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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 November 2018 (22.11.2018)

W!P O | PCT

(10) International Publication Number
WO 2018/213803 Al

(51) International Patent Classification:
A61K 39/00 (2006.01) *C12N 5/0784* (2010.01)
C12N 5/0783 (2010.01)

(21) International Application Number:
PCT/US2018/033539

(22) International Filing Date:
18 May 2018 (18.05.2018)

(25) Filing Language: English

(26) Publication Langiuage: English

(30) Priority Data:
62/508,754 19 May 2017 (19.05.2017) US

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

(54) Title: IMMUNOGENIC NEOANTIGEN IDENTIFICATION

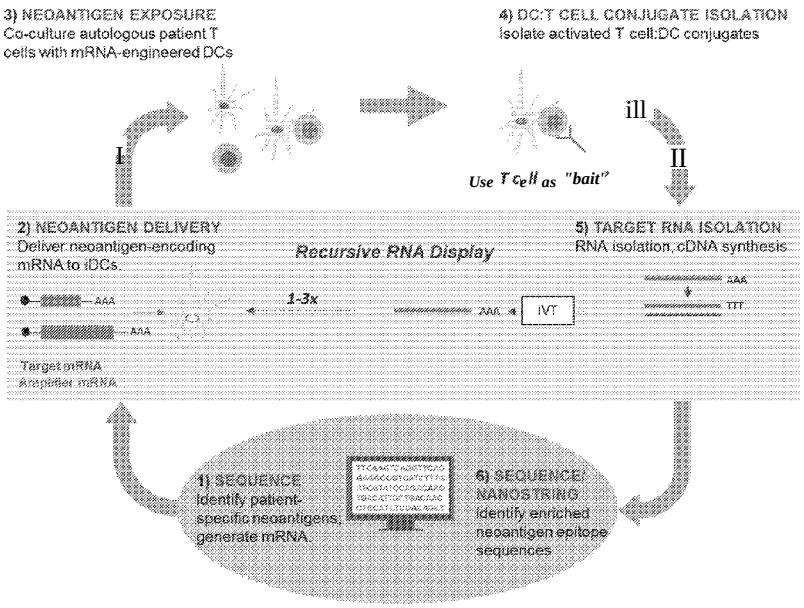

FIG. 1

(57) Abstract: The present invention provides methods and compositions for identifying immunogenic neoantigens and methods of usinjz such neoantigens for the treatment of disease and for producing therapeutics for the treatment of disease.

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MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

IMMUNOGENIC NEOANTIGEN IDENTIFICATION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/508,754, filed on May 19, 2017, which application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] The introduction of a foreign pathogenic antigen into an individual elicits an immune response consisting of two major components, the cellular and humoral immune responses, mediated by two functionally distinct populations of lymphocytes known as T and B cells, respectively. T cells respond to antigen stimulation by producing lymphokines which leads to activation of various other immune cells, including B cells. One T cell subset, $CD8^+$ cytotoxic T lymphocytes (CTLs), is capable of acquiring antigen-specific cytotoxic effector function and can directly kill antigen-positive target cells. Another T cell subset, $CD4^+$ T helper T cells, both supports CTL activity as well as induces B-cells to generate antigen-specific secretory proteins called antibodies that directly bind and neutralize antigens.

[0003] Immunogenic epitopes are currently being investigated for use in cancer therapy. While cancer cells originate from normal tissue, somatic mutations can result in a number of changes in the cancer proteome. Personalized cancer therapies custom tailored to target antigens that are unique to a given patient are also currently being investigated. These treatments are designed to leave healthy cells unharmed and can provide remission to cancer patients. However, many antigens, e.g., those resulting from somatic cancer mutations, do not induce an immune response when administered to a patient; thus, not every antigen can be used as a target for therapy. For effective immunotherapy, there is a critical need for identification of antigens that are likely to be immunogenic. There are computational prediction algorithms that can predict immunogenic T cell epitopes from mutated protein sequences, but these algorithms are only predictions and are limited in their positive predictive value. Immunogenic epitope prediction for individual patients using their specific antigens is particularly challenging. Efficient methods useful for immunogenic epitope identification are yet to be developed and remain a challenging pursuit.

SUMMARY

[0004] Tumor antigens can be tumor-associated self-antigens (e.g., cancer-testis antigens, differentiation antigens) and antigens derived from shared or patient-specific mutant proteins (neoantigens). High avidity T cells specific for tumor-associated self-antigens might be eliminated by self tolerance mechanisms in the thymus; thus, mutant neoantigens may be more immunogenic because they look like foreign epitopes. Successful neoantigen immunotherapy can hinge on selection of neoantigen epitopes that can stimulate a desired T cell response.

[0005] In some aspects, provided herein is A method for identifying a subject-specific and tumor-specific polypeptide sequence or polynucleotide sequence encoding the subject-specific and tumor-specific polypeptide sequence for preparing an immunogenic compositon, the method comprising: contacting an antigen presenting cell (APC) of a plurality of APCs with a T cell of a plurality of T cells, forming one or

more APC:T cell conjugates, wherein the APC expresses a target polypeptide encoded by a target polynucleotide; isolating an APC:T cell conjugate from non-conjugated APCs, non-conjugated T cells, or both; and sequencing the target polynucleotide that encodes the target polypeptide from the APC of the APC:T cell conjugate; wherein the target polypeptide comprises a neoantigen sequence encoded by a nucleic acid of a tumor cell sample of a subject and that is not encoded by a nucleic acid of a non-tumor cell sample of the subject.

[0006] In some embodiments, the method further comprises preparing an immunogenic compositon or a vaccine composition comprising (i) a peptide comprising a neoantigen sequence that is encoded by a target polynucleotide from the APC of the APC:T cell conjugate or (ii) a polynucleic acid encoding a neoantigen sequence that is encoded by a target polynucleotide from the APC of the APC:T cell conjugate. In some embodiments, the method further comprises preparing a therapeutic composition comprising (i) T cell receptor (TCR) or cell comprising a TCR, wherein the TCR is specific for a complex of an HLA protein expressed by the subject and a peptide with a neoantigen sequence that is encoded by a target polynucleotide from the APC of the APC:T cell conjugate or (ii) one or more APCs comprising a peptide with a neoantigen sequence that is encoded by a target polynucleotide from the APC of the APC:T cell conjugate. In some embodiments, the method comprises predicting target polypeptides that bind to a protein encoded by an HLA allele of the subject and/or predicting predicting target polypeptides that are presented by a protein encoded by an HLA allele of the subject. In some embodiments, predicting comprises predicting binding affinities of a plurality of candidate target polpeptide sequences to a protein encoded by an HLA allele expressed by the subject. In some embodiments, predicting comprises using a program implemented on computer system.

[0007] In some aspects, provided herein is a method comprising: contacting an antigen presenting cell (APC) with: an amplifier polynucleotide that is an in vitro transcribed RNA comprising a sequence encoding an amplifier polypeptide that transcribes a target polynucleotide, and the target polynucleotide, wherein the target polynucleotide is an in vitro transcribed RNA comprising a sequence encoding a target polypeptide; and expressing the target polypeptide in the APC.

[0008] In some aspects, provided herein is a method comprising: contacting an antigen presenting cell (APC) with: an amplifier polynucleotide comprising a sequence encoding an amplifier polypeptide that transcribes a target polynucleotide, and the target polynucleotide which comprises a sequence encoding a target polypeptide comprising at most about 100 amino acids; and presenting the target polypeptide or a fragment thereof on the APC.

[0009] In some aspects, provided herein is a method comprising: contacting a cell with: a linear amplifier polynucleotide comprising a sequence encoding an amplifier polypeptide that transcribes a linear target polynucleotide; and the linear target polynucleotide, expressing a target polypeptide encoded by the linear target polynucleotide in the cell.

[0010] In some embodiments, the cell is an antigen presenting cell (APC). In some aspects, provided herein is a method comprising: contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T

cell conjugate, wherein the APC expresses a target polypeptide encoded by a target polynucleotide; and isolating the APC:T cell conjugate from non-conjugated APCs, non-conjugated T cells, or both.

[0011] In some aspects, provided herein is a method comprising: contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a target polypeptide encoded by a target polynucleotide; sequencing a target polynucleotide that encodes the target polypeptide from the APC of the APC:T cell conjugate; and selecting a sequenced target polynucleotide that encodes an immunogenic target polypeptide.

[0012] In some aspects, provided herein is a method comprising contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a polypeptide encoded by a target polynucleotide that is an in vitro transcribed target RNA; and enriching the target polynucleotide from cellular nucleic acids of the APC of the APC:T cell conjugate.

[0013] In some aspects, provided herein is a method comprising: contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a target polypeptide encoded by a target polynucleotide; and selectively enriching for the target polynucleotide from the APC of the APC:T cell conjugate, wherein the enriching comprises transcribing or amplifying the target polynucleotide using a primer that hybridizes to a heterologous promoter region of the target polynucleotide.

[0014] In some aspects, provided herein is a method comprising: contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a target polypeptide comprising at most about 100 amino acids encoded by a target polynucleotide, wherein the target polynucleotide is an in vitro transcribed target RNA, and wherein the APC and/or the T cell is from a subject with a disease or condition; and identifying target RNA enriched from cellular RNA isolated from the APC of the APC:T cell conjugate to encode for an immunogenic polypeptide.

[0015] In some aspects, provided herein is a method comprising: contacting a population of antigen presenting cells (APCs) with a population of T cells, thereby forming a plurality of APC:T cell conjugates each comprising a single APC and at least one T cell, wherein a plurality of APCs of the population of APCs express a target polypeptide encoded by a target polynucleotide sequence; and partitioning a first APC:T cell conjugate of the plurality of APC:T cell conjugates into a first vessel and a second APC:T cell conjugate of the plurality of APC:T cell conjugates into a second vessel; wherein the APC of the first APC:T cell conjugate comprises a first APC polynucleotide and the APC of the second APC:T cell conjugate comprises a second APC polynucleotide; and wherein the T cell of the first APC:T cell conjugate comprises at least one first T cell polynucleotide and the T cell of the second APC:T cell conjugate comprises at least one second T cell polynucleotide.

[0016] In some embodiments, the APC comprises an amplifier polynucleotide comprising a sequence encoding an amplifier polypeptide that transcribes the target polynucleotide. In some embodiments, the amplifier polynucleotide is linear. In some embodiments, the amplifier polynucleotide is an in vitro transcribed RNA. In some embodiments, the target polynucleotide is an in vitro transcribed RNA. In some embodiments, the target polynucleotide is DNA. In some embodiments, the target polynucleotide is linear. In

some embodiments, the target polypeptide comprises at most about 100 amino acids. In some embodiments, the target polypeptide comprises at least about 20 amino acids. In some embodiments, the target polypeptide comprises from about 20 to about 90 amino acids. In some embodiments, the target polypeptide or a fragment thereof is presented on the APC. In some embodiments, the method further comprises contacting the antigen presenting cell (APC) with a T cell. In some embodiments, the method further comprises forming an APC:T cell conjugate. In some embodiments, the method further comprises isolating an APC:T cell conjugate from non-conjugated APCs, non-conjugated T cells, or both. In some embodiments, the method further comprises sequencing a target polynucleotide that encodes the target polypeptide from an APC of an APC:T cell conjugate. In some embodiments, the method further comprises identifying a target polynucleotide to encode for an immunogenic target polypeptide or selecting an immunogenic target polypeptide. In some embodiments, the immunogenic target polypeptide elicits in an immune response when administered to a subject with a disease or condition. In some embodiments, the immunogenic target polypeptide elicits an immune response when administered to a subject with a disease or condition. In some embodiments, the immunogenic target polypeptide increases an immune response when administered to a subject with a disease or condition. In some embodiments, the method further comprises contacting the immunogenic target polypeptide or a nucleic acid encoding the immunogenic target polypeptide to a plurality of cells. In some embodiments, the plurality of cells comprises a plurality of APCs. In some embodiments, the method further comprises administering one or more cells of the plurality of APCs to a subject. In some embodiments, the method further comprises contacting a plurality of T cells to the plurality of APCs. In some embodiments, the plurality of APCs is from a subject. In some embodiments, the plurality of APCs is from a cell line. In some embodiments, the plurality of APCs is engineered cells. In some embodiments, the plurality of T cells is from a subject. In some embodiments, the plurality of T cells is from a cell line. In some embodiments, the plurality of T cells is engineered cells. In some embodiments, the method further comprises expanding one or more T cells of the plurality of T cells. In some embodiments, the method further comprises administering one or more cells of the plurality of T cells to a subject. In some embodiments, the plurality of T cells and the plurality of APCs are from the same subject. In some embodiments, the method is performed ex vivo. In some embodiments, the method further comprises enriching the target polynucleotide from cellular RNA from the APC of the APC:T cell conjugate. In some embodiments, the method further comprises selectively enriching for the target polynucleotide from an APC of an APC:T cell conjugate, wherein the enriching comprises transcribing or amplifying the target polynucleotide using a primer that hybridizes to a heterologous promoter region of the target polynucleotide. In some embodiments, the APC is from a subject with a disease or condition. In some embodiments, the target polypeptide comprises a neoantigen or a neoepitope. In some embodiments, the target polypeptide comprises an immunogenic peptide or an immunogenic epitope. In some embodiments, the target polypeptide comprises a mutant peptide. In some embodiments, the target polypeptide comprises a cancer peptide, a viral peptide, an infectious disease peptide, or a non-cancer disease-associated peptide. In some embodiments, the non-cancer disease-associated peptide is a peptide associated with a disease selected from the group consisting of diabetes, Crohn's, ulcerative colitis and IBD, and arthritis. In some embodiments, the

target polypeptide activates a T cell or stimulates a T cell to proliferate. In some embodiments, the target polypeptide activates a $CD4^+$ T cell or a $CD8^+$ T cell. In some embodiments, the target polypeptide stimulates a $CD4^+$ T cell or a $CD8^+$ T cell to proliferate. In some embodiments, the target polypeptide stimulates a $CD4^+$ T cell or a $CD8^+$ T cell to proliferate when an APC comprising an MHC bound to the target pepide is contacted to the $CD4^+$ T cell or the $CD8^+$ T cell. In some embodiments, the T cell is from a subject with a disease or condition. In some embodiments, the target polynucleotide is incorporated into the genome of the APC. In some embodiments, the genome of the APC comprises the target polynucleotide. In some embodiments, the APC is a CRISPR-Cas engineered APC comprising the target polynucleotide. In some embodiments, the target polynucleotide further comprises a sequence encoding a reporter tag or wherein the target polpeptide is linked to a reporter tag. In some embodiments, the reporter tag comprises a reporter tag that can be detected in a cell optically. In some embodiments, the reporter tag comprises a flourescent reporter tag. In some embodiments, the reporter tag comprises a fluorescent protein, e.g., GFP. In some embodiments, isolating an APC:T cell conjuagte comprises isolating APC:T cell conjugates comprising the reporter tag. In some embodiments, APC:T cell conjugates without the reporter tag are not isolated. In some embodiments, the method comprises selecting a target polynucleotide of an APC:T cell conjugate. In some embodiments, the selecting comprises selecting a target polynucleotide of an APC:T cell conjugate. In some embodiments, the APC:T cell conjugate comprises a purality of APC:T cell conjugates and the selecting comprises selecting a target polynucleotide based on a frquency of the target polynucleotide or a barcode of a target polynucloetide in the purality of APC:T cell conjugates. In some embodiments, the T cell comprises an engineered T cell. In some embodiments, the engineered T cell is engineered to express a TCR of interest. In some embodiments, the method comprises identifying a target polypeptide that interacts with a TCR of interest expressed by the engineered T cell. In some embodiments, the target polynucleotide comprises one or more target polynucleotides encoding a library of noantigens. In some embodiments, the method comprises identifying a neoantigen of the library of neoantigens that binds to a TCR of interest expressed by the engineered T cell. In some embodiments, the APC is a first APC, and wherein the method further comprises expressing a polypeptide from a selectively transcribed target RNA in a second antigen presenting cell (APC); contacting the second APC to a second T cell, thereby forming a second APC:T cell conjugate; isolating cellular RNA from the second APC of the second APC:T cell conjugate; and enriching for the target RNA from the cellular RNA. In some embodiments, the method further comprises identifying a target RNA enriched from cellular RNA isolated from an APC of an APC:T cell conjugate to encode for a polypeptide that elicits an immune response when administered to a subject with a disease or condition. In some embodiments, the isolated APC:T cell conjugate is isolated by flow cytometry or magnetic beads. In some embodiments, the method further comprises isolating polynucleotides from the APC and/or T cell of the APC:T cell conjugate. In some embodiments, the isolated polynucleotides comprise cellular RNA. In some embodiments, the isolated polynucleotides comprise mRNA. In some embodiments, the isolated polynucleotides comprise the target polynucleotide. In some embodiments, the method further comprises reverse transcribing a target polynucleotide from the APC of an APC:T cell conjugate.

[0017] In some embodiments, the method comprises transcribing or amplifying a target polynucleotide using a primer that hybridizes to a heterologous promoter region of the target polynucleotide. In some embodiments, the APC comprises a plurality of APCs. In some embodiments, the APC is a dendritic cell (DC). In some embodiments, the dendritic cell is an immature dendritic cell (iDC). In some embodiments, the T cell comprises a plurality of T cells. In some embodiments, the T cell and the APC are from the same subject. In some embodiments, the target polynucleotide comprises a plurality of target polynucleotides. In some embodiments, the target polynucleotide is RNA. In some embodiments, the target polynucleotide is mRNA. In some embodiments, the target polynucleotide encodes a candidate immunogenic neoantigen. In some embodiments, the target polynucleotide and the amplifier polynucleotide are mixed at a molar concentration ratio of from about 1:2 to 1:1000 or from about 1:20 to 1:1000 or from abour 1:2 to 1:100 before contacting with the APC. In some embodiments, the method is performed in vitro or ex vivo. In some embodiments, the target polynucleotide and the amplifier polynucleotide are delivered into the APC by nucelofection or transfection or electroporation. In some embodiments, the amplifier polynucleotide is transcribed from an amplifier expression polynucleotide comprising a sequence encoding the amplifier polynucleotide. In some embodiments, the target polynucleotide is transcribed from a target expression polynucleotide comprising a sequence encoding the target polynucleotide. In some embodiments, the amplifier expression polynucleotide and/or the target expression polynucleotide is a vector. In some embodiments, the target expression polynucleotide comprises a target polynucleotide ID barcode sequence. In some embodiments, the target polynucleotide ID barcode sequence is barcoded to the sequence encoding the target polynucleotide or the target polypeptide encoded by the target polynucleotide. In some embodiments, the target polynucleotide ID barcode sequence of each target polynucleotide encoding a different target polypeptide is unique.

[0018] In some aspects, provided herein is a method comprising: contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a polypeptide encoded by a target polynucleotide that is an in vitro transcribed target RNA; isolating the APC:T cell conjugate from non-conjugated APCs, non-conjugated T cells, or both within at most about 12 hours after contacting the APC with the T cell. In some embodiments, the isolation is performed within at most about 6 hours after contacting the APC with the T cell. In some embodiments, the contacting comprises contacting the APC to a T cell, wherein the T cell is not activated prior to the contacting. In some embodiments, the contacting comprises contacting the APC to a T cell, wherein the T cell is not activated by an exogenous agent prior to the contacting step. In some embodiments, the APC:T cell conjugate is isolated using a positive selection. In some embodiments, the positive selection comprises contacting a mixture of the APC:T cell conjugate and unconjugated T cells, unconjugated APC cells or both, with a T cell surface marker binding agent. In some embodiments, the T cell surface marker binding agent is a CD3, CD4, CD8, CD40L, or CD137 binding agent, or any combinations thereof. In some embodiments, the positive selection comprises contacting a mixture of the APC:T cell conjugate and unconjugated T cells, unconjugated APC cells or both, with an APC surface marker binding agent. In some embodiments, the APC surface marker binding agent is a CD11c, CD80, or CD86 binding agent, or any combinations thereof. In some embodiments, the APC:T cell is isolated using a

negative selection. In some embodiments, the negative selection comprises contacting a mixture of the APC:T cell conjugate and unconjugated T cells, unconjugated APC cells or both, with a negative selection binding agent selected from the group consisting of a B cell, natural killer cell, monocyte, platelet, dendritic cell, granulocyte or erythrocyte negative selection binding agent, or any combination thereof. In some embodiments, isolating an APC:T cell conjugate comprises sorting APC:T cell conjugates from single cells. In some embodiments, isolating an APC:T cell conjugate comprises flow cytometry. In some embodiments, isolating an APC:T cell conjugate comprises immunopurifying the APC:T cell conjugate. In some embodiments, the immunopurifying comprises contacting a binding agent specifc to an APC to the APC:T cell conjugate. In some embodiments, the immunopurifying comprises contacting a binding agent specifc to a T cell to the APC:T cell conjugate. In some embodiments, isolating an APC:T cell conjugate comprises contacting a mixture of the APC:T cell conjugate and unconjugated T cells, unconjugated APC cells or both, with magnetic beads. In some aspects, provided herein is a method comprising: contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a target polypeptide encoded by a target polynucleotide, wherein the target polynucleotide contains a region comprising a probe binding region and a reporter barcode region; binding the target polynucleotide with a probe and a reporter probe. In some embodiments, the capture probe directly hybridizes with the capture probe binding region of the target polynucleotide. In some embodiments, the reporter probe directly hybridizes with the vector barcode region. In some embodiments, the capture probe indirectly binds to the capture probe binding region through an intermediate oligonucleotide. In some embodiments, the reporter probe indirectly binds to the vector barcode region through an intermediate oligonucleotide. In some embodiments, the method further comprises comprising enriching the target polynucleotide by affinity using the capture probe. In some embodiments, the method further comprises detecting the target polynucleotide on a surface. In some embodiments, the capture probe comprises a region that binds to the capture probe binding region of the target polynucleotide, and another region that is modified with a chemical such that the capture probe bound with the target polynucleotide is immobilized on the surface. In some embodiments, the chemical for modifying of the capture probe is chosen from the group consisting of biotin, digoxigenin, primary amine, thiol, NHS group, maleimide, azide, alkyne, halogen, dibenzocyclooctyl, trans-cyclooctene, tetrazine, aldehydes, hydrazide, and alkoxyamines. In some embodiments, the reporter probe comprises a vector barcode specific region that binds to the vector barcode region of the target polynucleotide, and another region to which is attached a plurality of label monomers that create a unique label for the reporter probe, said label having a detectable signal that distinguishes one reporter probe from the other reporter probe. In some embodiments, each of said label monomers is selected from the group consisting of a fluorochrome moiety, a fluorescent moiety, a dye moiety or a chemiluminescent moiety. In some embodiments, each of said label monomers comprises a fluorescent moiety. In some embodiments, the capture probe binding region is at least 20 bp in length. In some embodiments, the vector barcode region is at least 20 bp in length. In some embodiments, the method further comprises isolating the APC:T cell conjugate from non-conjugated APCs, non-conjugated T cells, or both. In some embodiments, the method further comprises isolating total RNA

