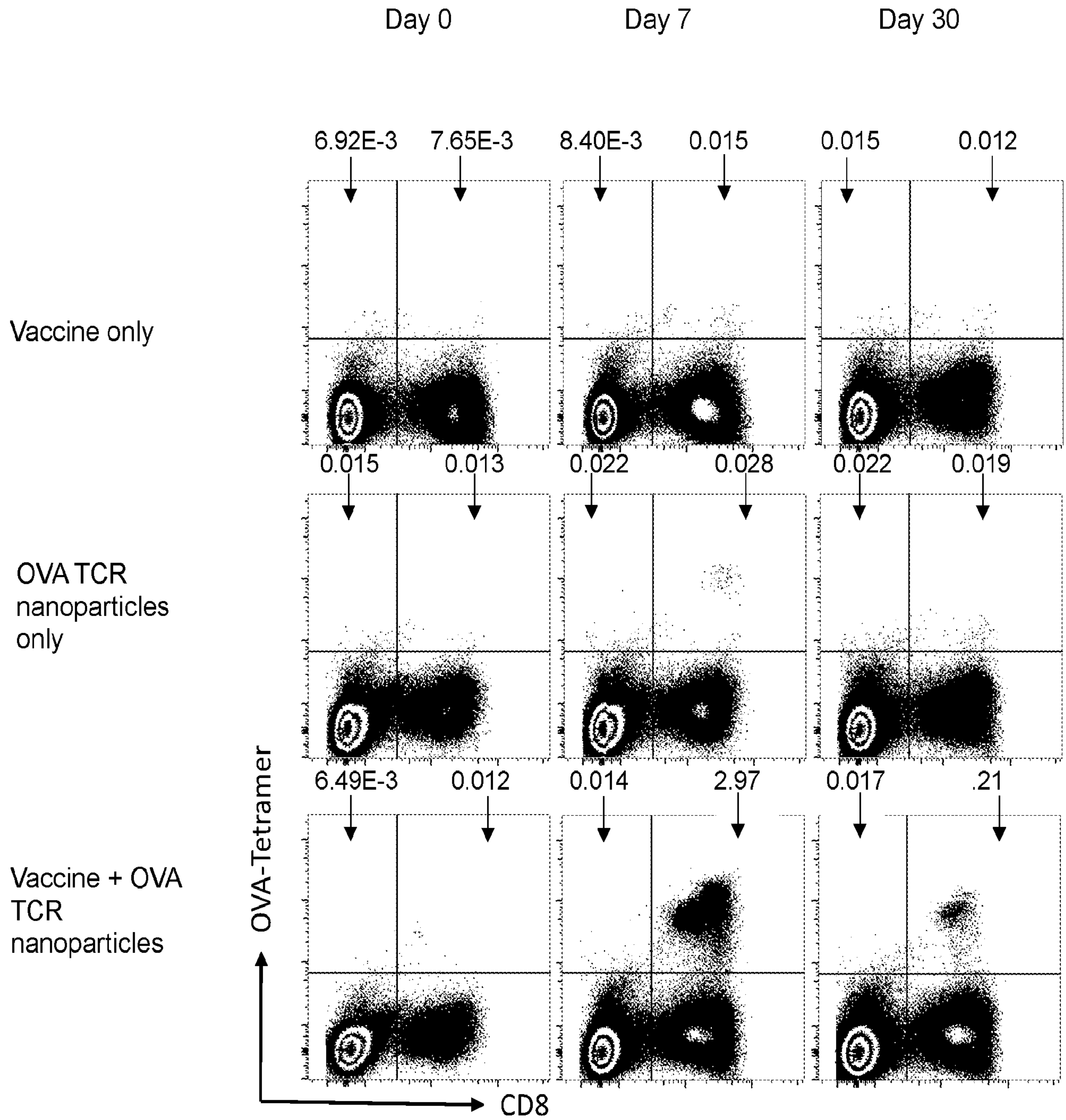


FIG. 3C

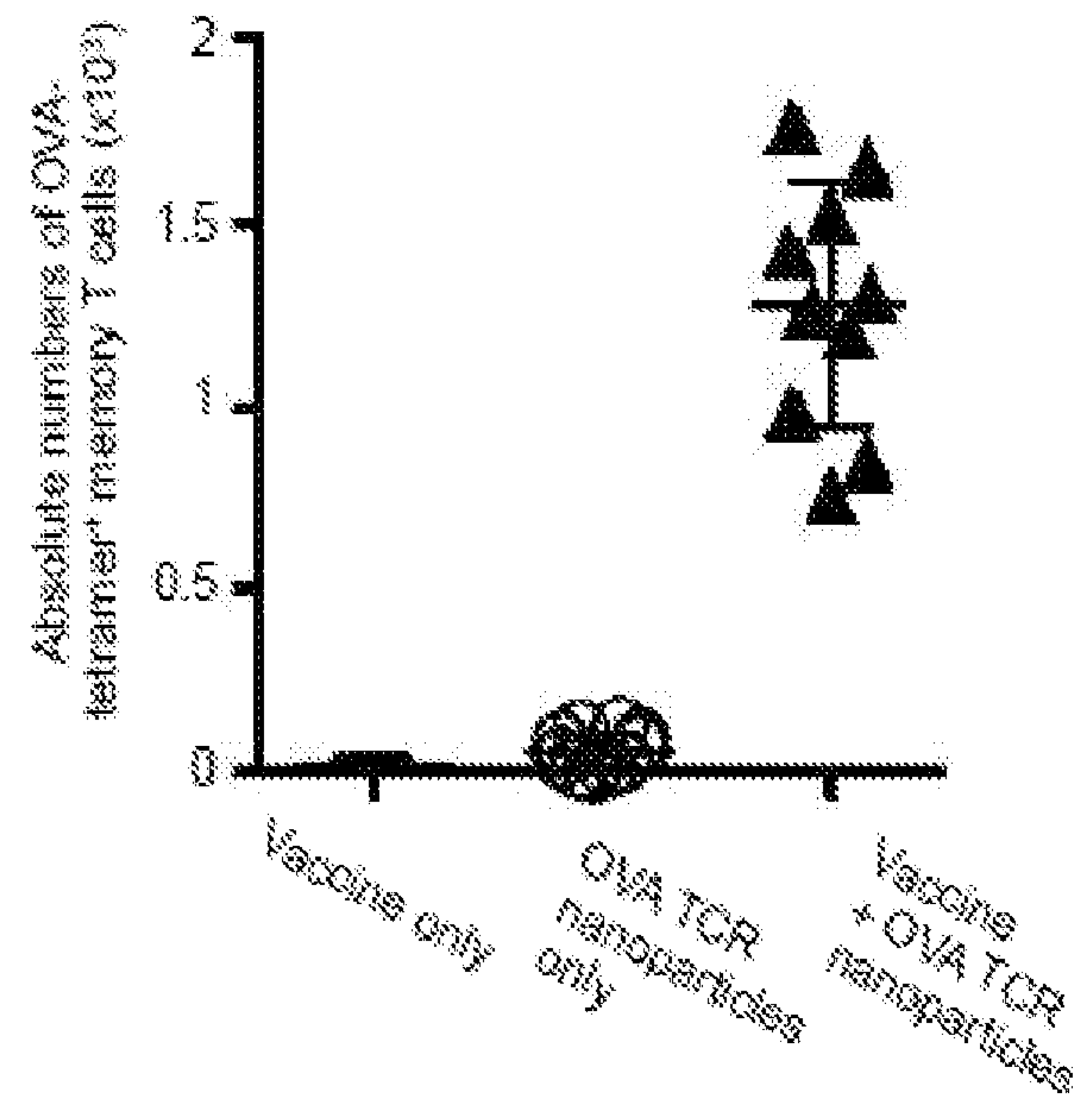


FIG. 4A

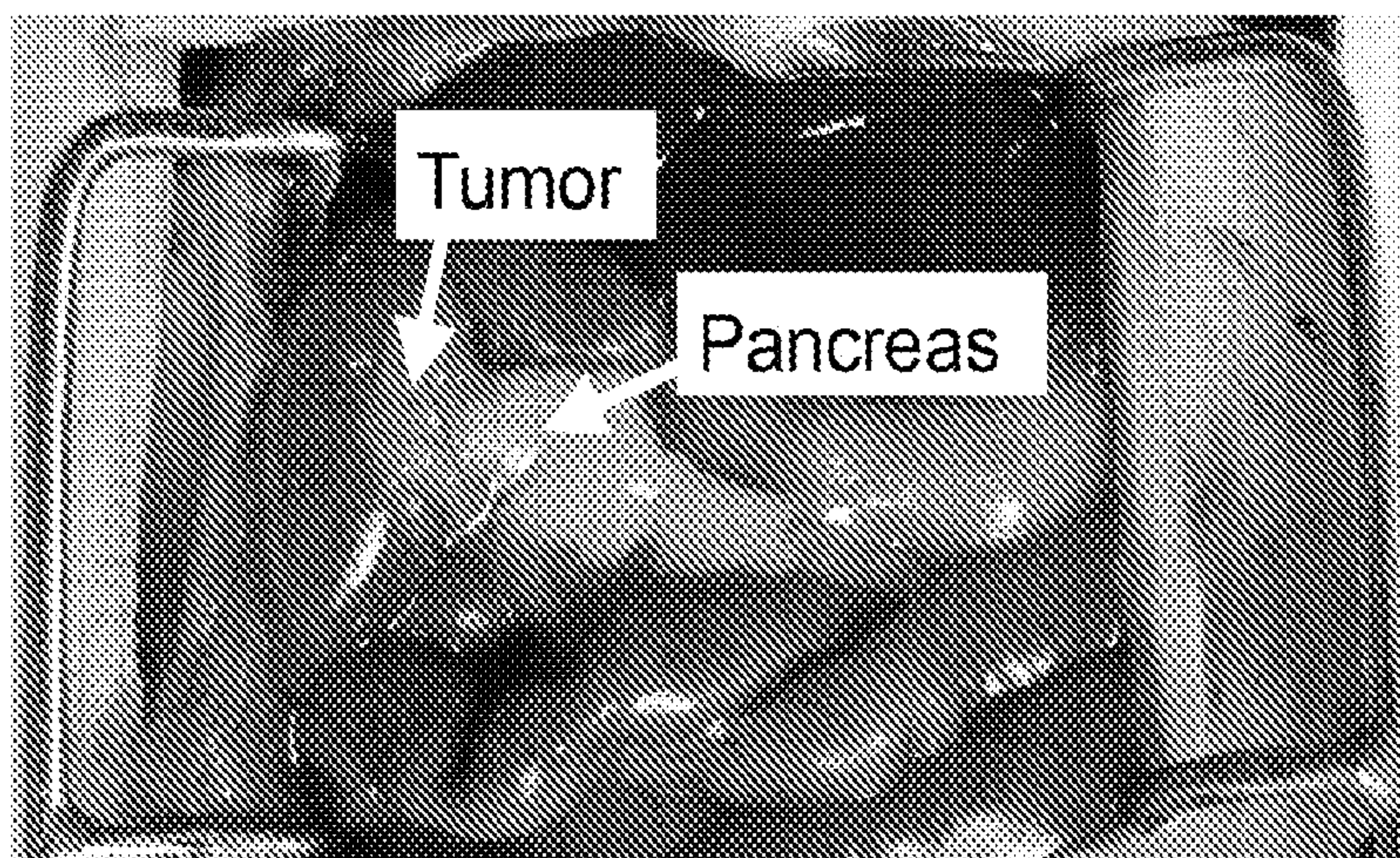


FIG. 4B

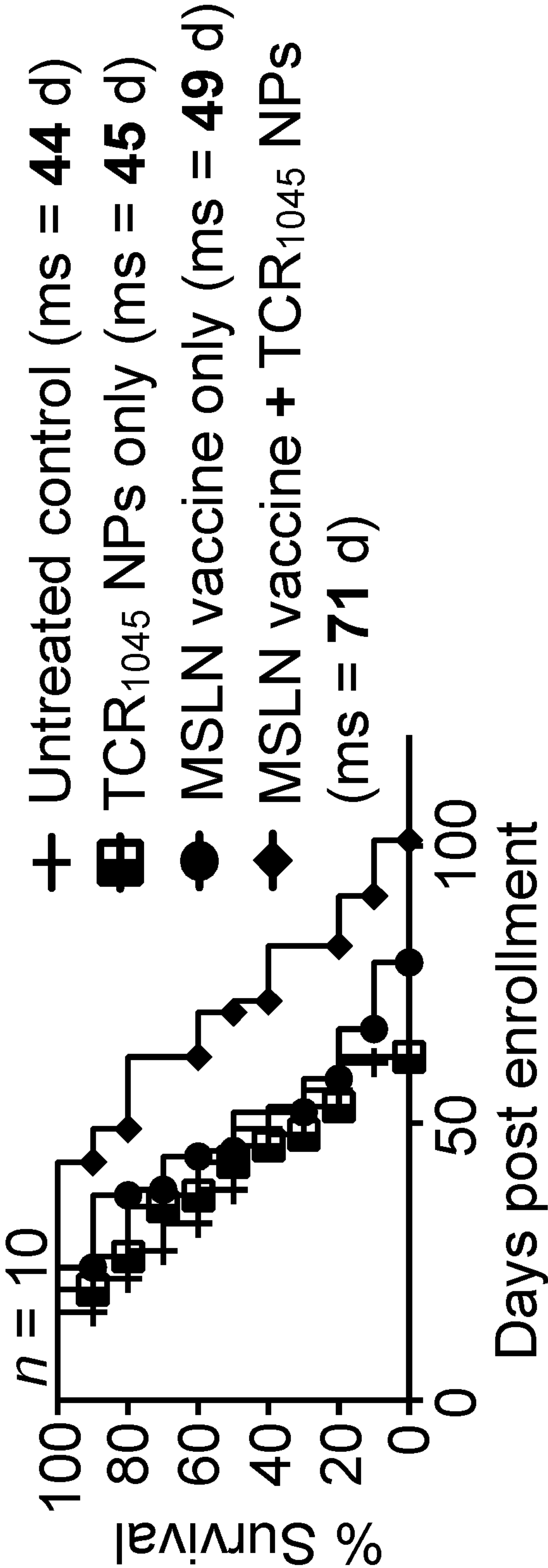


FIG. 5

Representative gene sequence encoding the CD4 transmembrane domain

ttcagcagaagcgccgacgcccctgcctaccagcagggccagaatcagctgtacaacgagctgaacctgggcagaa
gggaagagtacgacgtcctggataagcggagagggccgggaccctgagatgggcggcaagcctcggcggaagaac
ccccaggaaggcctgtataacgaactgcagaaagacaagatggccgagggcctacagcgagatcggcataagggc
gagcggagggcggggcaagggccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacgacgcc
ctgcacatgcaggccctgcccccaagg(SEQ ID NO: 40)

FIG. 6

Representative cDNA sequence encoding a murine codon-optimized piggyBac transposase (GenBank accession number: EF587698)

atgggcagcagcctggacgacgagcacatcctgagcgccctgctgcagagcgacgacgagctggtcggcgaggaca
gcgacagcgagatcagcgaccacgtgagcgaggacgacgtgcagtccgacaccgaggaggccttcacgacgaggt
gcacgaggtgcagcctaccagcagcggctccgagatcctggacgagcagaacgtgatcgagcagcccggcagctcc
ctggccagcaacaggatcctgaccctgccccagaggaccatcaggggcaagaacaagcactgctgggtccacctcaa
gagcaccaggcggagcaggggtgtccgccctgaacatcgtgagaagccagagggggccccaccaggatgtgcaggaa
catctacgacccccctgctgtgcttcaagctgttcttcaccgacgagatcatcagcgagatcgtgaagtggaccaacgccga
gatcagcctgaagaggcgggagagcatgaccggcgccaccttcagggacaccaacgaggacgagatctacgccttct
tcggcatcctgggtgatgaccgccgtgaggaaggacaaccacatgagcaccgacgacctgttcgacagatccctgagcat
gggtgtacgtgagcgtgatgagcagggacagattcgacttcctgatcagatgcctgaggatggacgacaagagcatcagg
cccaccctgcgggagaacgacgtgttcacccccgtgagaaagatctgggacctgttcacccagtgcatccagaact
acaccctggcgcccacctgaccatcgacgagcagctgctgggcttcaggggcaggtgccccttcaggatgtatatcccc
aacaagcccagcaagtacggcatcaagatcctgatgatgtgcgacagcggcaccaagtacatgatcaacggcatgcc
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acggcagctgcagaaacatcacctgcgacaactgggtcaccagcatccccctggccaagaacctgctgcaggagccct
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ggcgtgggcaccagcatgttctgcttcgacggccccctgaccctgggtgtcctacaagcccaagcccgccaagatgggtgt
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a (SEQ ID NO: 142)

FIG. 1