from the APC:T cell conjugate. In some embodiments, the APC is a first APC, wherein the method further comprises: contacting a second APC with a second T cell, thereby forming a second APC:T cell conjugate, wherein the APC expresses a second target polypeptide encoded by a second target polynucleotide, wherein the second target polynucleotide contains a second barcode having a second capture probe binding region and a second vector barcode region. In some embodiments, the first capture probe binding region of the first target polynucleotide has the same sequence as the second capture probe binding region of the second target polynucleotide. In some embodiments, the first vector barcode region of the first target polynucleotide has a different sequence from the second vector barcode region of the second target polynucleotide such that the vector barcode region is unique to each polynucleotide encoding a different neoantigen. In some embodiments, the identity of the polynucleotide is determined within at most about 10, or 9, or 8, or 7, or 6, or 5, or 4, or 3, or 2 days. In some embodiments, the identity of the polynucleotide is determined in about 24 hours. In some embodiments, the amplifier polynucleotide is self-replicating. In some embodiments, the amplifier polynucleotide comprises a viral promoter. In some embodiments, the amplifier polynucleotide encodes a viral polypeptide. In some embodiments, the viral polypeptide is a viral nonstructural protein. In some embodiments, the viral polypeptide is selected from the group consisting of nsPl , nsP2, nsP3, nsP4, and combinations thereof. In some embodiments, the viral polypeptide transcribes the target polynucleotide. In some embodiments, the target polynucleotide is not a self-replicating RNA. In some embodiments, the target polynucleotide comprises a viral subgenomic promoter. In some embodiments, the target polynucleotide is a target mRNA that encodes a neoantigen. In some embodiments, the target polynucleotide comprises a barcode sequence. In some embodiments, the target polynucleotide comprises more than one barcode sequence. In some embodiments, the barcode sequence is a degenerate sequence. In some embodiments, the barcode sequence is a known sequence. In some embodiments, the barcode sequence comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 45, or 50 nucleotides. In some embodiments, the barcode sequence is located 3' to the sequence encoding the target polypeptide. In some embodiments, the barcode sequence is located 5' to the sequence encoding the target polypeptide. In some embodiments, a first target polynucleotide encoding a first target polypeptide comprises a first barcode sequence and a second target polynucleotide encoding the first target polypeptide comprises a second barcode sequence that is different from the first barcode sequence. In some embodiments, the amplifier polynucleotide and the target polynucleotide lack one or more sequences encoding viral structural proteins that are required for viral particle formation in the cell. In some embodiments, the amplifier polynucleotide, the target polynucleotide, or both do not encode a viral structural protein. In some embodiments, the amplifier polynucleotide, the target polynucleotide, or both comprise a 5' UTR region. In some embodiments, the 5' UTR region comprises a region comprising a sequence from a genome of a virus. In some embodiments, the amplifier polynucleotide, the target polynucleotide, or both comprise a 3' UTR region. In some embodiments, the 3' UTR region comprises a region comprising a sequence from a genome of a virus. In some embodiments, the virus is an alphavirus. In some embodiments, the alphavirus is selected from the group consisting of Eastern Equine Encephalitis virus (EEE), Venezuelan Equine Encephalitis virus (VEE), Western Equine Encephalitis virus

(WEE), Sindbis virus, South African Arbovirus No. 86, Semliki, and Forest virus. In some embodiments, the amplifier polynucleotide, the target polynucleotide, or both comprise a sequence encoding a 3' poly(A) tail. In some embodiments, the method further comprises partitioning a first APC:T cell conjugate of a plurality of APC:T cell conjugates into a first vessel and a second APC:T cell conjugate of the plurality of APC:T cell conjugates into a second vessel; wherein the APC of the first APC:T cell conjugate comprises a first APC polynucleotide and the APC of the second APC:T cell conjugate comprises a second APC polynucleotide; and wherein the T cell of the first APC:T cell conjugate comprises at least one first T cell polynucleotide and the T cell of the second APC:T cell conjugate comprises at least one second T cell polynucleotide; and optionally, wherein the first vessel comprises a first vessel barcoded polynucleotide comprising a first vessel barcode sequence, and optionally, wherein the second vessel comprises a second vessel barcoded polynucleotide comprising a second vessel barcode sequence. In some embodiments, the first and/or the second APC:T cell conjugate comprises a single APC and at least two T cells. In some embodiments, the method further comprises extracting the first APC polynucleotide from the APC of the first APC:T cell conjugate. In some embodiments, the method further comprises extracting the at least one first T cell polynucleotide from the T cell of the first APC:T cell conjugate. In some embodiments, the method further comprises extracting the second APC polynucleotide from the APC of the second APC:T cell conjugate. In some embodiments, the method further comprises extracting the at least one second T cell polynucleotide from the T cell of the second APC:T cell conjugate. In some embodiments, the extracting comprises lysing. In some embodiments, the extracting comprises extracting after the partitioning. In some embodiments, the at least one first and/or second T cell polynucleotide and the first and/or second APC polynucleotide are extracted simultaneously or during a same reaction. In some embodiments, the first and/or second APC polynucleotide is an exogenous polynucleotide. In some embodiments, the first and/or second APC polynucleotide is RNA. In some embodiments, the at least one first and/or second T cell polynucleotide is an endogenous polynucleotide. In some embodiments, the at least one first and/or second T cell polynucleotide is RNA. In some embodiments, the at least one first and/or second T cell polynucleotide is a T cell receptor (TCR) chain. In some embodiments, the at least one first and/or second T cell polynucleotide is a TCRa chain, a TCRβ chain, a TCRγ chain, a TCR5 chain, or a combination thereof. In some embodiments, the at least one first and/or second T cell polynucleotide comprises a TCR chain variable sequence. In some embodiments, the at least one first and/or second T cell polynucleotide comprises a sequence specific to a T cell characteristic. In some embodiments, the T cell characteristic is a T cell immunophenotype, such as a T cell immunophenotype, such as a $CD4^+$ T cell or a $CD8^+$ T cell. In some embodiments, the at least one first T cell polynucleotide comprises at least two T cell polynucleotides and/or the at least one second T cell polynucleotide comprises at least two T cell polynucleotides. In some embodiments, the at least two T cell polynucleotides comprises a TCRa chain and a TCRβ chain. In some embodiments, the at least two T cell polynucleotides comprises a TCRy chain and a TCR5 chain. In some embodiments, the APC:T cell conjugate comprises a plurality of APC:T cell conjugates, wherein the method comprises selecting a target polynucleotide of an APC:T cell conjugate based on a frequency of a T cell polynucleotide in the plurality of APC:T cell conjugates, wherein the T cell

polynucleotide comprises a variable domain of a TCRa chain, a TCRβ chain, a TCRγ chain, a TCR5 chain, or a combination thereof. In some embodiments, the method further comprises reverse transcribing the first and/or second APC polynucleotide, the at least one first and/or second T cell polynucleotide, or a combination thereof. In some embodiments, the first vessel comprises a first vessel barcoded polynucleotide comprising a first vessel barcode sequence. In some embodiments, the second vessel comprises a second vessel barcoded polynucleotide comprising a second vessel barcode sequence. In some embodiments, the first vessel barcode sequence is not the same as the second vessel barcode sequence.

[0019] In some embodiments, the method further comprises attaching a vessel barcode sequence of a first vessel barcoded polynucleotide to the first APC polynucleotide or a complement thereof, thereby forming a first vessel barcoded APC polynucleotide. In some embodiments, the method further comprises attaching a vessel barcode sequence of a second vessel barcoded polynucleotide to the second APC polynucleotide or a complement thereof, thereby forming a second vessel barcoded APC polynucleotide. In some embodiments, the method further comprises attaching a vessel barcode sequence of the first vessel barcoded polynucleotide to the at least one first T cell polynucleotide or a complement thereof, thereby forming at least one first vessel barcoded T cell polynucleotide. In some embodiments, the method further comprises attaching a vessel barcode sequence of the second vessel barcoded polynucleotide to the at least one second T cell polynucleotide or a complement thereof, thereby forming at least one second vessel barcoded T cell polynucleotide. In some embodiments, the first vessel barcode sequence attached to first APC polynucleotide is the same as the first vessel barcode sequence attached to the least one first T cell polynucleotide. In some embodiments, the second vessel barcode sequence attached to the second APC polynucleotide is the same as the second vessel barcode sequence attached to the at least one second T cell polynucleotide. In some embodiments, the vessel barcode sequence of the first vessel barcoded polynucleotide and the vessel barcode sequence of the second vessel barcoded polynucleotide are different. In some embodiments, the vessel barcode of each vessel barcoded polynucleotide in any single vessel of the plurality of vessels is unique to the vessel barcode of each vessel barcoded polynucleotide in any other single vessel of the plurality of vessels. In some embodiments, the first and/or second vessel barcode sequence is attached after the partitioning. In some embodiments, the first and/or second vessel barcode sequence is attached after the extracting. In some embodiments, the method further comprises amplifying the first and/or second vessel barcoded APC polynucleotide, the at least one first and/or second vessel barcoded T cell polynucleotide, or a combination thereof. In some embodiments, the method further comprises sequencing the first and/or second vessel barcoded APC polynucleotide or an amplified product thereof, the at least one first and/or second vessel barcoded T cell polynucleotide or an amplified product thereof, or a combination thereof. In some embodiments, the sequencing comprises high-throughput sequencing. In some embodiments, the sequencing generates one or more APC polynucleotide sequence reads comprising a sequence of the first and/or second vessel barcoded APC polynucleotide or an amplified product thereof. In some embodiments, the one or more APC polynucleotide sequence reads comprise the vessel barcode sequence or complement thereof of the first vessel barcoded polynucleotide and/or the vessel barcode sequence or complement thereof of the second

vessel barcoded polynucleotide. In some embodiments, the sequencing generates one or more T cell polynucleotide sequence reads comprising a sequence of the at least one first and/or second vessel barcoded T cell polynucleotide or an amplified product thereof. In some embodiments, the one or more T cell polynucleotide sequence reads comprise the vessel barcode sequence or complement thereof of the first vessel barcoded polynucleotide and/or the vessel barcode sequence or complement thereof of the second vessel barcoded polynucleotide.

[0020] In some aspects, provided herein is a method comprising: forming a plurality of vessels each comprising a single APC:T cell conjugate from a sample comprising a plurality of APC:T cell conjugates, and a vessel barcoded polynucleotide comprising a vessel barcode sequence; producing: a complementary APC polynucleotide that is complementary to an APC polynucleotide from the APC of the APC:T cell conjugate, a first complementary T cell polynucleotide that is complementary to a first T cell polynucleotide from the T cell of the APC:T cell conjugate, and optionally, a second complementary T cell polynucleotide that is complementary to a second T cell polynucleotide from the T cell of the APC:T cell conjugate wherein the complementary APC polynucleotide, the first complementary T cell polynucleotide and optionally, the second complementary T cell polynucleotide comprise the vessel barcode sequence or a complement thereof. In some embodiments, the producing comprises reverse transcribing. In some embodiments, the producing comprises reverse transcribing with a primer comprising the vessel barcode sequence. In some embodiments, the producing comprises attaching the vessel barcoded polynucleotide or a complement thereof to the complementary APC polynucleotide, the first complementary T cell polynucleotide and optionally, the second complementary T cell polynucleotide. In some embodiments, the producing comprises hybridizing the vessel barcoded polynucleotide or a complement thereof to the APC polynucleotide, the first T cell polynucleotide and optionally, the second T cell polynucleotide. In some embodiments, the producing comprises extending a primer hybridized to the APC polynucleotide, the first T cell polynucleotide and optionally, the second T cell polynucleotide. In some embodiments, the steps are performed in the single vessel. In some embodiments, the steps are performed in a single reaction. In some embodiments, the method further comprises lysing the APC and/or the T cell of the APC:T cell conjugate. In some embodiments, the APC and/or the T cell is lysed in the vessel. In some embodiments, the plurality of vessels comprises a plurality of wells. In some embodiments, the vessels are water-in-oil droplets. In some embodiments, the plurality of vessels comprises an emulsion. In some embodiments, each vessel of the plurality of vessels is from about 0.01 picoliters to 10 microliters in volume. In some embodiments, the vessel barcode of a vessel barcoded polynucleotide or amplicon thereof in a first vessel of the plurality of vessels is a different than the vessel barcode of a vessel barcoded polynucleotide or amplicon thereof in a second vessel of the plurality of vessels. In some embodiments, a vessel barcode of each vessel barcoded polynucleotide and amplicon thereof in any single vessel of the plurality of vessels is unique to the vessel barcode of each vessel barcoded polynucleotide and amplicon thereof in any other single vessel of the plurality of vessels. In some embodiments, the method further comprises binning or grouping sequences comprising the same vessel barcode. In some embodiments, the method further comprises binning or grouping sequences comprising the same vessel barcode using a

computer or algorithm. In some embodiments, the method further comprises determining the number of sequence reads containing the same vessel barcode. In some embodiments, the method further comprises selecting a target polypeptide based on the number of sequence reads comprising a sequence encoding the target polypeptide. In some embodiments, the method further comprises pairing a TCR chain of the T cell of the APC:T cell conjugate to the target polynucleotide of the APC of the same APC:T cell conjugate. In some embodiments, the pairing comprises pairing a barcode of the TCR chain to a barcode of the target polynucleotide. In some embodiments, the selected TCR binds to an epitope with a KD of less than about or equal to lxlO^{-7}, lxlO^{-8}, lxlO^{-9}, lxlO^{-10}, lxlO^{-11}, or lxlO^{-12} M.

[0021] In some embodiments, the method comprises: contacting a first antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the first APC expresses a target polypeptide encoded by a target RNA; enriching for the target RNA from cellular RNA from the APC of the APC:T cell conjugate; contacting the enriched target RNA or a copy thereof with a second APC; expressing a target polypeptide encoded by the enriched target RNA in the second APC; and optionally repeating the above steps. In some embodiments, the second APC is matched to the first APC. In some embodiments, the first APC and the second APC are from the same subject. In some embodiments, the method comprises: expressing a polypeptide from an in vitro transcribed target RNA in an antigen presenting cell (APC); contacting the APC with a T cell, thereby forming an APC:T cell conjugate; isolating cellular RNA from the APC of the APC:T cell conjugate; and enriching for the target RNA from the cellular RNA. In some embodiments, the method comprises: delivering a target mRNA polynucleotide encoding a target polypeptide to an antigen presenting cell (APC); contacting the APC to a T cell, thereby forming an APC:T cell conjugate; isolating the APC:T cell conjugate; isolating cellular mRNA from the APC of the APC:T cell conjugate; selectively enriching for the target RNA polynucleotide from the cellular RNA; and identifying the enriched target RNA to encode for a polypeptide that elicits an immune response when administered to a subject with a disease or condition.

[0022] In some embodiments, the method comprises: providing isolated T cells from a subject with a disease or condition, providing APCs from the subject, wherein the APCs express a candidate immunogenic neoantigen from an exogenous target polynucleotide, contacting the T cells with the APCs, isolating an APC:T cell conjugate, and identifying a candidate immunogenic neoantigen expressed in the APC of the isolated APC:T cell conjugate as an immunogenic neoantigen of the subject with a disease or condition. In some embodiments, the method further comprises loading an APC with a target polypeptide selected or identified by a method described herein.

[0023] In some aspects, provided herein is an isolated, purified, antibody or TCR identified by a method described herein.

[0024] In some aspects, provided herein is an isolated, purified, TCRβ , or TCR5 identified by a method described herein.

[0025] In some aspects, provided herein is an isolated, purified, TCRa or TCRγ identified by a method described herein.

[0026] In some aspects, provided herein is an isolated, purified, Fat>2 fragment of a TCR identified by a method described herein.

[0027] In some aspects, provided herein is an isolated, purified, Fv fragment of a TCR identified by a method described herein.

[0028] In some aspects, provided herein is a method of treating a subject in need thereof, comprising administering a selected TCR described herein, or a fragment thereof, to a subject in need thereof. In some embodiments, the TCR or fragment thereof is identified from the subject in need thereof.

[0029] In some aspects, provided herein is a composition comprising an APC:T cell conjugate isolated from non-conjugated APCs and T cells, wherein the APC of the APC:T cell conjugate comprises an in vitro transcribed target RNA, and wherein the in vitro transcribed target RNA comprises a sequence encoding a polypeptide that interacts with the T cell of the APC:T cell conjugate.

[0030] In some aspects, provided herein is a composition comprising a plurality of vessels, each comprising a single APC:T cell conjugate from a sample comprising a plurality of APC:T cell conjugates, a vessel barcoded polynucleotide comprising a vessel barcode sequence; an APC polynucleotide from the APC of the single APC:T cell conjugate, a first T cell polynucleotide from the T cell of the single APC:T cell conjugate; and optionally, a second T cell polynucleotide from the T cell of the single APC:T cell conjugate wherein the APC polynucleotide, the first T cell polynucleotide, and optionally, the second T cell polynucleotide each comprise the vessel barcode sequence or a complement thereof.

[0031] In some aspects, provided herein is an antigen presenting cell (APC) comprising: a first in vitro transcribed RNA comprising a sequence encoding a first polypeptide, wherein the first polypeptide amplifies a second in vitro transcribed RNA; the second in vitro transcribed RNA, and a second polypeptide expressed from the second in vitro transcribed RNA, wherein the second polypeptide or a fragment thereof interacts with a T cell when presented by the APC.

[0032] In some aspects, provided herein is an antigen presenting cell (APC) comprising a linear amplifier polynucleotide comprising a sequence encoding a first polypeptide that amplifies a linear target polynucleotide; the linear target polynucleotide; and a second polypeptide expressed from the linear target polynucleotide, wherein the second polypeptide or a fragment thereof interacts with a T cell when presented by the APC.

[0033] In some aspects, provided herein is an antigen presenting cell (APC) comprising: an amplifier polynucleotide comprising a sequence encoding a first polypeptide that amplifies a target polynucleotide, and the target polynucleotide, wherein the target polynucleotide comprises a sequence encoding a second polypeptide comprising at most about 100 amino acids; and the second polypeptide, wherein the second polypeptide is expressed from the target polynucleotide, wherein the second polypeptide or a fragment thereof interacts with a T cell when presented by the APC.

[0034] In some aspects, provided herein is a method of treating a subject with a disease or condition comprising administering to the subject a peptide or polypeptide comprising a sequence of an immunogenic neoantigen identified according to a method described herein.

[0035] In some aspects, provided herein is a method of treating a subject with a disease or condition, comprising administering to the subject a polynucleotide comprising a sequence that encodes an immunogenic neoantigen identified according to a method described herein.

[0036] In some aspects, provided herein is a method of treating a subject with a disease or condition comprising administering to the subject an antibody or binding fragment thereof, wherein the antibody or binding fragment thereof specifically binds to an immunogenic neoantigen identified according to a method described herein.

[0037] In some aspects, provided herein is a method of treating a subject with a disease or condition, comprising administering to the subject a virus comprising a polynucleotide sequence encoding an immunogenic neoantigen identified according to a method described herein.

[0038] In some aspects, provided herein is a method of treating a subject with a disease or condition, comprising administering to the subject a T cell comprising a TCR that specifically binds to an immunogenic neoantigen identified according to a method described herein.

[0039] In some aspects, provided herein is a method of treating a subject with a disease or condition, comprising administering to the subject an adoptive T cell therapy comprising a T cell that specifically binds to an immunogenic neoantigen identified according to a method described herein.

[0040] In some aspects, provided herein is a method of treating a subject with a disease or condition, comprising administering to the subject a T cell expanded ex vivo in the presence of an APC comprising an immunogenic neoantigen identified according to a method described herein.

[0041] In some aspects, provided herein is a method of treating a subject with a disease or condition, comprising administering to the subject an APC comprising an immunogenic neoantigen identified according to a method described herein.

[0042] In some aspects, provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a peptide or polypeptide comprising a sequence of an immunogenic neoantigen identified according to a method described herein.

[0043] In some aspects, provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a polynucleotide comprising a sequence that encodes an immunogenic neoantigen identified according to a method described herein.

[0044] In some aspects, provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or binding fragment thereof, wherein the antibody or binding fragment thereof specifically binds to an immunogenic neoantigen identified according to a method described herein.

[0045] In some aspects, provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a virus comprising a polynucleotide sequence encoding an immunogenic neoantigen identified according to a method described herein.

[0046] In some aspects, provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a T cell comprising a TCR that specifically binds to an immunogenic neoantigen identified according to a method described herein.

[0047] In some aspects, provided herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an APC comprising an immunogenic neoantigen identified according to a method described herein.

[0048] In some aspects, provided herein is a method of treating a subject with a disease or condition comprising administering to the subject a pharmaceutical composition described herein.

[0049] In some aspects, provided herein is an immunogenic neoantigen identified according to a method described herein.

[0050] In some aspects, provided herein is A therapeutic, immunogenic or vaccine composition comprising: (a) a peptide comprising a neoantigen sequence that is encoded by the target polynucleotide from the APC of the APC:T cell conjugate identifed according to the methods described herein; (b) a polynucleotide encoding the peptide comprising a neoantigen sequence that is encoded by the target polynucleotide from the APC of the APC:T cell conjugate identifed according to the methods described herein; (c) one or more APCs comprising a peptide comprising a neoantigen sequence that is encoded by the target polynucleotide from the APC of the APC:T cell conjugate identifed according to the methods described herein; or (d) a T cell receptor (TCR) specific for a peptide in complex with an HLA protein, wherein the peptide comprises a neoantigen sequence that is encoded by the target polynucleotide from the APC of the APC:T cell conjugate identifed according to the methods described herein.

INCORPORATION BY REFERENCE

[0051] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] **FIG. 1** is a schematic representation of an exemplary Rapid Functional T cell (RaFT) assay experimental workflow.

[0053] **FIG. 2** is a schematic representation of an exemplary Rapid Functional T cell (RaFT) assay experimental workflow.

[0054] **FIG. 3** is a schematic representation of an exemplaryVenezuelan Equine Encephalitis (VEE)-derived RNA replicon vector components used to express neoantigens in the RaFT including amplifier mRNA and target mRNA components.

[0055] **FIG. 4A** is a schematic representation of an empty target mRNA construct.

[0056] **FIG. 4B** is a schematic representation of an exemplary assembly scheme for target mRNA.

[0057] **FIG. 5** depicts an example of paired antigen:TCR single conjugate sequencing scheme of RaFT.

[0058] **FIG. 6** depicts an example of molecular components of RaFT amplifier RNA and target RNA derived from alphavirus (e.g., VEE).

[0059] **FIG. 7** depicts sequence elements of exemplary amplifier and target mRNA constructs.

[0060] FIG. 8 is a schematic representation of tag pool generation from epitope of interest. Each tag pool can contain, e.g., 5 to 50,000 uniquely barcode tagged vectors.

[0061] FIG. 9 depicts an example of *in vitro* RNA synthesis workflow.

[0062] FIG. 10 depicts experimental data for amplifiertarget titration, showing that the RaFT amplifier RNA molecule has activity on the RaFT target RNA molecule.

[0063] FIG. 11 is a schematic representation of RNA delivery and conjugate formation process.

[0064] FIG. 12A depicts experimental data for RaFT conjugate flow sorting.

[0065] FIG. 12B depicts experimental data for RaFT conjugate flow sorting.

[0066] FIG. 12C depicts experimental data for RaFT conjugate flow sorting.

[0067] FIG. 13A depicts an exemplary workflow for target RNA recovery from DC:T cell conjugates and RNA regeneration for recursive rounds of RAFT.

[0068] FIG. 13B depicts an exemplary target regeneration workflow using RNA recovered from DC:T cell conjugates for recursive rounds of RaFT.

[0069] FIG. 14 depicts exemplary experimental data of FACS analysis using multimer reagents that recognizes T cells specific to the indicated viral antigens that are previously identified neoantigens (PINs) in samples initially taken from a donor subject without indution *(ex vivo)* and samples taken from the same donor subject induced for 14 days. The data is compared to RaFT assay analysis of DC:T cell conjugates isolated at the indicated time points for the indicated viral antigens.

[0070] FIG. 15A depicts exemplary experimental data of FACS analysis using multimer reagents that recognizes T cells specific to the indicated neoantigens that are previously identified neoantigens (PINs) in samples initially taken from a donor subject without indution *(ex vivo)* and samples taken from the same donor subject induced for 14 days. The data is compared to RaFT assay analysis of DC:T cell conjugates isolated at the indicated time points for the indicated antigens.

[0071] FIG. 15B depicts pie charts of exemplary experimental data of T cells from a healthy donor induced with PIN peptide pools using a standard T cell induction protocol with no antibody treatment or with anti-PD-1 and anti-CTLA4 treatment for a period of two weeks Cells were then put through a recall assay for 24 hours with individual mutant ACTN4 peptide or wildtype ACTN4 24-mer peptides at 0.8 μM or 3.2 μM in concentration. $CD4^+$ T cells were analyzed by flow cytometry for expression of IFN-γ, TNF, CD 107a and 4-1BB. The percentage of $CD4^+$ cells expressing 1, 2, 3, or 4 of these markers are represented in the pie charts.

[0072] FIG. 16A depicts exemplary experimental FACS data analysis of a KRAS G12C neoantigen using the RaFT assay.

[0073] FIG. 16B depicts exemplary experimental FACS data analysis of a GATA3 neoantigen using the RaFT assay.

[0074] FIG. 16C depicts exemplary experimental sequencing data of the depicted shared neoantigens using the RaFT assay.

[0075] FIG. 17A depicts exemplary RaFT experimental sequencing data showing $CD8^+$ T cell responses to the indicated neoantigens.

[0076] FIG. 17B depicts exemplary RaFT experimental sequencing data showing $CD4^+$ T cell responses to the indicated neoantigens.

[0077] FIG. 18A depicts exemplary RaFT experimental sequencing data showing $CD8^+$ T cell responses to the indicated shared neoantigens.

[0078] FIG. 18B depicts exemplary FACS data analysis of $CD8^+$ T cells after a 2 week induction period using a multimer reagent that recognizes T cells specific to a TMRSS2:ERG neoantigen.

[0079] FIG. 18C depicts exemplary RaFT experimental sequencing data showing $CD4^+$ T cell responses to the indicated shared neoantigens.

[0080] FIG. 19 depicts exemplary FACS data analysis DC:T cell conjugate formation frequencies with or without antigen expression using a RaFT assay.

[0081] FIG. 20 depicts a graph of exemplary RaFT experimental data of $CD11c^+/CD3^+$ DC:T cell conjugate frequencies at the indicated times normalized to total background conjugate formation. The number of $CD11c^+/CD3^+$ DC:T cell conjugates increases overtime in the presence of antigens.

[0082] FIG. 21A depicts a graph of exemplary RaFT experimental data of $CD4^+$ DC:T cell conjugate frequencies at the indicated times normalized to total background conjugate formation. The number of $CD4^+$ DC:T cell conjugates increases over time in the presence of antigens.

[0083] FIG. 21B depicts a graph of exemplary RaFT experimental data of $CD8^+$ DC:T cell conjugate frequencies at the indicated times normalized to total background conjugate formation. The number of $CD8^+$ DC:T cell conjugates increases over time in the presence of antigens.

[0084] FIG. 22A depicts a graph of exemplary RaFT experimental data of 4-lBB^+ DC:T cell conjugate frequencies at the indicated times normalized to total background conjugate formation.

[0085] FIG. 22B depicts a graph of exemplary RaFT experimental data of $PD1^+$ DC:T cell conjugate frequencies at the indicated times normalized to total background conjugate formation.

[0086] FIG. 22C depicts a graph of exemplary RaFT experimental data of $CD69^+$ DC:T cell conjugate frequencies at the indicated times normalized to total background conjugate formation.

[0087] FIG. 22D depicts a graph of exemplary RaFT experimental data of $ICOS^+$ DC:T cell conjugate frequencies at the indicated times normalized to total background conjugate formation.

[0088] FIG. 22E depicts a graph of exemplary RaFT experimental data of $CD27^+$ DC:T cell conjugate frequencies at the indicated times normalized to total background conjugate formation.

[0089] FIG. 23 depicts exemplary sequencing methods that can be used for a RaFT assay.

[0090] FIG. 24 depicts an exemplary method to identify the neoantigens using labeled NanoString hybridization probes. The depicted method avoids intoridcution of PCR and sequence bias.

[0091] FIG. 25A depicts experimental data showing the specificity of RaFT assay. T cells induced against EBV YVL antigen for 12 days in culture are stained using YVL specific multimer reagents, and induced T cells (19.6%) are used as input cells in the sequencing assay shown in FIG. 25B.

[0092] FIG. 25B depicts experimental data showing the specificity of RaFT assay. Sequencing readout of RaFT assay shows that there is an enrichment of reads for YVL using induced T cells as input cells, but there is no enrichment of control antigens (e.g., GAS7 antigen and GSN antigen).

[0093] FIG. 26 depicts a schematic representation of RaFT Nanostring read-out design. The RNA molecule to be used for *in vitro* transcription is designed to include sequences for probe binding for use in NanoString read-out. Shown in this figure, Probe A is specific for each epitope, and Probe B is a universal probe specific for binding to NanoString cassette.

[0094] FIG. 27A depicts an analysis of NanoString probe specificity and sensitivity. A dose dependent read-out of the NanoString probes that are specific to the NanoString sequence transcribed from the RNA molecule.

[0095] FIG. 27B depicts experimental data showing that NanoString reads reduce in response to titration of input RNA amount. The data are shown in number of NanoString reads versus the amount of input RNA.

[0096] FIG. 27C depicts experimental data showing that NanoString reads reduce in response to titration of input RNA amount. The data are shown in number of target molecules per NanoString read versus the amount of input RNA.

[0097] FIG. 28A depicts experimental data showing dendritic cells infected with different amount of RNA molecules expressing GFP reporter vectors with different NanoString probe sequences. The "^g RNA mix" shown in this figure refers to NS2, NS3, and NS4 mixed at a ratio of 60:30: 10.

[0098] FIG. 28B depicts experimental data showing dendritic cells infected with different amount of RNA molecules expressing GFP reporter vectors with different NanoString probe sequences. RNAs were isolated from dendritic cell populations and processed using the RaFT protocol and NanoString read-out. A dose dependant curve of different nanostring sequences can be observed when different mRNA sequences were mixed at different amounts.

[0099] FIG. 29A depicts experimental data showing the enrichment of the specific epitope (YVL) by RaFT assay using NanoString as a readout.

[00100] FIG. 29B depicts experimental data showing the enrichment of the speciric epitope (YVL) by RaFT assay using sequencing as a readout.

[00101] FIG. 30 depicts an schematic overview of RaFT assay using mcirobeads conjugated with T cell marker to isolate APC:T cell conjugates.

[00102] FIG. 31 depicts an exemplary graph of 562 peptides with predicted affinity for select HLA Class I molecule between 1 nM and 100,000 nM (plotted on x-axis) that were synthesized. Actual affinity (IC_{50} (nM)) was measured as described (y-axis). Thick vertical and horizontal lines denote 500 nM cutoff between weak and very weak predicted and observed binders, respectively. Diagonal dotted line depicts line-of-best fit (Graphpad Prism) with R^2 of 0.45.

DETAILED DESCRIPTION OF THE INVENTION

[00103] It is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of

describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims

[00104] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[00105] The practice of some embodiments disclosed herein employ, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See for example Sambrook and Green, Molecular Cloning: A Laboratory Manual, 4th Edition (2012); the series Current Protocols in Molecular Biology (F. M. Ausubel, et al. eds.); the series Methods In Enzymology (Academic Press, Inc.), PCR 2: A Practical Approach (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications, 6th Edition (R.I. Freshney, ed. (2010)).

[00106] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[00107] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. The details of one or more particular embodiments are set forth in the description below.

[00108] Provided herein is a detailed description of the present disclosure, including different sections covering Definitions (I), Overview (II), Rapid Functional T cell Test (RaFT) (III), Identification of Neoantigen (IV), Target RNA and Amplifier RNA (V), APCs (VI), Delivering Neoantigen-Encoding RNAs to APCs (VII), T Cells (VIII), Culturing T cells with APCs Containing Neoantigen-Encoding Polynucleotides (IX), Linking Neoantigen Sequences to T cells (X), Barcodes (XI), Flow Cytometric Isolation of Conjugates (XII), Magnetic Bead Based Isolation of Conjugates (XIII), Isolating mRNA from APC:T Cell Conjugates (XIV), Generating cDNA from mRNA (XV), Identification of Immunogenic Neoantigen (XVI), Therapeutic

Compositions and Methods of Treatment (XVII), Pharmaceutical Compositions (XVIII), Cell for Use in the Invention (XIX), Method of Use (XX), and Computer-Implementaed Aspects (XXI).

I. Definitions

[00109] The term "neoantigen" or "neoepitope" refers to antigens that are not encoded in a normal, non-mutated host genome. A neoantigen refers to an antigen including one or more amino acid modifications compared to the parental antigen. For example, a neoantigen may be a tumor-associated neoantigen, wherein the term "tumor-associated neoantigen" can include a peptide or protein including amino acid modifications due to tumor-specific mutations. For example, a neoantigen may be a disease-associated neoantigen, wherein the term "disease-associated neoantigen" can include a peptide or protein including amino acid modifications due to disease-specific mutations. In some instances, a neoantigen represents either oncogenic viral proteins or abnormal proteins that arise as a consequence of somatic mutations. For example, a neoantigen can arise by the disruption of cellular mechanisms through the activity of viral proteins. Another example can be an exposure of a carcinogenic compound, which in some cases can lead to a somatic mutation. This somatic mutation can ultimately lead to the formation of a tumor/cancer.

[00110] The term "somatic mutation" refers to a genetic alteration acquired by a cell that is not inherited in the germline.

[00111] The term "immunogenic peptide" or "immunogenic epitope" refers to an antigen, such as a neoantigen, that modulates T cells or elicits an immune response when administered to a subject. For example, an immunogenic peptide can be comprised within a vaccine. For example, an immunogenic peptide can be a peptide that activates T cells. For example, an immunogenic peptide can be a peptide that stimulates T cell proliferation. For example, an immunogenic peptide can be a peptide that stimulates T cell proliferation *ex vivo*. For example, an immunogenic peptide can be a peptide that is boud to an MHC of an APC. For example, an immunogenic peptide can be a peptide that is bound to an MHC of an APC of an APC:T cell conjugate. For example, an immunogenic peptide can be a peptide loaded onto a dendridic cell (DC) that stimulates or activates $CD4^+$ and/or $CD8^+$ T-cell proliferation.

[00112] A "T-cell receptor" ("TCR") refers to a molecule, whether natural or partly or wholly synthetically produced, found on the surface of T lymphocytes (T-cells) that recognizes antigens bound to major histocompatibility complex (MHC) molecules. The ability of T-cells to recognize antigens associated with various cancers or infectious organisms is conferred by its TCR, which is made up of both an alpha (a) chain and a beta (β) chain or a gamma (γ) and a delta (δ) chain. The proteins which make up these chains are encoded by DNA, which employs a unique mechanism for generating the tremendous diversity of the TCR. This multi-subunit immune recognition receptor associates with the CD3 complex and binds peptides presented by the MHC class I and II proteins on the surface of antigen-presenting cells (APCs). Binding of a TCR to the antigenic peptide on the APC is a central event in T-cell activation, which begins at the point of contact between the T-cell and the APC.

[00113] "Affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as K_D. Affinity of a binding protein to a ligand such as affinity of an antibody for an epitope can be,

for example, from about 100 nanomolar (nM) to about 0.1 nM, from about 100 nM to about 1 picomolar (pM), or from about 100 nM to about 1 femtomolar (fM). The term "avidity" refers to the resistance of a complex of two or more agents to dissociation after dilution.

[00114] The term "epitope" refers to an antigenic determinant in a molecule such as an antigen, i.e., to a part in or fragment of the molecule that is recognized by the immune system. For example, an epitope is recognized by a T cell, in particular when presented in the context of MHC molecules. An epitope of a protein or a peptide, such as a tumor antigen, can comprise a continuous or discontinuous portion of the protein or the peptide, and can be between 5 and 100, 5 and 50, 8 and 30, or 10 and 25 amino acids in length. For example, the epitope may be 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 21, 22, 23, 24, or 25 amino acids in length.

[00115] "Specific" refers to a situation in which an antibody or TCR will not show any significant binding to molecules other than the antigen containing the epitope recognized by the antibody or TCR. The term is also applicable where for example, an antigen binding domain is specific for a particular epitope which is carried by a number of antigens, in which case the selected antibody, TCR, or antigen-binding fragment thereof carrying the antigen binding domain will be able to bind to the various antigens carrying the epitope. The terms "preferentially binds" or "specifically binds" mean that the antibodies, TCRs, or fragments thereof bind to an epitope with greater affinity than it binds unrelated amino acid sequences, and, if cross-reactive to other polypeptides containing the epitope, are not toxic at the levels at which they are formulated for administration to human use. In one aspect, such affinity is at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1000-fold greater than the affinity of the antibody, TCR, or fragment thereof for unrelated amino acid sequences.

[00116] The term "binding" refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions under physiological conditions, and includes interactions such as salt bridges and water bridges, as well as any other conventional means of binding.

[00117] The term "alphavirus" refers to the various species of alphaviruses such as Eastern Equine Encephalitis virus (EEE), Venezuelan Equine Encephalitis virus (VEE), Western Equine Encephalitis virus (WEE), Sindbis virus, South African Arbovirus No. 86, Semliki, Forest virus, and others (See Field's Virology, 4th Edition, Chapter 30: Alphaviruses pp. 917-962 by Griffin, **D**.E., Publisher: Lippincott Williams & Wilkins, New York (2001)).

[00118] The term "antigen-presenting cell" (APC), as used herein, refers to those cells that normally initiate the responses of naive and/or memory T cells to antigen.

[00119] "Complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick base paring or other non-traditional types. A percent complementarity can indicate the percentage of residues in a nucleic acid molecule which can form hydrogen

bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary, respectively). "Perfectly complementary" can mean that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. "Substantially complementary" refers to a degree of complementarity that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more nucleotides, or can refer to two nucleic acids that hybridize under stringent conditions. Sequence identity, such as for the purpose of assessing percent complementarity, may be measured by any suitable alignment algorithm, including but not limited to the Needleman-Wunsch algorithm (e.g., the EMBOSS Needle aligner available at www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html, optionally with default settings), the BLAST algorithm (see e.g., the BLAST alignment tool available at blast.ncbi.nlm.nih.gov/Blast.cgi, optionally with default settings), or the Smith-Waterman algorithm (see e.g., the EMBOSS Water aligner available at www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html, optionally with default settings). Optimal alignment may be assessed using any suitable parameters of a chosen algorithm, including default parameters.

[00120] The term "expression" relates to the production of RNA and/or peptides, polypeptides or proteins, e.g., by transcription and/or translation. Expression can comprise partial expression of nucleic acids. Expression can be transient or stable.

[00121] The term "expression control sequence" or "regulatory sequence" refers to promoters, ribosome-binding sequences and other control elements, which control the transcription of a nucleic acid or the translation of the derived RNA. Regulatory sequences can be controlled. The precise structure of regulatory sequences can vary depending on the species or depending on the cell type, but generally can comprise 5' untranscribed and 5' and 3' untranslated sequences, which are involved in the initiation of transcription or translation, such as TATA-box, capping-sequence, CAAT-sequence and the like. 5' untranscribed regulatory sequences can comprise a promoter region that can include a promoter sequence for transcriptional control of the functionally bound gene. Regulatory sequences can also comprise enhancer sequences or upstream activator sequences.

[00122] The term "haplotype" refers to the human leukocyte antigen (HLA) alleles found on one chromosome and the proteins encoded thereby. Haplotype may also refer to the allele present at any one locus within the MHC. Each class of MHC is represented by several loci: e.g., HLA-A (Human Leukocyte Antigen-A). HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-H, HLA-J, HLA-K, HLA-L, HLA-P and HLA-V for class I and HLA-DRA, HLA-DRB 1-9, HLA-DQA1, HLA-DQB 1, HLA-DPA1, HLA-DPB 1, HLA-DPB2, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB for class II. The terms "HLA allele" and "MHC allele" are used interchangeably herein.

[00123] "Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson Crick base pairing, Hoogstein binding, or in any other sequence specific manner

according to base complementarity. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of PCR, or the enzymatic cleavage of a polynucleotide by an endonuclease. A second sequence that is complementary to a first sequence can be referred to as the "complement" of the first sequence.

[00124] The term "target polynucleotide" refers to a nucleic acid molecule or polynucleotide in a starting population of nucleic acid molecules having a target sequence whose presence, amount, and/or nucleotide sequence, or changes in one or more of these, are desired to be determined. In general, a target sequence can refer to a nucleic acid sequence on a single strand of nucleic acid. The target sequence may be a portion of a gene, a regulatory sequence, genomic DNA, cDNA, RNA including mRNA, miRNA, rRNA, or others. The target sequence may be a target sequence from a sample or a secondary target such as a product of an amplification reaction.

[00125] The term "nonstructural proteins" (nsPs) refers to the polymerase function of a replicon. The nonstructural protein genes are used as part of the replicon RNA for autonomous replication.

[00126] The term "structural protein" refers to the encoded proteins which are required for replication of the RNA replicon, and can include the capsid protein, E1 glycoprotein, and E2 glycoprotein.

[00127] The terms "polynucleotide", "nucleotide sequence", "nucleic acid" and "oligonucleotide" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

[00128] The term "promoters" refers to nucleic acid sequences that control RNA polymerase and transcription factor binding, and can have a major effect on the efficiency of gene transcription, where a gene may be expressed in the cell, and/or what cell types a gene may be expressed in. Exemplary promoters include a cytomegalovirus (CMV) promoter, an elongation factor 1 alpha (EFla) promoter, a simian vacuolating virus (SV40) promoter, a phosphoglycerate kinase (PGK1) promoter, a ubiquitin C (Ubc) promoter, a human beta actin promoter, a CAG promoter, a Tetracycline response element (TRE) promoter, a UAS promoter, an Actin 5c (Ac5) promoter, a polyhedron promoter, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKIIa) promoter, a GAL1 promoter, a GAL 10 promoter, a TEF 1 promoter, a glyceraldehyde 3-phosphage dehydrogenase

(GDS) promoter, an ADH1 promoter, a CaMV35S promoter, an Ubi promoter, a human polymerase III RNA (HI) promoter, and a U6 promoter.

[00129] The term "RNA" relates to a molecule which comprises at least one ribonucleotide residue and can be entirely or substantially composed of ribonucleotide residues. RNA can comprise double-stranded RNA, single-stranded RNA, isolated RNA such as partially or completely purified RNA, essentially pure RNA, synthetic RNA, and recombinantly generated RNA such as modified RNA which differs from naturally occurring RNA by addition, deletion, substitution and/or alteration of one or more nucleotides. Such modifications can include addition of non-nucleotide material, such as to the end(s) of a RNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in RNA molecules can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These modified RNAs can be referred to as analogs or analogs of naturally-occurring RNA. A modification of RNA includes any modification of an RNA which is not naturally present in said RNA

[00130] "Ribonucleotide" relates to a nucleotide with a hydroxyl group at the 2'-position of a β-D-ribofuranosyl group. The term "mRNA" means "messenger-RNA" and refers to a "transcript" which is generated by using a DNA template and encodes a peptide or polypeptide. Typically, an mRNA can comprise a 5' UTR, a protein coding region, and a 3' UTR. mRNA may be generated, for example, by *in vitro* transcription from a DNA template.

[00131] The term "replicon RNA" refers to RNA which contains all of the genetic information required for directing its own amplification or self-replication within a permissive cell. To direct its own replication, the RNA molecule can encode polymerase, replicase, or other proteins which may interact with viral or host cell-derived proteins, nucleic acids or ribonucleoproteins to catalyze the RNA amplification process; and contain cis-acting RNA sequences required for replication and transcription of the subgenomic replicon-encoded RNA. These sequences may be bound during the process of replication to its self-encoded proteins, or non-self-encoded cell-derived proteins, nucleic acids or ribonucleoproteins, or complexes between any of these components.

[00132] The term "sequence" and its grammatical equivalents as used herein refers to a nucleotide sequence, which can be DNA or RNA; can be linear, circular or branched; and can be either single-stranded or double stranded. A sequence can be mutated. A sequence can be of any length, for example, between 2 and 1,000,000 or more nucleotides in length (or any integer value there between or there above), e.g., between about 100 and about 10,000 nucleotides or between about 200 and about 500 nucleotides.

[00133] The term "subgenomic RNA" refers to a RNA molecule of a length or size which is smaller than the genomic RNA from which it was derived. The subgenomic RNA can be transcribed from an internal promoter, whose sequences reside within the genomic RNA or its complement. Transcription of the subgenomic RNA may be mediated by viral-encoded polymerase(s) associated with host cell-encoded proteins, ribonucleoprotein(s), or a combination thereof.

[00134] The term "translation" relates to the production of peptides, polypeptides or proteins. Translation can refer to the process in the ribosomes of a cell by which a strand of messenger RNA directs the assembly of a sequence of amino acids to make a peptide, polypeptide or protein.

[00135] The term "transcription" relates to a process, wherein the genetic code in a DNA sequence is transcribed into RNA. Subsequently, the RNA may be translated into protein. Transcription can comprise *in vitro* transcription, wherein the term "/« *vitro* transcription" refers to a process wherein RNA, such as mRNA, is *in vitro* synthesized, such as in a cell-free system. Cloning vectors can be applied for the generation of transcripts. These cloning vectors are generally designated as transcription vectors and are encompassed by the term "vector".

[00136] The terms "major histocompatibility complex" and the abbreviation "MHC" includes MHC class I and MHC class II molecules and relates to a complex of genes which occurs in all vertebrates. MHC proteins or molecules can be important for signaling between lymphocytes and antigen presenting cells or diseased cells in immune reactions, wherein the MHC proteins or molecules bind peptides and present them for recognition by T cell receptors. The proteins encoded by the MHC can be expressed on the surface of cells, and display both self-antigens (peptide fragments from the cell itself) and non-self-antigens (e.g., fragments of invading microorganisms) to a T cell. The MHC region can be divided into three subgroups, class I, class II, and class III. MHC class I proteins can contain an a-chain and p2-microglobulin (not part of the MHC encoded by chromosome 15). They can present antigen fragments to cytotoxic T cells. MHC class II proteins can contain a- and β-chains and they can present antigen fragments to T-helper cells. MHC class III region can encode for other immune components, such as complement components and cytokines. The MHC can be both polygenic (there are several MHC class I and MHC class II genes) and polymorphic (there are multiple alleles of each gene).

[00137] The term "enriching" is used synonymously with "isolating" cells, means that the yield (fraction) of cells of one type is increased over the fraction of other types of cells as compared to the starting or initial cell population. In some cases, enriching refers to increasing the percentage by about 10%, by about 20%, by about 30%, by about 40%, by about 50% or greater than 50% of one type of cell in a population of cells as compared to the starting population of cells.

[00138] The term "peripheral blood lymphocytes" (PBL) and its grammatical equivalents as used herein can refer to lymphocytes that circulate in the blood (e.g., peripheral blood). Peripheral blood lymphocytes can refer to lymphocytes that are not localized to organs. Peripheral blood lymphocytes can comprise T cells, NK cells, B cell, or any combinations thereof.

[00139] The term "amino acid", as used herein, includes, without limitation, a-amino acids, natural amino acids, non-natural amino acids, and amino acid analogs. The term "a-amino acid" refers to a molecule containing both an amino group and a carboxyl group bound to a carbon which is designated the a-carbon. The term "β-amino acid" refers to a molecule containing both an amino group and a carboxyl group in a β configuration. "Naturally occurring amino acid" refers to any one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I,

L, K, M, F, P, S, T, W, Y and V. A table showing a summary of the properties of natural amino acids can be found, e.g., in U.S. Patent Application Publication No. 20130123169, which is herein incorporated by reference.

II. Overview

[00140] The cost and time of identifying and validating neoantigen epitopes has hindered development of efficacious immunogenic therapies, e.g, cancer vaccinations. Furthermore, neoantigens, such as cancer cell mutation variations, can be patient specific. The discovery of a mutant neoepitope has previously relied on laborious screening of a patient's tumor infiltrating lymphocytes for their ability to recognize an antigen from libraries constructed based on information from that patient's tumor exome sequence, or detection of mutant neoepitopes by mass spectrometry. However, public proteomic databases may not contain patient-specific mutations and may not allow for identification of mutant sequences. Although the use of predictive algorithms may have potential application in the identification of personalized neoantigen epitopes, the magnitude of predicted neoantigen epitopes can be very large and may not be conducive for high-throughput screening. Furthermore, due to evidence of the poor immunogenicity of many predicted epitopes, the utility of current methodologies can be questionable.

[00141] There is a need for methods to identify epitopes of neoantigen for use as an immunotherapy. There is also a need for methods to identify epitopes of neoantigen for for use in preparing immunotherapies. There is need for high-throughput methods to identify or for the selection of epitopes of neoantigens, such as for use as a personalized immunotherapy. The methods and compositions described herein can be provided to address these needs.

[00142] There is a need in the field of immunology to quickly 1) identify immunogenic antigens, and 2) pair immunogenic antigens with functional T cell receptor (TCR) sequences. The disclosure herein provides methods, compositions, and systems that can be used to meet these needs. In some embodiments, the methods described herein use autologous patient immune cells and next generation sequencing to simultaneously survey many patient-specific antigens for immunogenicity or ability to modulate T cell activity. In some embodiments, the methods described herein use an alphavirus-derived self-amplifying mRNA replicon system for expression of patient-specific antigens in primary human immune cells, and can also use primary patient immune cells to facilitate patient-specific neoantigen screening.

[00143] The present disclosure also provides non-limiting examples of applications where the methods and compositions described herein can be used. These examples include 1) functional ranking of *in silico*-predicted antigens to inform immunogenic antigen selection; 2) query both $CD4^+$ T cell responses and $CD8^+$ T cell responses to antigen specific responses to epitopes simultaneously; 3) link epitopes with cognate TCR sequences for development of T cell therapies; 4) rapid screening of low-frequency memory responses from patient peripheral blood to monitor responses to immunotherapy; 5) functional ranking of *in silico*-predicted antigens to inform immunogneoc antigen selection for any immunotherapy, including infectious disease immunotherapies.

III. Rapid Functional T cell Test (RaFT)

[00144] Described herein are methods, compositions, and systems useful for identifying neoantigen epitopes to which T cells respond (FIG. 2). For example, the methods, compositions, and systems described herein can be useful for identifying neoantigen epitopes to which T cells bind to form APC: T cell conjugates. For example, the methods, compositions, and systems described herein can be useful for identifying immunogenic neoantigens or noantigens that modulate T cell activity. Antigens can be presented by antigen presenting cells in functional assays. Functional assays can be performed using T cells that express subject specific TCRs. Neoantigens expressed in autologous tumor cells can be presented to T cells.

[00145] The fast and scalable methods and systems described herein can be used to interrogate T cells from a subject for reactivity to one or more candidate peptides, e.g., peptides from a subject with a disease or condition, viral antigens, tumor associated antigens and non-cancer disease-associated antigens. The methods, compositions, and systems described herein can be used to identify sequence features of antigens that are preferentially expressed by HLA class I and II molecules. The methods, compositions, and systems described herein can be used to identify epitopes that elicit immunostimulatory T cell responses. The methods, compositions, and systems described herein can be used to identify peptides that stimulate T cell activation. The methods, compositions, and systems described herein can be used to perform T cell receptor (TCR) sequencing for identifying TCRs and the antigen sequences to which they preferentially bind.

[00146] Generally, one or more candidate antigen sequences can be first determined, e.g., *in silico* (FIG. 1). Nucleotide sequences, such as DNA or RNA sequences encoding identified antigen sequences can then be synthesized. Neoantigen encoding sequences can then be assembled into a vector sequence, e.g., a self-amplifying mRNA vector, under control of a promoter, e.g., a replicon subgenomic promoter. RNA encoding neoantigen sequences can then be produced, e.g., by *in vitro* transcription. The RNA encoding neoantigen sequences can then be delivered, e.g., in a pooled fashion, to antigen presenting cells (APCs), e.g., primary dendritic cells (DCs). After the neoantigen sequences are processed by the APCs, the APCs presenting the processed neoantigen sequences can be contacted to T cells, e.g., autologous T cells. After exposure of the APCs to the T cells, APC:T cell conjugates can be isolated, e.g., by using T cell activation as a functional bait (FIG. 2). RNA, e.g., total polyadenylated mRNA, can then be isolated from one or more of a pool of the APC:T cell conjugates. Neoantigen-encoding replicon RNA can then be specifically synthesized, e.g., by *in vitro* transcription using constant sequence features on replicon molecules. The method can enrich for RNA sequences from APCs to which T cells specifically respond. Enriched RNA sequences can be sequenced to identify these RNA sequences from APCs to which the T cells specifically respond. To continue to narrow the immunogenic neoantigens by selective RNA enrichment, RNA sequences isolated from APC:T cell conjugates can be tested in a subsequent round of the RaFT method by re-introducing the RNA sequences to a new culture of matched APCs. In some embodiments, the RaFT method can be performed for one round. In some embodiments, the RaFT method can be performed for more than one round. In some embodiments, mRNA encoding a neoantigen sequence can be a linear mRNA. In some embodiments, the linear mRNA encoding a neoantigen sequence is *in vitro* transcribed. In some embodiments, the linear mRNA encoding a neoantigen sequence can be delivered into a APC.

IV. Identification of Neoantigens

[00147] The present invention relates to methods for identifying immunogenic neoantigens, such as neoantigens that activate T cells. Candidate immunogenic neoantigen sequences can be identified by any suitable method known in the art. The methods of the invention can be useful, for example, to produce therapies specific to a subject's disease or to produce vaccines to a disease. Candidate immunogenic neoantigens can be neoantigens previously identified. In some embodiments, candidate immunogenic neoantigens may not be previously identified. Candidate immunogenic neoantigens for use in the methods and compositions described herein can be specific to a subject. In some embodiments, candidate neoantigens for use in the methods and compositions described herein can be specific to a plurality of subjects.

[00148] In both animals and humans, mutated epitopes can be potentially effective in inducing an immune response or activating T cells. In one embodiment, the potentially immunogenic epitopes of an infectious agent in a subject, such as a virus, can be determined. In one embodiment, the potentially immunogenic mutated epitopes of a subject with a disease, such as cancer, can be determined. In some embodiments, a potentially immunogenic neoantigen for use in the methods described herein can be a differentiation antigen expressed in a tumor and cells of the type of tissue from which they are generated. In some embodiments, a potentially immunogenic neoantigen for use in the methods described herein can be a cancer/germ line antigens not expressed in another differentiated tissue. In some embodiments, a potentially immunogenic neoantigen for use in the methods described herein can be a mutated antigen. For example, a candidate immunogenic neoantigen for use in the methods described herein can comprise a missense-point mutation or a neoantigen of a fusion protein generated through tumor specific translocation of a gene segment. In some embodiments, a potentially immunogenic neoantigen for use in the methods described herein can be an over-expressed antigen. In some embodiments, a potentially immunogenic neoantigen can be found in tumors. For example, a potentially immunogenic neoantigen for use in the methods described herein can include a protein whose expression is strictly regulated in cells of differentiated normal tissue.

[00149] Potentially immunogenic mutated epitopes can be determined by genomic or exomic sequencing of tumor tissue and healthy tissue from a cancer patient using next generation sequencing technologies. For example, genes selected based on their mutation frequency and ability to act as a neoantigen can be sequenced using next generation sequencing technology. In one embodiment, sequencing data can be analyzed to identify potentially immunogenic mutated peptides that can bind to HLA molecules of the subject. In one embodiment, the data can be analyzed using a computer. In another embodiment the sequence data can be analyzed for the presence of neoantigens. In one embodiment, potentially immunogenic neoantigens can be determined by their affinity to MHC molecules.

[00150] Potentially immunogenic neoantigens can be determined by direct protein sequencing. For example, protein sequencing of enzymatic protein digests using multidimensional mass spectrometry techniques (e.g., tandem mass spectrometry (MS/MS)) can be used to identify potentially immunogenic neoantigens for use in the methods described herein.

[00151] High-throughput methods for *de novo* sequencing of unknown proteins may be used to identify potentially immunogenic neoantigens. For example, high-throughput methods for *de novo* sequencing of unknown proteins, such as meta-shotgun protein sequencing, may be used to analyze the proteome of a subject's tumor to identify potentially immunogenic expressed neoantigens.

[00152] Potentially immunogenic neoantigens may also be identified using MHC multimers to identify neoantigen-specific T cell responses. For example, high-throughput analysis of neoantigen-specific T cell responses in patient samples may be performed using MHC tetramer-based screening techniques. Tetramer-based screening techniques may be used for the initial identification of potentially immunogenic tumor specific neoantigens, or alternatively as a secondary screening protocol to assess what potentially immunogenic neoantigens a patient may have already been exposed to, thereby facilitating the selection of potentially immunogenic neoantigens for use in the methods described herein.

[00153] Potentially immunogenic neoantigens for use in the methods described herein can be known neoantigen sequences. For example, potentially immunogenic neoantigens for use in the methods described herein can be from a database of neoantigen sequences.

[00154] In aspects, the invention provides peptides or polynucloetides encoding peptides identified using the methods described herein (e.g., a peptide with a tumor specific mutation, a viral peptide, or peptide associated with a non-cancerous disease).

[00155] In some embodiments, mass spectrometry is not used to select or identify immunogenic antigens.

[00156] In some embodiments, an optical method is used to select or identify immunogenic antigens. In some embodiments, a barcoded probe is used to select or identify immunogenic antigens. In some embodiments, a barcoded probe comprising a target specific region and a barcoded region is used to select or identify immunogenic antigens. In some embodiments the target specific region comprises a nucleic acid sequence that hybridizes to or has at least about 90%, 95% or 100% sequence complementarity to a nucleic acid sequence of a target polynucleotide.

[00157] In some embodiments, a sequencing method is used to identify immunogenic antigens. Any suitable sequencing method can be used according to the invention, for example, Next Generation Sequencing (NGS) technologies. Third Generation Sequencing methods might substitute for the NGS technology in the future to speed up the sequencing step of the method. For clarification purposes: the terms "Next Generation Sequencing" or "NGS" in the context of the present invention mean all high throughput sequencing technologies which, in contrast to the "conventional" sequencing methodology known as Sanger chemistry, read nucleic acid templates randomly in parallel along the entire genome by breaking the entire genome into small pieces. Such NGS technologies (also known as massively parallel sequencing technologies) are able to deliver nucleic acid sequence information of a whole genome, exome, transcriptome (all transcribed sequences of a genome) or methylome (all methylated sequences of a genome) in very short time periods, e.g. within 1-2 weeks, for example, within **1-7** days or within less than 24 hours and allow, in principle, single cell sequencing approaches. Multiple NGS platforms which are commercially available or which are mentioned in the literature can be used in the context of the invention e.g. those described in detail in WO 2012/159643.

[00158] In certain embodiments, an antigen or epitope thereof can comprise, but is not limited to, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120 or greater amino acid residues, and any range derivable therein. In specific embodiments, an immunogenic antigen or epitope thereof is equal to or less than 100 amino acids.

[00159] In some embodiments, an antigen or epitope thereof for MHC Class I is 13 residues or less in length and usually consists of between about 8 and about 11 residues, particularly 9 or 10 residues. In some embodiments, an immunogenic antigen or epitope thereof for MHC Class II is 9-24 residues in length.

[00160] A longer immunogenic peptide can be designed in several ways. In some embodiments, when HLA-binding peptides are predicted or known, a longer immunogenic peptide could consist of (1) individual binding peptides with extensions of 2-5 amino acids toward the N- and C-terminus of each corresponding gene product; or (2) a concatenation of some or all of the binding peptides with extended sequences for each. In other embodiments, when sequencing reveals a long (> 10 residues) epitope sequence, e.g., a neoepitope present in a tumor (e.g. due to a frameshift, read-through or intron inclusion that leads to a novel peptide sequence), a longer neoantigenic peptide could consist of the entire stretch of novel tumor-specific amino acids as either a single longer peptide or several overlapping longer peptides. In some embodiments, use of a longer peptide is presumed to allow for endogenous processing by patient cells and can lead to more effective antigen presentation and induction of T cell responses. In some embodiments, two or more peptides can be used, where the peptides overlap and are tiled over the long neoantigenic peptide.

[00161] In some embodiments, neoantigens bind an HLA protein (e.g., HLA class I or HLA class II). In specific embodiments neoantigens bind an HLA protein with greater affinity than a corresponding wild-type peptide. In specific embodiments the neoantigenic peptide or polypeptide has an IC_{50} of at least less than 5000 nM, at least less than 500 nM, at least less than 100 nM, at least less than 50 nM or less.

[00162] In some embodiments, the neoantigenic peptides can be from about 8 and about 50 amino acid residues in length, or from about 8 and about 30, from about 8 and about 20, from about 8 and about 18, from about 8 and about 15, or from about 8 and about 12 amino acid residues in length. In some embodiments, the neoantigenic peptides can be from about 8 and about 500 amino acid residues in length, or from about 8 and about 450, from about 8 and about 400, from about 8 and about 350, from about 8 and about 300, from about 8 and about 250, from about 8 and about 200, from about 8 and about 150, from about 8 and about 100, from about 8 and about 50, or from about 8 and about 30 amino acid residues in length.

[00163] In some embodiments, the neoantigenic peptides can be at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more amino acid residues in length. In some embodiments, the neoantigenic peptides can be at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36,

37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500 or more amino acid residues in length. In some embodiments, the neoantigenic peptides can be at most 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or less amino acid residues in length. In some embodiments, the neoantigenic peptides can be at most 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, or less amino acid residues in length.

[00164] In some embodiments, the neoantigenic peptides has a total length of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, or at least 500 amino acids.

[00165] In some embodiments, the neoantigenic peptides has a total length of at most 8, at most 9, at most 10, at most 11, at most 12, at most 13, at most 14, at most 15, at most 16, at most 17, at most 18, at most 19, at most 20, at most 21, at most 22, at most 23, at most 24, at most 25, at most 26, at most 27, at most 28, at most 29, at most 30, at most 40, at most 50, at most 60, at most 70, at most 80, at most 90, at most 100, at most 150, at most 200, at most 250, at most 300, at most 350, at most 400, at most 450, or at most 500 amino acids.

[00166] In some embodiments, the neoantigenic peptides can have a pi value of about 0.5 and about 12, about 2 and about 10, or about 4 and about 8. In some embodiments, the neoantigenic peptides can have a pi value of at least 4.5, 5, 5.5, 6, 6.5, 7, 7.5, or more. In some embodiments, the neoantigenic peptides can have a pi value of at most 4.5, 5, 5.5, 6, 6.5, 7, 7.5, or less.

[00167] In some embodiments, the neoantigenic peptides can have an HLA binding affinity of between about lpM and about ImM, about IOOρM and about 500μM, about 500pM and about 10μM, about InM and about IμM, or about IOηM and about IμMIη some embodiments, the neoantigenic peptides can have an HLA binding affinity of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 700, 800, 900 μM, or more. In some embodiments, the neoantigenic peptides can have an HLA binding affinity of at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 700, 800, 900 μM.

[00168] In some embodiments, a neoantigenic peptide described herein can comprise carriers such as those well known in the art, e.g., thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acid residues such as poly L-lysine, poly L-glutamic acid, influenza virus proteins, hepatitis B virus core protein, and the like.

[00169] In some embodiments, a neoantigenic peptide described herein can be modified by terminal-NH2 acylation, e.g., by alkanoyl **(C_1-C_{20})** or thioglycolyl acetylation, terminal-carboxyl amidation, e.g., ammonia, methylamine, etc. In some embodiments these modifications can provide sites for linking to a support or other molecule.

[00170] In some embodiments, a neoantigenic peptide described herein can contain modifications such as but not limited to glycosylation, side chain oxidation, biotinylation, phosphorylation, addition of a surface active material, e.g. a lipid, or can be chemically modified, e.g., acetylation, etc. Moreover, bonds in the peptide can be other than peptide bonds, e.g., covalent bonds, ester or ether bonds, disulfide bonds, hydrogen bonds, ionic bonds, etc.

[00171] In some embodiments, a neoantigenic peptide described herein can contain substitutions to modify a physical property (e.g., stability or solubility) of the resulting peptide. For example, neoantigenic peptides can be modified by the substitution of a cysteine (C) with a-amino butyric acid ("B"). Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting a-amino butyric acid for C not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances. Substitution of cysteine with a-amino butyric acid can occur at any residue of aneoantigenic peptide, e.g., at either anchor or non-anchor positions of an epitope or analog within a peptide, or at other positions of a peptide.

[00172] In some embodiments, a neoantigenic peptide described herein can comprise amino acid mimetics or unnatural amino acid residues, e.g. D- or L-naphylalanine; D- or L-phenylglycine; D- or L-2-thieneylalanine; D- or L-l, -2, 3-, or 4-pyreneylalanine; D- or L-3 thieneylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine; D-(trifluoromethyl)-phenylglycine; D-(trifluoro-methyl)-phenylalanine; D-.rho.-fluorophenylalanine; D- or L-.rho.-biphenyl-phenylalanine; D- or L-.rho.-methoxybiphenylphenylalanine; D- or L-2-indole(allyl)alanines; and, D- or L-alkylalanines, where the alkyl group can be a substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or a non-acidic amino acid residues. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings. Modified peptides that have various amino acid mimetics or unnatural amino acid residues are particularly useful, as they tend to manifest increased stability in vivo. Such peptides can also possess improved shelf-life or manufacturing properties.

[00173] Peptide stability can be assayed in a number of ways. For instance, peptidases and various biological media, such as human plasma and serum, have been used to test stability. See, e.g., Verhoef, et al., Eur. J. Drug Metab. Pharmacokinetics 11:291 (1986). Half-life of the peptides described herein is conveniently determined using a 25% human serum (v/v) assay. The protocol is as follows: pooled human serum (Type AB, non-heat inactivated) is dilapidated by centrifugation before use. The serum is then diluted to 25% with RPMI-1640 or another suitable tissue culture medium. At predetermined time intervals, a small amount of reaction solution is removed and added to either 6% aqueous trichloroacetic acid (TCA) or ethanol. The cloudy reaction sample is cooled (4 °C) for 15 minutes and then spun to pellet the precipitated serum proteins. The presence of the peptides is then determined by reversed-phase HPLC using stability-specific chromatography conditions.

[00174] In some embodiments, a neoantigenic peptide described herein can be in solution, lyophylized, or can be in crystal form.

[00175] In some embodiments, a neoantigenic peptide described herein can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or can be isolated from natural sources such as native tumors or pathogenic organisms. Epitopes can be synthesized individually or joined directly or indirectly in a peptide. Although a neoantigenic peptide described herein will be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptide can be synthetically conjugated to be joined to native fragments or particles.

[00176] In some embodiments, a neoantigenic peptide described herein can be prepared in a wide variety of ways. In some embodiments, the peptides can be synthesized in solution or on a solid support according to conventional techniques. Various automatic synthesizers are commercially available and can be used according to known protocols. Further, individual peptides can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

[00177] Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes a peptide inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art. Thus, recombinant peptides, which comprise or consist of one or more epitopes described herein, can be used to present the appropriate T cell epitope.

[00178] In one aspect, the invention described herein also provides compositions comprising one, at least two, or more than two neoantigenic peptides. In some embodiments a composition described herein contains at least two distinct peptides. In some embodiments, the at least two distinct peptides are derived from the same polypeptide. By distinct polypeptides is meant that the peptide vary by length, amino acid sequence or both. The peptides are derived from any polypeptide known to or have been found to contain a tumor specific mutation.

[00179] In some embodiments, the isolated neoantigenic peptide is encoded by a gene with a point mutation resulting in an amino acid substitution of the native peptide.

V. Target RNA and Amplifier RNA

[00180] In line with an *in vitro* T cell functional assay described herein, the methods can employ a self-amplifying mRNA-based antigen system to deliver candidate immunogenic neoantigens to APCs. For example, the methods described herein can utilize an adaptable mRNA-based gene assembly system to synthesize and insert a candidate immunogenic neoantigen sequence into a self-replicating mRNA replicon. The methods described herein can utilize an adaptable mRNA-based gene assembly system to synthesize and insert two or more candidate immunogenic neoantigen sequences into a self-replicating mRNA replicon. For example, an adaptable mRNA-based gene assembly system can be synthesized containing 2, 3, 4, 5, 6, **7,** 8, 9, 10, 15, 20, 25, 30, 40, 50, 100, or more candidate immunogenic neoantigen sequences into a self-replicating mRNA replicon.

[00181] The methods described herein can utilize a self-amplifying mRNA-based antigen delivery system to deliver one or more individual candidate immunogenic neoantigen sequences into APCs. Such a self-amplifying mRNA-based antigen delivery system can be useful, for example, to deliver candidate

immunogenic neoantigen sequences into primary patient APCs quickly. A self-amplifying mRNA-based candidate immunogenic antigen delivery system can comprise a first vector and a second vector **(FIG. 3).** The first vector can comprise an amplifier vector. The amplifier vector can be used to produce amplifier RNA. The amplifier vector can be used to produce amplifier mRNA. For example, the amplifier vector can be used to produce amplifier mRNA that can be delivered into APCs. The second replicon vector can comprise a target vector **(FIG. 4A** and **FIG. 4B).** The target replicon vector can be used to produce target RNA comprising sequences encoding neoantigen epitopes. The target replicon vector can be used to produce target mRNA comprising sequences encoding candidate immunogenic neoantigen epitopes. For example, the target vector can be used to produce target mRNA that can be delivered into APCs where candidate immunogenic neoantigen epitopes can be translated and processed by the APCs. For example, the target vector can be used to produce target mRNA that can be delivered into APCs where candidate immunogenic neoantigen epitopes can be processed by the APCs and presented by the APCs.

[00182] The target mRNA encoding candidate neoantigen sequences can also be delivered into the APCs without the amplifer mRNA. In some embodiments, the target mRNA can be a linear mRNA. In some emboidments, the target mRNA can be *in vitro* transcribed. The target mRNA can be delivered into the APCs by a variety of means including microinjection, electroporation, and lipid-mediated transfection.

[00183] In some embodiments, a sequence encoding an amplifier RNA can be derived from a viral genome. For example, a sequence encoding an amplifier RNA can be from an alphavirus genome. For example, a sequence encoding an amplifier RNA can be from a positive-stranded alphavirus genome (e.g., Sindbis, Semliki Forest, and Venezuelan equine encephalitis viruses). For example, in Venezuelan Equine Encephalitis virus (VEE), the polymerase function is provided by nsPl, nsP2, nsP3 and nsP4 proteins translated as a single polyprotein.

[00184] Alphaviruses comprise a set of genetically, structurally, and serologically related arthropod-borne viruses of the Togaviridae family. Generally, an alphavirus genome is single-stranded RNA of positive polarity of almost 12-kb in length with the 5' two thirds of the genome encoding a number of nonstructural proteins (nsPl-4). These proteins are translated directly from the RNA. Alphavirus replicons are self-replicating RNAs that can be useful for directing the expression of heterologous gene products. Upon delivery into cells, they can be utilized by cellular translational machinery as usual cellular mRNAs, and viral nonstructural proteins can be translated to form replicative complexes. Together with cellular proteins, these nonstructural proteins form the RNA-dependent RNA polymerase for viral genome replication and transcription of subgenomic RNA. Replicon RNAs can be used as templates for the synthesis of full-length, minus-strand intermediates. These minus-strand intermediates can serve as templates for production of large quantities of positive-strand genomic and subgenomic RNAs. Subgenomic RNA can serves as a template for translation of all the structural proteins required for forming viral particles. Replicons can mimic the structure of cellular mRNAs in that they contain cap structure at the 5' end and poly(A)-tail at the 3' end.

[00185] A vector encoding an amplifier RNA can be derived from a viral vector comprising a viral genomic 5' UTR sequence, a viral sequence encoding one or more nsPs (e.g., nsPl-4), a viral genomic 3'UTR sequence, a

viral subgenomic 5'UTR sequence, a viral subgenomic promoter, a viral subgenomic 3'UTR sequence, and combinations thereof.

[00186] A vector encoding an amplifier RNA can comprise a viral genomic 5' UTR sequence. A vector encoding an amplifier RNA can comprise a viral sequence encoding one or more nsPs (e.g., nsPl-4). A vector encoding an amplifier RNA can comprise a viral genomic 3'UTR sequence. In some embodiments, a vector encoding an amplifier RNA can comprise a viral subgenomic promoter. In some embodiments, a vector encoding an amplifier RNA may not comprise a viral subgenomic promoter. A vector encoding an amplifier RNA can comprise a viral genomic 5' UTR sequence, viral sequence encoding one or more nsPs (e.g., nsPl-4), a viral subgenomic promoter, a viral genomic 3'UTR sequence, and combinations thereof.

[00187] A vector encoding an amplifier RNA can comprise a viral genomic promoter or subgenomic promoter and a vector encoding a target RNA can comprise the viral subgenomic promoter. For example, an amplifier mRNA replicon can replicate itself and have activity on target mRNA sequences that contains a recognizable subgenomic promoter sequence.

[00188] A vector encoding a target RNA can comprise a sequence encoding a neoantigen. A vector encoding a target RNA can comprise a viral subgenomic 5' UTR sequence. A vector encoding a target RNA can comprise a viral subgenomic promoter. A vector encoding a target RNA can comprise a viral subgenomic 3'UTR sequence. A vector encoding a target RNA can comprise a viral subgenomic 5' UTR sequence, viral subgenomic promoter, a viral subgenomic 3'UTR sequence, and combinations thereof.

[00189] In some cases, a target RNA vector may comprise a barcode or a barcode sequence. A barcode or barcode sequence relates to a natural or synthetic nucleic acid sequence comprised by a polynucleotide allowing for unambiguous identification of the polynucleotide and other sequences comprised by the polynucleotide having the same barcode sequence. The barcode can be any suitable length, such as 2 to 100 nucleotides in length. The barcode can have random sequences or pre-determined sequences. For example, a nucleic acid comprising a barcode can allow for identification of the encoded neoantigen. A barcode sequence can comprise a sequence of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 45, or 50 or more consecutive nucleotides. A target RNA vector can comprise two or more barcode sequences or compliments thereof. A barcode sequence can comprise a randomly assembled sequence of nucleotides. A barcode sequence can be a degenerate sequence. A barcode sequence can be a known sequence. A barcode sequence can be a predefined sequence.

[00190] In some embodiments, a barcode is a vessel barcode. A vessel barcode can comprise information that is unique to polynucleotides from a single cell or from a single vessel, compared to polynucleotides from a different single cell or from a different single vessel.

[00191] In some embodiments, a target RNA vector can comprise a barcode sequence that is 3' to the sequence encoding the neoantigen. In some embodiments, a target RNA vector can comprise a barcode sequence that is 5' to the sequence encoding the neoantigen. In some embodiments, a target RNA vector encoding a first neoantigen can comprise a first barcode sequence. In some embodiments, a target RNA vector encoding a second neoantigen can comprise a second barcode sequence that is different than the first barcode

sequence. In some embodiments, a target RNA vector encoding a second neoantigen can comprise a second barcode sequence that is the same as the first barcode sequence.

[00192] In some embodiments, a target RNA vector encoding a first neoantigen can comprise a first barcode sequence and each target RNA vector of a plurality of target RNA vectors that encodes a neoantigen sequence that is different from the first neoantigen sequence can comprise a barcode sequence that is different from the first barcode sequence. In some embodiments, a target RNA vector encoding a first neoantigen can comprise a first barcode sequence and each target RNA vector of a plurality of target RNA vectors that encodes a neoantigen that is different from the first neoantigen sequence can comprise a barcode sequence that is the same as the first barcode sequence.

[00193] In some embodiments, a first target RNA vector encoding a first neoantigen can comprise a first barcode sequence and a second target RNA vector encoding the first neoantigen sequence can comprise a second barcode sequence that is different from the first barcode sequence. In some embodiments, a target RNA vector encoding a first neoantigen can comprise a first barcode sequence and each target RNA vector of a plurality of target RNA vectors that encodes the first neoantigen sequence can comprise a barcode sequence that is different from the first barcode sequence.

[00194] In some cases, the methods and compositions for gene assembly may involve a combination of specifically synthesized building blocks and presynthesized building blocks. Libraries of presynthesized oligonucleotides may be stored and assembly processes for desired target nucleic acids may be optimized for maximum use of presynthesized oligonucleotides, minimizing the need for new synthesis. Specifically synthesized oligonucleotides may fill in parts of a target nucleic acid, for which there is no coverage in libraries of presynthesized oligonucleotides.

[00195] For example, after identification of a neoantigen sequence, the neoantigen sequence can be cloned into a target RNA vector. Neoantigen sequences can then be combined and assembled into a target polynucleotide, such as a target RNA vector. For example, neoantigen sequences can then be combined and assembled into a target RNA vector in a modular fashion. For example, neoantigen sequences can then be combined and assembled into a target RNA vector in a single cloning step. For example, neoantigen sequences can then be combined and assembled into a target RNA vector in the presence of a type IIS restriction endonuclease, T4 ligase, and ATP.

[00196] For example, the Golden-gate method (see, e.g., Engler et al. (2008) PLoS ONE, 3(1 1): e3647; Engler et al. (2009) PLoS ONE 4(5): e5553) can be used. The Golden-gate method can use Type lis endonucleases, whose recognition sites are distal from their cutting sites. Several different Type lis endonucleases are available, e.g., Bsal. The Golden-gate method can be advantageous by the use of a single Type lis endonuclease. The Golden-gate method is further described in U.S. Patent Pub. 2012/0258487, which is incorporated herein by reference in its entirety.

[00197] In some cases, a nucleic acid may readily bind to another nucleic acid (e.g., the nucleic acid comprises a sticky end or nucleotide overhang). For example, the nucleic acid may comprise an overhang at a first end of the nucleic acid. Generally, a sticky end or overhang may refer to a series of unpaired nucleotides at the end of

a nucleic acid. In some cases, the nucleic acid may comprise a single stranded overhang at one or more ends of the nucleic acid. In some cases, the overhang can occur on the 3' end of the nucleic acid. In some cases, the overhang can occur on the 5' end of the nucleic acid. The overhang can comprise any number of nucleotides. For example, the overhang can comprise 1 nucleotide, 2 nucleotides, 3 nucleotides, 4 nucleotides, or 5 or more nucleotides. In some cases, the nucleic acid may require modification prior to binding to another nucleic acid (e.g., the nucleic acid may need to be digested with an endonuclease). In some cases, modification of the nucleic acid may generate a nucleotide overhang, and the overhang can comprise any number of nucleotides. For example, the overhang can comprise 1 nucleotide, 2 nucleotides, 3 nucleotides, 4 nucleotides, or 5 or more nucleotides. In one example, the nucleic acid may comprise a restriction site, wherein digesting the nucleic acid at the restriction site with a restriction enzyme (e.g., NotI) can produce a 4 nucleotide overhang. In some cases, the modification can comprise generating a blunt end at one or more ends of the nucleic acid. Generally, a blunt end may refer to a double stranded nucleic acid wherein both strands terminate in a base pair. In one example, the nucleic acid may comprise a restriction site, wherein digesting the nucleic acid at the restriction site with a restriction enzyme (e.g., BsaI) can produce a blunt end.

[00198] Single stranded or double stranded oligonucleotides encoding a neoantigen can be synthesized and cloned into a target RNA vector. Where single stranded oligonucleotides are used, more than one oligonucleotide for some neoantigen sequences may be necessary because the upper limit of reliable ssDNA oligonucleotides synthesis length is ~140 bases and some neoantigen sequences, such as for neoORFs, are much longer than just 30 amino acids. Thus, the methods described herein contemplate a modular cloning system for generating target RNA vectors. For example, identified neoantigen sequences can be synthesized in DNA form and assembled in either arrayed or pooled fashion into flanking 5' and 3' sequences in a target RNA vector construct. For example, a modular cloning system using Type IIS restriction endonucleases (e.g., Golden Gate cloning) can be used to assemble neoantigen sequences for cloning into a target RNA vector. In some embodiments, neoantigen sequences (e.g., encoded by ssDNA oligonucleotides) can comprise TypeIIS restriction endonuclease sites. In some embodiments, neoantigen sequences can comprise acceptor sequence-compatible 5' and 3' end sequences (4 bp).

[00199] In some embodiments, the nucleic acid may comprise one or more restriction sites. A restriction site may generally refer to a specific peptide or nucleotide sequences at which site-specific molecules (e.g., proteases, endonucleases, or enzymes) may cut the nucleic acid. In one example, a nucleic acid may comprise one or more restriction sites, wherein cleaving the nucleic acid at the restriction site fragments the nucleic acid. In some embodiments, the nucleic acid may comprise at least one endonuclease recognition site. In some embodiments, the endonuclease recognition site may comprise a Type I endonuclease recognition site, a Type II endonuclease recognition site, a Type III endonuclease recognition site, a Type IV endonuclease recognition site, or a Type V endonuclease recognition site.

[00200] Non-limiting examples of endonuclease recognition sites can include an AatII recognition site, an Acc65I recognition site, an AccI recognition site, an AclI recognition site, an AatII recognition site, an Acc65I recognition site, an AccI recognition site, an AclI recognition site, an AfeI recognition site, an AflII

recognition site, an AgeI recognition site, an ApaI recognition site, an ApaLI recognition site, an ApoI recognition site, an AscI recognition site, an AseI recognition site, an AsiSI recognition site, an AvrII recognition site, a BamHI recognition site, a BclI recognition site, a BglII recognition site, a Bme 15801 recognition site, a BmtI recognition site, a BsaI recognition site, a BsaHI recognition site, a BsiEI recognition site, a BsiWI recognition site, a BspEI recognition site, a BspHI recognition site, a BsrGI recognition site, a BssHII recognition site, a BstBI recognition site, a BstZ17I recognition site, a BtgI recognition site, a ClaI recognition site, a DraI recognition site, an EaeI recognition site, an EagI recognition site, an EcoRI recognition site, an EcoRV recognition site, an FseI recognition site, an FsplI recognition site, an HaeII recognition site, an HinclI recognition site, a HindIII recognition site, an HpaI recognition site, a KasI recognition site, a KpnI recognition site, an MfeI recognition site, an MluI recognition site, an MscI recognition site, an MspAlI recognition site, an MfeI recognition site, an MluI recognition site, an MscI recognition site, an MspAlI recognition site, an NadI recognition site, a NarI recognition site, an NcoI recognition site, an NdeI recognition site, an NgoMIV recognition site, an NheI recognition site, a NotI recognition site, an NruI recognition site, an NsiI recognition site, an NsplI recognition site, a PadI recognition site, a PciI recognition site, a PmeI recognition site, a PmlI recognition site, a PsiI recognition site, a PspOMI recognition site, a PstI recognition site, a PvuI recognition site, a PvuII recognition site, a SadI recognition site, a SacII recognition site, a SalI recognition site, an SbfI recognition site, an SeaI recognition site, an SfcI recognition site, an SfoI recognition site, an SgrAI recognition site, an SmaI recognition site, an SmlI recognition site, an SnaBI recognition site, an SpeI recognition site, an SphI recognition site, an SsplI recognition site, an StuI recognition site, an SwaI recognition site, an XbaI recognition site, an XhoI recognition site, and an XmaI recognition site. In a particular example, the restriction site may comprise NotI endonuclease recognition site.

[00201] Non-limiting examples of TypellS endonuclease recognition sites can include a MnlI recognition site, an AlwI recognition site, a BbvI recognition site, a BedI recognition site, a BceAI recognition site, a BcoDI recognition site, a BsmAI recognition site, a BsmFI recognition site, a BspCNI recognition site, a BsrI recognition site, a BtsCI recognition site, a BtsI recognition site, a MutI recognition site, a FauI recognition site, a FokI recognition site, a HgaI recognition site, a HphI recognition site, a HpyAV recognition site, a MboII recognition site, a MlyI recognition site, a PleI recognition site, a SfaNI recognition site, an AcuI recognition site, a BbsI recognition site, a BcgI recognition site, a BciVI recognition site, a BfuAI recognition site, a BmrI recognition site, a BpmI recognition site, a BpuEI recognition site, a BsaI recognition site, a BsaI-HF recognition site, a BsaXI recognition site, a BseRI recognition site, a BsgI recognition site, a BsmBI recognition site, a BsmI recognition site, a BspMI recognition site, a BsrDI recognition site, a BtgZI recognition site, a BtsI recognition site, a EarI recognition site, an EciI recognition site, a MmeI recognition site, a NmeAIII recognition site, a BadI recognition site, a BspQI recognition site, a CspCI recognition site, and a SapI recognition site.

[00202] A method of generating a target RNA vector can comprise generating a repository of one or more 5' target vector element sequences (e.g., sequences 5' to a target RNA sequence in the assembled target RNA

vector) and comprising a subgenomic promoter. The subgenomic promoter can comprise a donor compatible 3' end sequence. A method of generating a target RNA vector can comprise generating a repository of one or more 3' target vector element sequences (e.g., sequences 3' to a target RNA sequence in the assembled target RNA vector) comprising a 3' UTR sequence. The 3' UTR sequence can comprise a donor compatible 3' end sequence.

[00203] A method of generating a target RNA vector can comprise obtaining two or more modular neoantigen sequences. A modular neoantigen sequence can comprise dsDNA oligonucleotides. A modular neoantigen sequence can comprise ssDNA oligonucleotides. A method of generating a target RNA vector can comprise amplifying a ssDNA oligonucleotide comprising a modular neoantigen sequence and obtaining dsDNA products. A method of generating a target RNA vector can comprise obtaining a dsDNA oligonucleotide comprising a modular neoantigen sequence.

[00204] A modular neoantigen sequence can comprise a length of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 or more bases. A modular neoantigen sequence can comprise a restriction endonuclease site. A modular neoantigen sequence can comprise 5' and 3' restriction endonuclease sites. A modular neoantigen sequence can comprise TypellS restriction endonuclease sites. A modular neoantigen sequence can comprise a 5' TypellS restriction endonuclease site. A modular neoantigen sequence can comprise a 3' TypellS restriction endonuclease site. A modular neoantigen sequence can comprise 3' and 5' TypellS restriction endonuclease sites. A modular neoantigen sequence can comprise an acceptor sequence-compatible 5' end sequence. A modular neoantigen sequence can comprise an acceptor sequence-compatible 3' end sequence. A modular neoantigen sequence can comprise acceptor sequence-compatible 5' end and 3' end sequences.

[00205] A method of generating a target RNA vector can comprise contacting a modular neoantigen sequence to a 5' target vector element sequence. A method of generating a target RNA vector can comprise contacting a modular neoantigen sequence to a 3' target vector element sequence. A method of generating a target RNA vector can comprise contacting a modular neoantigen sequence to a 5' target vector element sequence and a 3' target vector element sequence.

[00206] A method of generating a target RNA vector can comprise contacting two or more modular neoantigen sequences to each other. A method of generating a target RNA vector can comprise contacting two or more modular neoantigen sequences to a 5' target vector element sequence. A method of generating a target RNA vector can comprise contacting two or more modular neoantigen sequences to a 3' target vector element sequence. A method of generating a target RNA vector can comprise contacting two or more modular neoantigen sequences to a 5' target vector element sequence and a 3' target vector element sequence.

[00207] 3' target vector element sequences and/or 5' target vector element sequences can be combined with a modular neoantigen sequence at a ratio of about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, or about 1:4. 3' target vector element sequences and/or 5' target vector element sequences can be combined with two or more modular neoantigen sequences at a ratio of about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, or about 1:4.

[00208] Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) Meth. Enzymol. 218, Part I; Wu (ed.) (1979) Meth. Enzymol. 68; Wu et al. (eds.) (1983) Meth. Enzymol. 100 and 101; Grossman and Moldave (eds.) Meth. Enzymol. 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink (1982) Practical Methods in Molecular Biology; Glover (ed.) (1985) DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; Setlow and Hollaender (1979) Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York; and Ausubel et al. (1992) Current Protocols in Molecular Biology, Greene/Wiley, New York, NY, and in other sources referenced herein. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

Amplifier RNA

[00209] An amplifier RNA can replicate itself. Amplifier RNA can also have activity on target RNA sequences containing a recognizable promoter sequence, e.g., a subgenomic promoter sequence. In some embodiments, the target RNA may have no self-amplifying ability. Continued expression of target RNA can be dependent on the presence of an active amplifier RNA molecule. Any expression sequence of interest can be placed under control of a subgenomic promoter of a target RNA.

[00210] In some embodiments, an amplifier RNA can comprise a 5'cap. In some embodiments, an amplifier RNA can comprise a 5' UTR. In some embodiments, an amplifier RNA can comprise a 3' UTR. In some embodiments, an amplifier RNA can comprise a poly(A) tail. In some embodiments, an amplifier RNA can comprise an ORF encoding a nonstructural protein. In some embodiments, an amplifier RNA can comprise an ORF encoding a nonstructural protein selected from the group consisting of nsP1, nsP2, nsP3, nsP4, and combinations thereof. In some embodiments, an amplifier RNA can comprise a subgenomic region. In some embodiments, an amplifier RNA can comprise a subgenomic promoter.

[00211] In some embodiments, an amplifier RNA can comprise a deletion of a sequence encoding a viral structural protein. In some embodiments, an amplifier RNA can comprise a sequence encoding a neoantigen that replaces a sequence encoding a viral structural protein.

[00212] In some embodiments, an amplifier RNA can be 1-20 kilobases (kb) in length. For example, an amplifier RNA can be 1-20, 1-18, 1-16, 1-14, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 5-20, 5-19, 5-18, 5-16, 5-14, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 10-20, 10-18, 10-16, 10-14, 10-12, or 10-11 kb in length.

[00213] The nsPs within an amplifier RNA can encode the replication machinery to transcribe a genomic portion of an amplifier RNA. The nsPs within an amplifier RNA can encode the replication machinery to

transcribe a subgenomic portion of an amplifier RNA. The nsPs within an amplifier RNA can encode the replication machinery to transcribe both the genomic and subgenomic portions of an amplifier RNA. In some embodiments, a genomic region can be transcribed when nonstructural protein, such as nsPl, nsP2, nsP3, nsP4, and combinations thereof acts on an amplifier RNA genomic promoter. In some embodiments, a genomic region can be transcribed when nsP4 acts on an amplifier RNA genomic promoter.

[00214] In some embodiments, a subgenomic region is transcribed when nonstructural protein, such as nsPl, nsP2, nsP3, nsP4, and combinations thereof acts on an amplifier RNA subgenomic promoter. In some embodiments, a subgenomic region is transcribed when nsP4 acts on an amplifier RNA subgenomic promoter.

[00215] The nsPs within an amplifier RNA can encode the replication machinery to transcribe a genomic portion of a target RNA. The nsPs within an amplifier RNA can encode the replication machinery to transcribe a subgenomic portion of a target RNA. The nsPs within an amplifier RNA can encode the replication machinery to transcribe both the genomic and subgenomic portions of a target RNA. The nsPs within an amplifier RNA can encode the replication machinery to transcribe a subgenomic portion of a target RNA and its upstream 5' regions. The nsPs within a self-amplifying RNA can encode the replication machinery to transcribe a subgenomic portion of a target RNA and its upstream 5' regions in *trans.* The nsPs within an amplifier RNA can encode the replication machinery to transcribe a subgenomic portion of a target RNA and a neoantigen encoded by the target RNA. The nsPs within an amplifier RNA can encode the replication machinery to transcribe a subgenomic portion of a target RNA and a neoantigen encoded by the target RNA in *trans.*

[00216] An amplifier RNA can comprise a viral 5' UTR sequence, a viral sequence encoding one or more nsPs (e.g., nsPl-4), a viral 3'UTR sequence, a viral subgenomic 5' UTR sequence, a viral subgenomic promoter, a viral subgenomic 3'UTR sequence, and combinations thereof.

[00217] An amplifier RNA can comprise a viral 5' UTR sequence. An amplifier RNA can comprise a viral sequence encoding one or more nsPs (e.g., nsPl-4). An amplifier RNA can comprise a viral genomic 3'UTR sequence. In some embodiments, an amplifier RNA can comprise a viral subgenomic promoter. In some embodiments, an amplifier RNA may not comprise a viral subgenomic promoter. An amplifier RNA can comprise a viral genomic 5' UTR sequence, viral sequence encoding one or more nsPs (e.g., nsPl-4), a viral subgenomic promoter, a viral genomic 3'UTR sequence, and combinations thereof.

[00218] An amplifier RNA can comprise a viral promoter or subgenomic promoter and a target RNA can comprise the viral subgenomic promoter. For example, an amplifier RNA can replicate itself and have activity on target RNA sequences that contains a recognizable subgenomic promoter sequence.

TargetRNA

[00219] The methods disclosed herein may comprise a target nucleic acid that encodes a neoantigen. Generally, a polynucleotide encoding a neoantigen may refer to a linear polymer comprising multiple nucleotide subunits. In any of the cases disclosed herein, the polynucleotide encoding a neoantigen may comprise DNA, RNA, or a hybrid of DNA and RNA. In some cases, the polynucleotide encoding a

neoantigen may be single stranded. For example, a target RNA can encode a neoantigen. In some cases, the polynucleotide encoding a neoantigen may be double stranded.

[00220] A target RNA can comprise a sequence encoding a neoantigen. A target RNA can comprise a viral subgenomic 5' UTR sequence. A target RNA can comprise a viral subgenomic promoter. A target RNA can comprise a viral subgenomic 3'UTR sequence. A target RNA can comprise a viral subgenomic 5' UTR sequence, viral subgenomic promoter, a viral subgenomic 3'UTR sequence, and combinations thereof.

[00221] A polynucleotide sequence encoding a neoantigen may comprise any number of nucleotides. In some cases, a polynucleotide sequence encoding a neoantigen may comprise less than about 100, 100, 200, 300, 400, 500, 1000, 5000, 10,000, 20,000, 30,000, 40,000, or 50,000 nucleotides. In some cases, a polynucleotide sequence encoding a neoantigen may comprise between about 50 and about 300 nucleotides. In some cases, a polynucleotide sequence encoding a neoantigen may comprise between about 300 and about 500 nucleotides. In some cases, a polynucleotide sequence encoding a neoantigen may comprise between about 500 and about 5000 nucleotides. In some cases, a polynucleotide sequence encoding a neoantigen may comprise between about 5000 and about 10,000 nucleotides. The neoantigen encoded by a polynucleotide sequence can have 20 to 100 amino acid resides. In some embodiments, the neoantigen encoded by a polynucleotide sequence can have a length of at least 20 amino acids, or at least 30 amino acids, or at least 40 amino acids, or at least 50 amino acids, or at least 60 amino acids, or at least 70 amino acids, or at least 80 amino acids, or at least 90 amino acids, or at least 100 amino acids. In some embodiments, the neoantigen encoded by a polynucleotide can have a length of at most 100 amino acids.

[00222] In some embodiments, a subgenomic region of a target RNA can be transcribed when nonstructural protein expressed from an amplifier RNA, such as nsP l, nsP2, nsP3, nsP4, and combinations thereof acts on a target RNA subgenomic promoter. In some embodiments, a subgenomic region can be transcribed when nsP4 expressed from an amplifier RNA acts on a target RNA subgenomic promoter. In some embodiments, a subgenomic region of a target RNA can be transcribed when nonstructural protein expressed from an amplifier RNA, such as nsPl, nsP2, nsP3, nsP4, and combinations thereof acts in *trans* on a target RNA subgenomic promoter.

[00223] In some embodiments, a nucleic acid may comprise promoter regions, barcodes, restriction sites, cleavage sites, endonuclease recognition sites, primer binding sites, selectable markers, unique identification sequences, resistance genes, linker sequences, or any combination thereof. In some aspects, these sites may be useful for enzymatic digestion, amplification, sequencing, targeted binding, purification, providing resistance properties (e.g., antibiotic resistance), or any combination thereof.

[00224] In some embodiments, a target RNA can be about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000, 18,000, 19,000, or 20,000 bases or base-pairs in length. In some embodiments, a target RNA can be at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 11,000, 12,000, 13,000, 14,000, 15,000,

16,000, 17,000, 18,000, 19,000, or 20,000 bases or base-pairs in length. In some embodiments, a target RNA can be at most about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 11,000, 12,000, 13,000, 14,000, 15,000, 16,000, 17,000, 18,000, 19,000, or 20,000 bases or base-pairs in length. In some embodiments, a target RNA can be from about 10-20, 10-30, 10-40, 10-30, 10-40, 10-50, 10-60, 10-70, 10-80, 10-90, 10-100, 50-60, 50-70, 50-80, 50-90, 50-100, 100-200, 100-300, 100-400, 100-300, 100-400, 100-500, 100-600, 100-700, 100-800, 100-900, 100-1000, 500-600, 500-700, 500-800, 500-900, 500-1000, 1000-2000, 1000-3000, 1000-4000, 1000-3000, 1000-4000, 1000-5000, 1000-6000, 1000-7000, 1000-8000, 1000-9000, 1000-10000, 5000-6000, 5000-7000, 5000-8000, 5000-9000, or 5000-10000 bases or base-pairs in length. In some embodiments, the average length of the target RNAs can be less than about 100, 200, 300, 400, 500, or 800 base pairs, or less than about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 nucleotides, or less than about 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 kilobases.

[00225] The target RNAs as described herein can be modified. A modification can be made at any location of a target RNA. More than one modification can be made to a single target RNA. A target RNA can undergo quality control after a modification. In some cases, quality control may include PAGE, HPLC, MS, or any combination thereof.

[00226] A modification can be a substitution, insertion, deletion, chemical modification, physical modification, stabilization, purification, or any combination thereof.

[00227] A target RNA can also be modified by 5'adenylate, 5' guanosine-triphosphate cap, 5'N^{7}-Methylguanosine-triphosphate cap, 5'triphosphate cap, 3'phosphate, 3'thiophosphate, 5'phosphate, 5'thiophosphate, Cis-Syn thymidine dimer, trimers, C12 spacer, C3 spacer, C6 spacer, dSpacer, PC spacer, rSpacer, Spacer 18, Spacer 9, 3'-3' modifications, 5'-5' modifications, abasic, acridine, azobenzene, biotin, biotin BB, biotin TEG, cholesteryl TEG, desthiobiotin TEG, DNP TEG, DNP-X, DOTA, dT-Biotin, dual biotin, PC biotin, psoralen C2, psoralen C6, TINA, 3'DABCYL, black hole quencher 1, black hole quencher 2, DABCYL SE, dT-DABCYL, IRDye QC-1, QSY-21, QSY-35, QSY-7, QSY-9, carboxyl linker, thiol linkers, 2'deoxyribonucleoside analog purine, 2'deoxyribonucleoside analog pyrimidine, ribonucleoside analog, **2'-0-**methyl ribonucleoside analog, sugar modified analogs, wobble/universal bases, fluorescent dye label, 2'fluoro RNA, 2'**-0** -methyl RNA, methylphosphonate, phosphodiester DNA, phosphodiester RNA, phosphothioate DNA, phosphorothioate RNA, UNA, pseudouridine-5 '-triphosphate, 5-methylcytidine-5 '-triphosphate, or any combination thereof. In some cases, a modification can be modification is permanent. In other cases, a modification is transient. In some cases, multiple modifications can be made to a target RNA. A target RNA modification may alter physio-chemical properties of a nucleotide, such as their conformation, polarity, hydrophobicity, chemical reactivity, base-pairing interactions, or any combination thereof. A modification can also be a phosphorothioate substitute. In some cases, a natural phosphodiester bond may be susceptible to rapid degradation by cellular nucleases and; a modification of internucleotide linkage using phosphorothioate (PS) bond substitutes can be more stable towards hydrolysis by cellular degradation. A modification can

increase stability in a target RNA. A modification can also enhance biological activity. In some cases, a phosphorothioate enhanced RNA target RNA can inhibit RNase A, RNase Tl, calf serum nucleases, or any combinations thereof. These properties can allow the use of PS-RNA target RNAs to be used in applications where exposure to nucleases is of high probability *in vivo* or *in vitro*. For example, phosphorothioate (PS) bonds can be introduced between the last 3-5 nucleotides at the 5' or 3' end of a target RNA which can inhibit exonuclease degradation. In some cases, phosphorothioate bonds can be added throughout an entire target RNA to reduce attack by endonucleases.

[00228] In some cases, a target RNA may comprise a barcode or a barcode sequence. A barcode or barcode sequence relates to a natural or synthetic nucleic acid sequence comprised by a polynucleotide allowing for unambiguous identification of the polynucleotide and other sequences comprised by the polynucleotide having the same barcode sequence. For example, a nucleic acid comprising a barcode can allow for identification of the encoded neoantigen. A barcode sequence can comprise a sequence of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 45, or 50 or more consecutive nucleotides. A target RNA can comprise two or more barcode sequences or compliments thereof. A barcode sequence can comprise a randomly assembled sequence of nucleotides. A barcode sequence can be a degenerate sequence. A barcode sequence can be a known sequence. A barcode sequence can be a predefined sequence.

[00229] In some embodiments, a target RNA comprises a 3' barcode sequence. In some embodiments, a target RNA can comprise a 5' barcode sequence. In some embodiments, a target RNA encoding a first neoantigen can comprise a first barcode sequence. In some embodiments, a target RNA encoding a second neoantigen can comprise a second barcode sequence that is different than the first barcode sequence. In some embodiments, a target RNA encoding a second neoantigen can comprise a second barcode sequence that is the same as the first barcode sequence.

[00230] In some embodiments, a target RNA encoding a first neoantigen can comprise a first barcode sequence and each target RNA of a plurality of target RNAs that encodes a neoantigen sequence that is different from the first neoantigen sequence can comprise a barcode sequence that is different from the first barcode sequence. In some embodiments, a target RNA encoding a first neoantigen can comprise a first barcode sequence and each target RNA of a plurality of target RNAs that encodes a neoantigen that is different from the first neoantigen sequence can comprise a barcode sequence that is the same as the first barcode sequence.

[00231] In some embodiments, a first target RNA encoding a first neoantigen can comprise a first barcode sequence and a second target RNA encoding the first neoantigen sequence can comprise a second barcode sequence that is different from the first barcode sequence. In some embodiments, a target RNA encoding a first neoantigen can comprise a first barcode sequence and each target RNA of a plurality of target RNAs that encodes the first neoantigen sequence can comprise a barcode sequence that is different from the first barcode sequence.

VI. APCs

[00232] Antigen presenting cells (APC) are cells that present peptide fragments of protein antigens in association with MHC molecules on their cell surface. Some APCs may activate antigen specific T cells. Antigen presenting cells can be loaded with MHC class I presented peptides by introducing into the cells a nucleic acid, such as RNA, encoding a neoantigen peptide or polypeptide comprising the peptide to be presented.

[00233] From a biological perspective, in order for a somatic mutation to generate an immune response several criteria need to be satisfied: the allele containing the mutation should be expressed by the cell, the mutation should be in a protein coding region and nonsynonymous, the translated protein should be cleaved by the proteasome and an epitope containing the mutation should be presented by the MHC complex, the presented epitope should be recognized by a T cell receptor (TCR) and, finally, the TCR-pMHC complex should launch a signaling cascade that activates the T cell.

[00234] Processing and presentation of peptide-MHC class I molecules can involve a series of sequential stages comprising: protease-mediated digestion of proteins; peptide transport into the endoplasmic reticulum (ER) mediated by the transporter associated with antigen processing (TAP); formation of peptide-MHC I molecules using newly synthesized MHC I molecules; and transport of peptide-MHC I molecules to the cell surface.

[00235] Professional antigen-presenting cells can be very efficient at internalizing antigen, either by phagocytosis or by receptor-mediated endocytosis, and then displaying a fragment of the antigen, bound to a class II MHC molecule, on their membrane. The T cell can recognize and interact with the antigen-class II MHC molecule complex on the membrane of the APC. An additional co-stimulatory signal can then be produced by the APC, leading to activation of the T cell. The expression of co-stimulatory molecules can be a defining feature of professional antigen-presenting cells. Exemplary professional APCs can include, but are not limited to, dendritic cells (DCs), macrophages, and **B**-cells. Professional APCs may express high levels of MHC class II, ICAM-1 and **B7-2**.

[00236] Monocytes and **B** cells have been shown to be competent APCs, although their antigen presenting capacities appear to be limited to the re-activation of previously sensitized T cells. These cell types may not be capable of directly activating functionally naive or unprimed T cell populations.

[00237] The main types of professional antigen-presenting cells can be dendritic cells, which have the broadest range of antigen presentation, and are probably the most important antigen-presenting cells among others including macrophages, **B**-cells, and certain activated epithelial cells. Dendritic cells are capable of both activating naive and previously primed T cells. Mature dendritic cells may be CDllb $^{+}$, CDllc $^{+}$, HLA-DR^{+}, CD80^{+}, CD86^{+}, CD54^{+}, CD3^{-}, CD19^{-}, CD14^{-}, and/or CDla^{+}. Dendritic cells (DCs) can be leukocyte populations that present antigens captured in peripheral tissues to T cells via both MHC class II and I antigen presentation pathways. Dendritic cells can be potent inducers of immune responses and the activation of these cells can be a critical step for the induction of antitumoral immunity. Dendritic cells can be categorized as "immature" and "mature" cells, which can be used as a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible

intermediate stages of differentiation. Immature dendritic cells can be characterized as antigen presenting cells with a high capacity for antigen uptake and processing, which correlates with the high expression of Fey receptor and mannose receptor. The mature phenotype can be typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1 BB). Dendritic cell maturation can be referred to as the status of dendritic cell activation at which such antigen-presenting dendritic cells lead to T cell priming, while presentation by immature dendritic cells results in tolerance. Dendritic cell maturation can be caused by biomolecules with microbial features detected by innate receptors (bacterial DNA, viral RNA, endotoxin, etc.), pro-inflammatory cytokines (TNF, IL-1, IFNs), ligation of CD40 on the dendritic cell surface by CD40L, and substances released from cells undergoing stressful cell death. The dendritic cells can be derived by culturing bone marrow cells *in vitro* with cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor alpha.

[00238] Typically, non-professional antigen-presenting cells do not constitutively express the MHC class II proteins required for interaction with naive T cells; these are typically expressed only upon stimulation of the non-professional antigen-presenting cells by certain cytokines such as IFNy.

[00239] The source of antigen-presenting cell (APC) can be typically a tissue source comprising APCs or APC precursors that are capable of expressing and presenting neoantigen peptides *in vitro.* In some embodiments, APCs are capable of proliferating and becoming professional APCs when loaded with target RNA and/or treated with the necessary cytokines or factors.

[00240] In one aspect, APC precursor cells are capable of proliferating and maturing *in vitro* into dendritic cells (DC). While many tissue sources may be used, typical tissue sources can comprise spleen, thymus, tissue biopsy, tumor, afferent lymph, lymph nodes, bone marrow, apheresis or leukapheresis product, and/or peripheral blood. In certain embodiments, apheresis product, bone marrow and peripheral blood can be preferred sources. Fetal tissue, fetal or umbilical cord blood, which is also rich in growth factors, may also be used as a source of blood for obtaining APC and/or precursor APC. Exemplary precursor cells may be, but are not limited to, embryonic stem cells, $CD34^+$ cells, monocyte progenitors, monocytes, and pre-B-cells. For example, APCs may be derived from precursor cells comprising monocytes or $CD34^+$ cells.

[00241] In one aspect, the source of APCs and/or precursor APCs can be an apheresis or leukapheresis product. Cells can be collected using apheresis procedures known in the art (e.g., Bishop et al., Blood, vol. 83, No. 2, pp. 610-616 (1994)). Apheresis product typically can contain lymphocytes, including T cells, monocytes, granulocytes, B-cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In another embodiment of the invention, the cells can be washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution may lack calcium and may lack magnesium or may lack many if not all divalent cations. A washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through"

centrifuge. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[00242] APCs can be prepared from a variety of sources, including human and non-human primates, other mammals, and vertebrates. In certain embodiments, APCs can be prepared from blood of a human or non-human vertebrate. APCs can also be isolated from an enriched population of leukocytes. Populations of leukocytes can be prepared by methods known to those skilled in the art. Such methods typically include collecting heparinized blood, apheresis or leukopheresis, preparation of buffy coats, resetting, centrifugation, density gradient centrifugation (e.g., using Ficoll, colloidal silica particles, and sucrose), differential lysis non-leukocyte cells, and filtration. A leukocyte population can also be prepared by collecting blood from a subject, defibrillating to remove the platelets and lysing the red blood cells. The leukocyte population can optionally be enriched for monocytic dendritic cell precursors.

[00243] Blood cell populations can be obtained from a variety of subjects, according to the desired use of the enriched population of leukocytes. The subject can be a healthy subject. Alternatively, blood cells can be obtained from a subject in need of immunostimulation, such as, for example, a cancer patient or other patient for which immunostimulation will be beneficial. Likewise, blood cells can be obtained from a subject in need of immune suppression, such as, for example, a patient having an autoimmune disorder (e.g., rheumatoid arthritis, diabetes, lupus, multiple sclerosis, and the like). A population of leukocytes also can be obtained from an HLA -matched healthy individual.

[00244] When blood is used as a source of APC, blood leukocytes may be obtained using conventional methods that maintain their viability. According to one aspect of the invention, blood can be diluted into medium that may or may not contain heparin or other suitable anticoagulant. The volume of blood to medium can be about 1 to 1. Cells can be concentrated by centrifugation of the blood in medium at about 1000 rpm (150 g) at 4° C. Platelets and red blood cells can be depleted by resuspending the cells in any number of solutions known in the art that will lyse erythrocytes, for example ammonium chloride. For example, the mixture may be medium and ammonium chloride at about 1:1 by volume. Cells may be concentrated by centrifugation and washed in the desired solution until a population of leukocytes, substantially free of platelets and red blood cells, is obtained. Any isotonic solution commonly used in tissue culture may be used as the medium for separating blood leukocytes from platelets and red blood cells. Examples of such isotonic solutions can be phosphate buffered saline, Hanks balanced salt solution, and complete growth media. APCs and/or APC precursor cells may also purified by elutriation.

[00245] In one embodiment, isolation of APCs and/or precursor APCs can be performed by preincubating ficolled whole blood or apheresed peripheral blood with one or more varieties of irrelevant or non-antibody coupled paramagnetic particles (approx. 1 vial of beads or 4×10^9 beads to one batch of cells (typically from about 5xl0 ^{8} to about 2×10^{10} cells) for about 30 minutes to 2 hours at 22 to 37° C, followed by magnetic removal of cells which have attached to or engulfed the paramagnetic particles. Such separation can be performed using standard methods available in the art. For example, any magnetic separation methodology

may be used including a variety of which are commercially available, (e.g., DYNAL® Magnetic Particle Concentrator (DYNAL MPC®)). Assurance of isolation can be monitored by a variety of methodologies known to those of ordinary skill in the art, including flow cytometric analysis of cells before and after said isolation.

[00246] APCs may be cultured to form a primary culture in an appropriate culture container or vessel in an appropriate culture medium. In certain embodiments, the culture medium can be supplemented with one or more cytokines. The appropriate culture container or vessel may be any container with tissue culture compatible surface. Examples include various bags, flasks, roller bottles, petri dishes and multi-well containing plates made for use in tissue culture. Surfaces treated with a substance, for example collagen or poly-L-lysine, or antibodies specific for a particular cell type to promote cell adhesion may also be used provided they allow for the differential attachment of cells as described below. Surfaces may also be chemically treated, for example by ionization. Cells can be plated at an initial cell density from about 10^5 to 10^7 cells/cm^2. In one aspect, cells can be plated at 10^6 cells/cm^2.

[00247] In one embodiment, the primary cultures from the selected tissue source are allowed to incubate at about 37° C under standard tissue culture conditions of humidity, CO_2, and pH until a population of cells has adhered to the substrate sufficiently to allow for the separation of nonadherent cells. Some immature APCs in blood initially are nonadherent to plastic, particularly immature DC, in contrast to monocytes, so that the precursors can be separated after overnight culture. Monocytes and fibroblasts can comprise the majority of adherent cells and usually adhere to the substrate within about 30 minutes to about 24 hours. In certain aspects, nonadherent cells can be separated from adherent cells between about 1 to 16 hours. Nonadherent cells may be separated at about 1 to 2 hours. Any method which does not dislodge significant quantities of adherent cells may be used to separate the adherent from nonadherent cells. In certain aspects, the cells can be dislodged by simple shaking or pipetting. In certain aspects, pipetting can be most preferred.

[00248] Adherent cells comprising precursor APCs (e.g., monocytes) isolated according to the methods of the invention can be incubated at about 37° C under standard tissue culture conditions of humidity, $C0_2$, and pH until a population of cells has reached an immature APC stage. In certain aspects, according to the present invention, adherent cells can be incubated for a period of between 4 hours and 7 days. However, one of ordinary skill in the art will readily appreciate that incubation times and conditions may vary. Immature APC may be $CD14^-$ or $CD14^+$ depending on the origin of the precursor cells. Immature APC may also express CDla, CD40, CD86, CD54, and intermediate levels of MHC class II (levels of marker expression on sample cells can be compared by flow cytometric analysis to levels of expression on MHC class II-negative cells and cells known to express high levels of MHC class II). Immature APCs typically do not express CCR7.

[00249] In certain aspects of the present invention, it is not necessary to separate T cells from APCs. For example, in one embodiment, PBMC comprising APC and T cells can be exposed to antigen as described herein and the resulting antigen-specific T cells further expanded as described herein.

[00250] In certain aspects of the present invention, it is not required that the APCs or the T cells described herein be derived from an autologous source. Thus, the APCs and T cells can be obtained from a matched or

unmatched donor, or from a cell line, a T cell line, or other cells grown *in vitro*. Methods for matching haplotypes are known in the art. Furthermore, the APCs and T cells or supernatant therefrom may be obtained from a xenogeneic source, for example, mouse, rat, non-human primate, and porcine cells may be used.

[00251] Suitable preparations of APCs include, for example, dendritic cells and monocytes. In other embodiments, the APCs can be activated non-nominal APCs, such as, for example, B cells, cells, or epithelial or endothelial cells. The APCs can be immature or mature. The APCs and T cells are typically co-cultured for about 6 to about 48 hours, although greater and lesser times are within the scope of the present invention. Co-culturing typically can be performed for a sufficient time to allow activation of T cells, but less than the time required for the differentiation and/or maturation of a significant number of immature APCs or APC precursors.

[00252] In certain embodiments, monocytic dendritic cell precursors can be isolated, for example, by contacting enriched leukocytes or monocytes with a monocytic dendritic cell precursor adhering substrate. Briefly, when a population of enriched leukocytes or monocytes is contacted with the substrate, the monocytic dendritic cell precursors, or monocytes, in the cell population can adhere to the substrate. Other leukocytes can exhibit reduced binding affinity to the substrate, thereby allowing monocytic dendritic cell precursors to be preferentially enriched on the surface of the substrate.

[00253] Suitable substrates include particulate substrates, such as, for example, glass particles, plastic particles, glass-coated plastic particles, glass-coated polystyrene particles, microcapillary tubes, and microvillous membrane. The surface of the substrate can optionally be treated to enhance adherence of monocytic dendritic cell precursors to the substrate. The surface of the substrate can be coated with, for example, proteins, cytokines, plasma, and/or monocyte-binding proteins.

[00254] After contacting the leukocyte- or monocyte-enriched cell population with the monocytic dendritic cell precursor adhering substrate, the monocytic dendritic cell precursors adhere to the substrate to form complexes comprising monocytic dendritic cell precursors on the substrate. Monocytic dendritic cell precursor binding can be monitored, for example, by antibody detection using anti-cell surface marker antibodies, such as, for example, anti-CD 14 antibodies, by FACS forward and side scatter analysis, and the like. In some embodiments, the leukocyte population can be contacted with the substrate for about 5 to about 300 minutes, more typically about 30 to about 120 minutes.

[00255] The monocytic dendritic cell precursor complexes can optionally be washed with a suitable washing buffer to remove non-specifically bound leukocytes. Suitable washing buffers include tissue culture media, phosphate buffered saline, Dulbecco's phosphate buffered saline, and the like. The media can be supplemented with amino acids, vitamins, and/or hormones to promote the viability and/or proliferation of the monocytic dendritic cell precursors. The efficacy of washing can be monitored by FACS forward and side scatter analysis of the washing buffer, by staining eluted cells for cell surface markers, and the like. Typically, the complexes can be washed several times to remove non-specifically bound leukocytes.

[00256] The adhered monocytic dendritic cell precursors can be eluted from the substrate. For example, the precursors can be eluted from the substrate by treatment with phosphate buffered saline containing 0.4%

EDTA or other non-toxic chelating agent. The monocytic dendritic cell precursors typically can be eluted from the substrate without the use of trypsin or other proteases.

[00257] In other embodiments, the dendritic cells can be isolated according to other methods known to the skilled artisan (e.g., O'Doherty et al., J. Exp. Med. 178:1067-76 (1993); Young and Steinman, J. Exp. Med. 171:1315-32 (1990); Freudenthal and Steinman, Proc. Natl. Acad. Sci. USA 87:7698-702 (1990); Macatonia, et al., Immunol. 67:285-89 (1989); Markowicz and Engleman, J. Clin. Invest. 85:955-61 (1990); U.S. Pat. Nos. 5,994,126 and 5,851,756). Methods for immuno-selecting dendritic cells include, e.g., using antibodies to cell surface markers associated with dendritic cell precursors, such as anti-CD34 and/or anti-CD14 antibodies coupled to a substrate (e.g., Bernhard et al., Cancer Res. 55:1099-104 (1995); Caux at al., Nature 360:258-61 (1992)) or associated with fully differentiated dendritic cells, such as, CDllc, CD54, CD83, CD80, and CD86.

[00258] In other embodiments, the APCs can be non-nominal APCs under inflammatory or otherwise activated conditions. For example, non-nominal APCs can include epithelial cells stimulated with interferon-gamma, T cells, B cells, and/or monocytes activated by factors or conditions that induce APC activity. Such non-nominal APCs can be prepared according to methods known in the art.

[00259] The APCs can be cultured, expanded, differentiated and/or, matured, as desired, according to the according to the type of APC. The APCs can be cultured in any suitable culture vessel, such as, for example, culture plates, flasks, culture bags, and bioreactors.

[00260] In certain embodiments, APCs can be cultured in suitable culture or growth medium to maintain and/or expand the number of APCs in the preparation. The culture media can be selected according to the type of APC isolated. For example, mature APCs, such as mature dendritic cells, can be cultured in growth media suitable for their maintenance and expansion. The culture medium can be supplemented with amino acids, vitamins, antibiotics, divalent cations, and the like. In addition, cytokines, growth factors and/or hormones, can be included in the growth media. For example, for the maintenance and/or expansion of mature dendritic cells, cytokines, such as granulocyte/macrophage colony stimulating factor (GM-CSF) and/or interleukin 4 (IL-4), can be added. In other embodiments, immature APCs can be cultured and/or expanded. Immature dendritic cells can they retain the ability to uptake target mRNA and process new antigen. In an exemplary embodiment, immature dendritic cells can be cultured in media suitable for their maintenance and culture. The culture medium can be supplemented with amino acids, vitamins, antibiotics, divalent cations, and the like. In addition, cytokines, growth factors and/or hormones, can be included in the growth media.

[00261] Other immature APCs can similarly be cultured or expanded according to methods known to the skilled artisan. Preparations of immature APCs can be matured to form mature APCs. Maturation of APCs can occur during or following exposure to target mRNA. In certain embodiments, preparations of immature dendritic cells can be matured. Suitable maturation factors include, for example, cytokines TNF-a, bacterial products (e.g., BCG), and the like. In another aspect, isolated APC precursors can be used to prepare preparations of immature APCs. APC precursors can be cultured, differentiated, and/or matured, as is known to the skilled artisan.

[00262] In certain embodiments, monocytic dendritic cell precursors can be cultured in the presence of suitable culture media supplemented with amino acids, vitamins, cytokines, and/or divalent cations, to promote differentiation of the monocytic dendritic cell precursors to immature dendritic cells.

VII. Delivering Neoantigen-Encoding RNAs to APCs

[00263] In some aspects, the methods disclosed herein can comprise introducing into the cell one or more nucleic acids (e.g., a first nucleic acid or a second acid). A person of skill in the art will appreciate that a nucleic acid may generally refer to a substance whose molecules consist of many nucleotides linked in a long chain. Non-limiting examples of the nucleic acid include an artificial nucleic acid analog (e.g., a peptide nucleic acid, a morpholino oligomer, a locked nucleic acid, a glycol nucleic acid, or a threose nucleic acid), a circular nucleic acid, a DNA, a single stranded DNA, a double stranded DNA, a genomic DNA, a plasmid, a plasmid DNA, a viral DNA, a viral vector, a gamma-retroviral vector, a lentiviral vector, an adeno-associated viral vector, an RNA, short hairpin RNA, psiRNA and/or a hybrid or combination thereof. In some embodiments, a method may comprise a nucleic acid, and the nucleic acid is synthetic. In some embodiments, a sample may comprise a nucleic acid, and the nucleic acid may be fragmented. In some embodiments, a method may comprise a nucleic acid, and the nucleic acid can be *in vitro* transcribed mRNA.

[00264] In one embodiment, RNA that is to be introduced into a cell can be obtained by *in vitro* transcription of an appropriate DNA template, e.g., a target RNA vector or an amplifier RNA vector. Target RNA and amplifier RNAs can be delivered to APCs for expression and processing by the APCs. Upon delivery of target RNA and amplifier RNA to an APC, the neoantigen-encoding RNAs can be transcribed within the APC. Once an amplifier RNA transcript (mRNA) and the target RNA transcript (mRNA) are present in an APC, a neoantigen sequence can be translated to produce the encoded neoantigen polypeptides. In some embodiments, target mRNA can be introduced into a cell (e.g., a APC) without an amplifier mRNA. These neoantigen peptides can be subsequently processed by the APCs, leading to display of neoantigen epitopes on the surface of the APCs.

[00265] Terms such as "transferring", "introducing" or "transfecting" are used interchangeably herein and refer to the introduction of nucleic acids, in particular exogenous or heterologous nucleic acids, in particular mRNA into a cell.

[00266] Cells can be transfected with any carriers with which RNA can be associated, e.g., by forming complexes with the RNA or forming vesicles in which the RNA is enclosed or encapsulated, resulting in increased stability of the RNA compared to naked RNA. Carriers useful according to the invention include, for example, lipid-containing carriers such as cationic lipids, liposomes, in particular cationic liposomes, and micelles, and nanoparticles. Cationic lipids may form complexes with negatively charged nucleic acids. Any cationic lipid may be used according to the invention.

[00267] A plurality of target polynucleotides encoding for a plurality of neoantigen sequences can be introduced into cells, e.g., APCs. For example, at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200 or more target polynucleotides encoding for a plurality of neoantigen sequences can be introduced into cells. For example, a plurality of target polynucleotides encoding for at least about 2, 3, 4, 5, 6,

7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200 or more neoantigen sequences can be introduced into cells. In some embodiments, the plurality of target polynucleotides can comprise from about 2-1000 target polynucleotides. For example, the plurality of target RNAs can comprise from about 2-900, 2-800, 2-700, 2-600, 2-500, 2-400, 2-300, 2-200, 2-100, 25-900, 25-800, 25-700, 25-600, 25-500, 25-400, 25-300, 25-200, 25-100, 100-1000, 100-900, 100-800, 100-700, 100-600, 100-500, 100-400, 100-300, 100-200, 200-1000, 200-900, 200-800, 200-700, 200-600, 200-500, 200-400, 200-300, 300-1000, 300-900, 300-800, 300-700, 300-600, 300-500, 300-400, 400-1000, 400-900, 400-800, 400-700, 400-600, 400-500, 500-1000, 500-900, 500-800, 500-700, 500-600, 600-1000, 600-900, 600-800, 600-700, 700-1000, 700-900, 700-800, 800-1000, 800-900, or 900-1000 target RNAs. In some embodiments, the plurality of neoantigen sequences can comprise from about 2-1000 neoantigen sequences. For example, the plurality of neoantigen sequences can comprise from about 2-900, 2-800, 2-700, 2-600, 2-500, 2-400, 2-300, 2-200, 2-100, 25-900, 25-800, 25-700, 25-600, 25-500, 25-400, 25-300, 25-200, 25-100, 100-1000, 100-900, 100-800, 100-700, 100-600, 100-500, 100-400, 100-300, 100-200, 200-1000, 200-900, 200-800, 200-700, 200-600, 200-500, 200-400, 200-300, 300-1000, 300-900, 300-800, 300-700, 300-600, 300-500, 300-400, 400-1000, 400-900, 400-800, 400-700, 400-600, 400-500, 500-1000, 500-900, 500-800, 500-700, 500-600, 600-1000, 600-900, 600-800, 600-700, 700-1000, 700-900, 700-800, 800-1000, 800-900, or 900-1000 neoantigen sequences.

[00268] Different copy numbers of target RNA and amplifier RNA may be delivered into the APCs. In some embodiments, the target RNA can be delivered on average at 1 to 20 copies per cell. In some embodiments, the target RNA can be delivered on average at 1 copy, 2 copies, 3 copies, 4 copies, 5 copies, 6 copies, 7 copies, 9 copies, 10 copies, 11 copies, 12 copies, 13 copies, 14 copies, 15 copies, 16 copies, 17 copies, 18 copies, 19 copies, or 20 copies per cell. In some embodiments, the target RNA can be delivered on average at 1-3 copies, 3-5 copies, 5-10 copies, 10-15 copies, or 15-20 copies. In some embodiments, the target RNA can be delivered on average at least 1 copy, at least 2 copies, at least 3 copies, at least 4 copies, at least 5 copies, at least 6 copies, at least 7 copies, at least 8 copies, at least 9 copies, or at least 10 copies per cell. In some embodiments, the amplifier RNA can be delivered on average at 1 to 20 copies per cell. In some embodiments, the amplifier RNA can be delivered on average at 1 copy, 2 copies, 3 copies, 4 copies, 5 copies, 6 copies, 7 copies, 9 copies, 10 copies, 11 copies, 12 copies, 13 copies, 14 copies, 15 copies, 16 copies, 17 copies, 18 copies, 19 copies, or 20 copies per cell. In some embodiments, the amplifier RNA can be delivered on average at 1-3 copies, 3-5 copies, 5-10 copies, 10-15 copies, or 15-20 copies. In some embodiments, the amplifier RNA can be delivered on average at least 1 copy, at least 2 copies, at least 3 copies, at least 4 copies, at least 5 copies, at least 6 copies, at least 7 copies, at least 8 copies, at least 9 copies, or at least 10 copies per cell.

[00269] The amplifier RNA and target RNA can be pre-mixed at different ratios (e.g., based on molar concentration) before delivering into APCs. In some embodiments, the target RNA and amplifier RNA can be pre-mixed at ratio 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, 1:25, 1:26, 1:27, 1:28, 1:29, 1:30, 1:31, 1:32, 1:33, 1:34, 1:35, 1:36, 1:37, 1:38, 1:39, 1:40, 1:41, 1:42, 1:43, 1:44, 1:45, 1:46, 1:47, 1:48, 1:49, 1:50, 1:51, 1:52, 1:53, 1:54, 1:55, 1:56, 1:57, 1:58, 1:59, 1:60, 1:61, 1:62, 1:63, 1:64, 1:65, 1:66, 1:67, 1:68, 1:69, 1:70, 1:71, 1:72, 1:73, 1:74, 1:75,

1:76, 1:77, 1:78, 1:79, 1:80, 1:81, 1:82, 1:83, 1:84, 1:85, 1:86, 1:87, 1:88, 1:89, 1:90, 1:91, 1:92, 1:93, 1:94, 1:95, 1:96, 1:97, 1:98, 1:99, or 1:100. In some embodiments, the target RNA and amplifier RNA can be pre-mixed at ratio within the range of 1:1-1:5, 1:5-1:10, 1:10-1:15, 1:15-1:20, 1:20-1:25, 1:25-1:30, 1:30-1:35, 1:35-1:40, 1:40-1:45, 1:45-1:50, 1:50-1:55, 1:55-1:60, 1:60-1:65, 1:65-1:70, 1:70-1:75, 1:75-1:80, 1:80-1:85, 1:85-1:90, or 1:95-1:100. In some embodiments, the target RNA and amplifier RNA can be pre-mixed at ratio within the range of 1:100-1:200, 1:200-1:300, 1:300-1:400, or 1:400-1:500.

[00270] In some embodiments, an APC containing a sequence encoding an amplifier RNA and a target RNA encoding a neoantigen can express the neoantigen for a longer period of time than an APC containing a sequence encoding a non-amplifier RNA and the target RNA encoding the neoantigen expresses the neoantigen.

[00271] In some embodiments, an APC containing a sequence encoding an amplifier RNA and a target RNA encoding a neoantigen can express the neoantigen at a higher level than an APC containing a sequence encoding a non-amplifier RNA and the target RNA encoding the neoantigen expresses the neoantigen.

[00272] In some embodiments, an APC containing an amplifier RNA and a target RNA encoding a neoantigen can express the neoantigen for a longer period of time than an APC containing a non-amplifier RNA and the target RNA encoding the neoantigen expresses the neoantigen.

[00273] In some embodiments, an APC containing an amplifier RNA and a target RNA encoding a neoantigen can express the neoantigen at a higher level than an APC containing a non-amplifier RNA and the target RNA encoding the neoantigen expresses the neoantigen.

VIII. T cells

[00274] T cells belong to a group of white blood cells known as lymphocytes, and play a central role in cell-mediated immunity. T cells include $CD4^+$ T cells (helper T cells) and $CD8^+$ T cells (cytotoxic T cells). $CD4^+$ T cells can assist other white blood cells in immunologic processes, including maturation of B-cells and activation of cytotoxic T cells and macrophages. $CD4^+$ T cells are activated when presented with peptide antigens by MHC class II molecules expressed on the surface of antigen presenting cells (APCs). Once activated, the T cells can divide rapidly and secrete cytokines that regulate the active immune response. $CD8^+$ T cells can destroy virally infected cells and tumor cells, and can also be implicated in transplant rejection. $CD8^+$ T cells can recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body. Most T cells have a T cell receptor (TCR). The ability of T cells to recognize antigens associated with various cancers or infectious organisms is conferred by its TCR, which is made up of both an alpha (a) chain and a beta (β) chain or a gamma (γ) and a delta (δ) chain. The proteins which make up these chains are encoded by DNA, which employs a unique mechanism for generating the diversity of the TCR. This multi-subunit immune recognition receptor can associate with the CD3 complex and bind peptides presented by the MHC class I and II proteins on the surface of antigen-presenting cells (APCs). The first signal in activation of T cells can be provided by binding of the T cell receptor to a short peptide presented by the MHC on another cell. This ensures that only a T cell with a TCR specific to that peptide is activated. The partner cell is usually an antigen presenting cell such as a professional

antigen presenting cell, usually a dendritic cell in the case of naive responses, although B-cells and macrophages can be important APCs. Binding of a TCR to the antigenic peptide on the APC can be a central event in T cell activation, which occurs at an immunological synapse at the point of contact between the T cell and the APC.

[00275] Each TCR contains variable complementarity determining regions (CDRs), as well as framework regions (FRs) and a constant region. The amino acid sequence of the third complementarity-determining region (CDR3) loops of the a and β chain variable domains is largely determines the sequence diversity of αβ T cells arising from recombination between variable (νβ), diversity (Dβ), and joining (Jβ) gene segments in the β chain locus, and between analogous Vα and Jα gene segments in the a chain locus, respectively. The existence of multiple such gene segments in the TCR a and β chain loci allows for a large number of distinct CDR3 sequences to be encoded. Independent addition and deletion of nucleotides at the νβ-Dβ, Dβ-Jβ, and Va-Ja junctions during the process of TCR gene rearrangement further increases CDR3 sequence diversity. In this respect, immunocompetence is reflected in the diversity of TCRs. The γδ TCR is distinctive from the αβ TCR in that it encodes a receptor that interacts closely with the innate immune system. TCRγδ, is expressed early in development, has specialized anatomical distribution, has unique pathogen and small-molecule specificities, and has a broad spectrum of innate and adaptive cellular interactions. Early in ontogeny, as the restricted subsets of TCRγδ cells populate various tissues prenatally, a biased pattern of TCRγ V and J segment expression is established.

[00276] T cells can be prepared according to methods known in the art. T cells can be an enriched T cell preparation, an APC-depleted cell preparation, or a substantially purified T cell preparation. T cells can be a mixed T cell population or a purified T cell subset. T cells can be an enriched T cell preparation containing a number or percentage of T cells that is increased with respect to an isolated population of T cells.

[00277] T cells, or a subset of T cells, can be obtained from various lymphoid tissues. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells (PBMCs), bone marrow, thymus, tissue biopsy, tumor, lymph node tissue, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen tissue, lymphoid tissue, and tumors.

[00278] The method can comprise isolating T cells from a subject. The method can comprise obtaining T cells isolated from a subject. T cells can be obtained from T cell lines. T cells can be obtained from autologous sources. T cells can be obtained from allogeneic sources. T cells may also be obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, and pig.

[00279] T cells can be an APC-depleted cell preparation. T cells can be substantially free of APCs. For example, T cells can comprise T cells separated from over 75% of APCs. In an exemplary embodiment, peripheral blood mononuclear cells (PBMCs) can be obtained from blood, e.g., in heparinized vials. PBMCs can be separated from red blood cells by centrifugation and PBMCs recovered from the interface. The recovered PBMCs optionally can be washed (e.g., with PBS).

[00280] T cell purification can be achieved, for example, by positive or negative selection including, but not limited to, the use of antibodies directed to CD2, CD3, CD4, CD5, CD8, CD14, CD19, and/or MHC class II

molecules. A specific T cell subset, such as $CD28^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, and/or $CD45RO^+$ T cells, can be isolated by positive or negative selection techniques. For example, $CD3^+$, $CD28^+$ T cells can be positively selected using CD3/CD28 conjugated magnetic beads. In one aspect of the present invention, enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells.

[00281] For example, a T cell sample can comprise cells from a subject's circulating blood and can be obtained by apheresis or leukapheresis. A T cell sample may contain lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets. Undesirable components of the T cell sample can be removed and the remaining T cells can be suspended in culture media. For example, cells can be washed to remove the plasma fraction. For example, T cells can be isolated from peripheral blood lymphocytes by lysing the red blood cells and by centrifugation through a PERCOLL™ gradient.

IX. Culturing T cells with APCs Containing Neoantigen Encoding Polynucleotides

[00282] The method can further comprise contacting APCs containing neoantigen-encoding RNAs to T cells to form APC:T cell conjugates. In some embodiments, the method can comprise co-culturing APCs and T cells (e.g., autologous APCs and T cells) and enriching for expanded memory and *de novo* responses.

[00283] The presentation of antigens to T cells can be carried out by specialized cell populations referred to as antigen presenting cells (APCs). T cells can recognize antigens that have been processed or degraded into small fragments and presented on APCs. Additionally, antigenic fragments can be presented to T cells in association with major histocompatibility complex (MHC)-encoded class I or class II molecules (HLA in humans). Class I gene products can be found on all somatic cells, and class II gene products can be mostly expressed on cells of various hematopoietic lineages and are involved in cell-cell interactions in the immune system. MHC-encoded proteins can function as receptors for processed antigenic fragments on the surface of APC. APCs can be important to elicit an effective immune response. APCs not only present antigens to T cells with antigen-specific receptors, but also provide the signals necessary for T cell activation. Such signals involve a variety of cell surface molecules, as well as the production of cytokines and/or growth factors.

[00284] The interaction between a T cell and an antigenic fragment can occur when the MHC molecules on the APC and the responding T cells are the same. A T cell specific for a particular antigenic epitope can express a receptor having low affinity for self MHC proteins. When the MHC proteins on an APC are occupied by the epitope, they can have a higher affinity for the T cell and antigen-specific T cell activation can occur. The specificity of T cell immune responses for antigens is a function of the T cell receptor (TCRs) unique receptors expressed by these cells.

[00285] In some aspects, the method can comprise determining a time point of optimal T cell stimulation when cultured with APCs. In some aspects, the method can comprise determining an optimal time point for formation of APC:T cell conjugates when T cells are cultured with APCs. For example, APCs derived from blood can be pulsed with CEF and these CEF-pulsed APCs can be co-cultured with T cells for various amounts of time (e.g., 30 minutes or 1, 2, 4, 6, 8, or 24 hours).

[00286] In some aspects, the method comprises: (a) contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a polypeptide encoded by a target polynucleotide that is an *in vitro* transcribed target RNA; (b) isolating the APC:T cell conjugate from non-conjugated APCs, non-conjugated T cells, or both within at most about 12 hours after the step of contacting. In some embodiments, an APC:T cell conjugate can be isolated about 30 min, or about 1 hour, or about 2 hours, or about 3 hours, or about 4 hours, or about 5 hours, or about 6 hours, or about 7 hours, or about 8 hours, or about 9 hours, or about 10 hours, or about 11 hours, or about 12 hours after the step of contacting. . In some embodiments, an APC:T cell conjugate can be isolated within at most 30 min, at most 1 hour, at most 2 hours, at most 3 hours, at most 4 hours, at most 5 hours, at most 6 hours, at most 7 hours, at most 8 hours, at most 9 hours, at most 10 hours, at most 11 hours, at most 12 hours after the step of contacting.

[00287] The CEF control peptides are 8-12 amino acids in length, with sequences derived from the human Cytomegalovirus, Epstein-Barr Virus and Influenza Virus (CEF). These peptides can be used in the stimulation of IFN gamma release from $CD8^+$ T cells in individuals with defined ULA types, they are useful in applications such as ELISPOT, intracellular cytokine and CTL assays.

[00288] At each time point, the cells can be fixed, e.g., using 1% PFA. Fluorophore labeled antibodies specific for APC and T cell surface proteins can then be contacted to the fixed cells and a cell sorter can be used to determine the frequency or amount of APC:T cell conjugates at each time point. In some aspects, after determining the optimal time point for T cell activation or APC:T cell conjugate formation, the optimal amount of fixative for stabilizing APC:T cell conjugates can be determined by repeating the exemplary method above with varying concentrations of fixative (e.g., 0-1% PFA) at the chosen time point.

[00289] APCs derived from blood can be pulsed with sequence-tagged mRNA encoding CEF peptide sequences and these CEF-pulsed APCs can be co-cultured with T cells for the optimal time determined above. Conjugates can then be isolated using magnetic beads comprising an agent specific to T cells (e.g., a CD3/CD40L/CD137 antibody). The culture and conjugates bound the magnetic beads can then be fixed at various fixative concentrations. Fluorophore labeled antibodies specific for APC and T cell surface proteins can then be contacted to the culture and beads. The beads can then be separated from the culture. The isolated conjugate fraction bound to the beads and the fraction not bound to the beads (flow-through) can be analyzed for conjugate enrichment. qPCR can be performed on the isolated conjugate fraction bound to the beads and the fraction not bound to the beads (flow-through) to determine relative enrichment for APCs containing CEF-encoding mRNA using sequence tags. Iterative refinements of the above processes can be used to determine the optimal conditions for capturing APC:T cell interactions that are antigen-specific.

[00290] T cell activation can be determined during and/or following co-culturing of the T cells and the APCs. Suitable assays for T cell activation include, for example, DNA replication assays (e.g., ^{3}H-thymidine incorporation), extracellular and/or cytokine production assays (e.g., ELISA, flow cytometry, and the like), and T cell activation marker assays (e.g., flow cytometry). Specific activation of $CD4^+$ or $CD8^+$ T cells may be detected in a variety of ways. Methods for detecting specific T cell activation include detecting the proliferation of T cells, the production of cytokines (e.g., lymphokines), or the generation of cytolytic activity.

[00291] Activation of T cells can be correlated with T cell proliferation, such as DNA replication, which can be measured, for example, by labeled thymidine incorporation (e.g., ^{3}H-thymidine or other suitable label). Co-cultures of T cells and APCs can be pulsed with the label (e.g., ^{3}H-thymidine, about 1 μCi/well) for about 6 to about 24 hours. The cells can then be collected (e.g., using a cell harvester) and the incorporated radioactivity measured by liquid scintillation spectroscopy. In certain embodiments, the APCs can be inactivated prior to co-culturing with the T cells to prevent APC DNA replication. Alternatively, the T cells can be separated from the APCs prior to determining the amount of label incorporated.

[00292] T cell activation also can be measured by extracellular or intracellular cytokine production, such as, for example, IFNγ and/or IL-2 production, and the like. Extracellular cytokine production can be measured by determining changes in levels of one or more cytokines in culture media. Typically an immunoassay (e.g., ELISA assay, sandwich assay, immunoprecipitation assay, or Western blotting) can be used, although other assays can also be suitable. For intracellular cytokine levels, immunoassays or other assays can be used. The T cells can optionally be separated from the APCs (e.g., by collection based on expression of T cell markers), prior to assay for intracellular cytokine levels.

[00293] In additional embodiments, T cell activation can be determined by modulation of T cell activation markers. Such markers include, for example, CD25 (Interleukin 2 receptor alpha chain), CD69 (VEA or AIM), CD44 (Pgp-1), CD125 (IL-2 receptor beta chain), and the like. The modulation of T cell activation markers can be measured, for example, by determining changes in protein levels or mRNA levels. For example, changes in protein levels can be determined by flow cytometry using labeled antibodies against the T cell activation markers, transcription factors or other proteins associated with T cell activation, by immunoassay, such as, ELISA or Western blotting. Changes in mRNA levels can be determined for the message encoding the T cell activation markers, transcription factors, and the like. mRNA levels can be determined by, for example, Northern blotting, polymerase chain reaction (e.g., RT-PCR), other hybridization assays (e.g., assays using GeneChip® probe arrays), or other assays. (e.g., U.S. Pat. Nos. 5,445,934; 5,532,128; 5,556,752; 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,436,327; 5,472,672; 5,527,681; 5,529,756; 5,545,531; 5,554,501; 5,561,071; 5,571,639; 5,593,839; 5,599,695; 5,624,711; 5,658,734; and 5,700,637).

X. Linking Neoantigen Sequences to T cells

[00294] The method can comprise linking neoantigen sequences with a functional T cell response. Thus, the methods described herein can utilize T cells as functional baits. In some embodiments, a functional T cell response can comprise a *de novo* T cell response. In some embodiments, a functional T cell response can comprise a memory T cell response. In some embodiments, the method can comprises identifying a T cell from a subject capable of being activated by a presented neoantigen.

[00295] The method can comprise determining a neoantigen sequence that activates a particular T cell. In one embodiment, neoantigen sequences can be linked with a functional T cell response by isolating APC:T cell conjugates. In some embodiments, the method can comprise sorting T cell conjugated to APCs from

unconjugated T cells. For example, the method can comprise performing flow cytometry to specifically isolate only T cells in conjugation with APCs.

[00296] The method can comprise linking neoantigen sequences with TCR sequences. For example, T cells in conjugation with cognate APCs can be interrogated to link neoantigen sequences with T cell receptor sequences of the T cells conjugated to cognate APCs.

[00297] In one aspect, a method can comprise identifying an immunogenic neoantigen sequence by analyzing an antigen sequence of an isolated APC:T cell conjugate. Generally, DC:T cell conjugates can be isolated using a solid surface (e.g., magnetic beads) coated with an anti-T cell agent (e.g., an anti-T cell antibody) or flow cytometric sorting.

[00298] The method can comprise isolating one or more APC:T cell conjugates, such as one or more individual APC:T cell conjugates. For example, APC:T cell conjugates can be isolated after forming APC:T cell conjugates. For example, APC:T cell conjugates can be isolated from unconjugated APCs. For example, APC:T cell conjugates can be isolated from unconjugated T cells. For example, APC:T cell conjugates can be isolated from unconjugated APCs and unconjugated T cells. For example, individual APC:T cell conjugates can be isolated from other APC:T cell conjugates. For example, a first APC:T cell conjugate can be isolated from a second APC:T cell conjugate.

[00299] The method can also comprise partitioning APC-T cell conjugates into individual compartments or vessels, for example, water-in-oil emulsions/droplets **(FIG. 5).** In some embodiments, the method can comprise partitioning a plurality of APC:T cell conjugates into a plurality of compartments or vessels after the conjugate formation without isolating the APC:T cell conjugates. In some embodiments, the method can comprise partitioning a plurality of APC:T cell conjugates into a plurality of compartments or vessels after isolating one or more APC:T cell conjugates. After partitioning, some of the compartments or vessels may contain one APC:T cell conjugate, some of the compartments or vessels may contain at least one APC:T cell conjugate, or some of the compartments or vessels may not contain at least one APC:T cell conjugate. In some embodiments, each vessel or compartment can further comprise a unique barcode sequence (i.e. compartment barcode or vessel barcode). The polynucleotide sequences from the APC:T cell conjugate within the compartment or vessel can be labeled with the unique barcode sequence. The polynucleotide sequence encoding the neoantigen share the same unique barcode sequence with the polynucleotide sequences encoding the TCR chains of the matching T cell, therefore, the neoantigen sequences can be linked to the T cells.

[00300] In some embodiments, the method can comprise identifying an immunogenic neoantigen sequence from an APC:T cell conjugate, wherein the T cell of an APC:T cell conjugate is a T cell that is not stimulated in an antigen-specific manner. In some embodiments, the method can comprise identifying a neoantigen presented by the APC of an APC:T cell conjugate as a potentially immunogenic neoantigen. In some embodiments, the method can comprise identifying a neoantigen presented by the APC of an APC:T cell conjugate as an immunogenic neoantigen.

Single APC:T cell Conjugate Barcoding

[00301] The single APC:T cell conjugate barcoding can be performed using various methods in the art performed for single cell barcoding. For single cell conjugate barcoding with a vessel barcode, vessels, such as water-in-oil emulsions, can be created in such way that resulting vessels contain 1 cell conjugate or less per vessel. The vessels can be created in such way that resulting vessels also contain 1 vessel barcode per vessel.

[00302] In some aspects, single cells or cell conjugates can be isolated inside an emulsion, which can act as a compartment. The cells can be lysed and transcripts from the cell or cell conjugate can be barcoded. Each of the transcripts can be fused with a vessel barcode, in such way that when two or more RNA transcripts are detected with the same vessel barcode, they can be determined to have originated from the same starting cell or cell conjugate. This can be applied to many different types of sequences. One particular application can be linking Vγ and Vδ chains of TCR sequences. Another particular application can be linking neoantigen sequences and TCR chain sequences.

[00303] One or more single cells or cell conjugates can be isolated in one or more emulsions, in the presence of a vessel barcode, so that one droplet of the one or more emulsions can contain a maximum of 1 cell (or cell conjugate) or less. Cells can be lysed chemically by a buffer contained in an emulsion or by freeze thaw, thereby releasing the contents of a cell in an emulsion.

[00304] RNAs of a single cell can be reverse transcribed into cDNA. All reverse transcription buffers, enzymes, and nucleotides can be present when forming an emulsion. In some embodiments, a primer can be generalized (such as polynucleotide comprising a poly dT sequence) to target all mRNA. In some embodiments, DNA can be used. In some embodiments, more than 2 RNAs can be targeted.

[00305] In some embodiments, a vessel barcode can be linked to a RNA during reverse transcription.

XI. Barcodes

[00306] A barcode can have a length within a range of from 2 to 36 nucleotides, or from 4 to 36 nucleotides, or from 6 to 30 nucleotides, or from 8 to 20 nucleotides, or from 2 to 20 nucleotides, or from 4 to 20 nucleotides, or from 6 to 20 nucleotides. In some embodiments, a barcode can have a length within a range of from 3 to 10 nucleotides, or from 10 to 50 nucleotides, or from 50 to 100 nucleotides. In certain aspects, the melting temperatures of barcodes within a set are within 10 °C of one another, within 5 °C of one another, or within 2 °C of one another. In certain aspects, the melting temperatures of barcodes within a set are not within 10 °C of one another, within 5 °C of one another, or within 2 °C of one another. In other aspects, barcodes are members of a minimally cross-hybridizing set. For example, the nucleotide sequence of each member of such a set can be sufficiently different from that of every other member of the set that no member can form a stable duplex with the complement of any other member under stringent hybridization conditions. In some embodiments, the nucleotide sequence of each member of a minimally cross-hybridizing set differs from those of every other member by at least two nucleotides. Barcode technologies are described in Winzeler et al. (1999) Science 285:901; Brenner (2000) Genome Biol. 1:1 Kumar et al. (2001) Nature Rev. 2:302; Giaever et al. (2004) Proc. Natl. Acad. Sci. USA 101:793; Eason et al. (2004) Proc. Natl. Acad. Sci. USA 101:11046; and Brenner (2004) Genome Biol. 5:240.

[00307] A vessel barcode can comprise any length of nucleotides. For example a vessel barcode can comprise at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 nucleotides. For example a vessel barcode can comprise at most about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 nucleotides. In some embodiments, a vessel barcode has a particular length of nucleotides. For example, a vessel barcode can be about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 nucleotides in length.

[00308] In some embodiments, each a vessel barcode in a plurality of vessel barcodes has at least about 2 nucleotides. For example, each a vessel barcode in a plurality of vessel barcodes can be at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 nucleotides in length. In some embodiments, each a vessel barcode in a plurality of vessel barcodes has at most about 1000 nucleotides. For example, each a vessel barcode in a plurality of vessel barcodes can be at most about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 nucleotides in length. In some embodiments, each a vessel barcode in a plurality of vessel barcodes has the same length of nucleotides. For example, each a vessel barcode in a plurality vessel barcodes can be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 nucleotides in length. In some embodiments, one or more vessel barcodes in a plurality of vessel barcodes have a different length of nucleotides. For example one or more first vessel barcodes in a plurality of vessel barcodes can have about, or at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 nucleotides and one or more second vessel barcodes in a plurality of vessel barcodes can have about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 200, 500, or 1000 nucleotides, wherein the number of nucleotides of the one or more first vessel barcodes is different than the one or more second vessel barcodes.

[00309] The number of different vessel barcodes can be less than the total number of molecules to be labeled in a plurality of vessels. In some embodiments, the number of different vessel barcodes is at least about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times less than the total number of molecules to be labeled in a plurality of vessels.

[00310] The number of amplified product molecules from a vessel barcoded polynucleotide molecule in a single vessel can be in excess of the number of different molecules to be labeled in the single vessel. In some embodiments, the number of amplified product molecules from a vessel barcoded polynucleotide molecule in

a single vessel is at least about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times greater than the number of different molecules to be labeled in the single vessel.

[00311] The number of vessel barcoded polynucleotide molecules in a single vessel can be less than the number of different molecules to be labeled in the single vessel. In some embodiments, the number of vessel barcoded polynucleotide molecules in a single vessel is at least about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times less than the number of different molecules to be labeled in the single vessel.

[00312] The number of vessel barcoded polynucleotide molecules in a single vessel can be one molecule. The number of unamplified vessel barcoded polynucleotide molecules in a single vessel can be one molecule.

[00313] The vessel barcodes in a population of vessel barcodes can have at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more different sequences. For example, the vessel barcodes in a population can have at least 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, 1,000,000 or more different sequences. Thus, a plurality of vessel barcodes can be used to generate at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more different sequences from one or more polynucleotides, such as target polynucleotides. For example, a plurality of vessel barcodes can be used to generate at least 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, lxlO^{6}, 2xl0^{6}, 3xl0^{6}, 4xl0^{6}, 5xl0^{6}, 6xl0^{6}, 7xl0^{6}, 8xl0^{6}, 9xl0^{6}, lxlO^{7}, 2xl0^{7}, 3xl0^{7}, 4xl0^{7}, 5xl0^{7}, 6xl0^{7}, 7xl0^{7}, 8xl0^{7}, 9xl0^{7}, lxlO^{8}, 2xl0^{8}, 3xl0^{8}, 4xl0^{8}, 5xl0^{8}, 6xl0^{8}, 7xl0^{8}, 8xl0^{8}, 9xl0^{8}, lxlO^{9}, 2xl0^{9}, 3xl0^{9}, 4xl0^{9}, 5xl0^{9}, 6xl0^{9}, 7xl0^{9}, 8xl0^{9}, 9xl0^{9}, lxlO^{10}, 2xl0^{10}, 3xl0^{10}, 4xl0^{10}, 5xl0^{10}, 6xl0^{10}, 7xl0^{10}, 8xl0^{10}, 9xl0^{10}, lxlO^{11}, 2xlO^{11}, 3xl0^{11}, 4xlO^{11}, 5xl0^{11}, 6xlO^{11}, 7xlO^{11}, 8xl0^{11}, 9xlO^{11}, lxlO^{12}, 2xl0^{12}, 3xl0^{12}, 4xl0^{12}, 5xl0^{12}, 6xl0^{12}, 7xl0^{12}, 8xl0^{12}, 9xl0^{12} or more different sequences from one or more polynucleotides, such as target polynucleotides. For example, a plurality of vessel barcodes can be used to generate at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, lxlO^{6}, 2xl0^{6}, 3xl0^{6}, 4xl0^{6}, 5xl0^{6}, 6xl0^{6}, 7xl0^{6}, 8xl0^{6}, 9xl0^{6}, lxlO^{7}, 2xl0^{7}, 3xl0^{7}, 4xl0^{7}, 5xl0^{7}, 6xl0^{7}, 7xl0^{7}, 8xl0^{7}, 9xl0^{7}, lxlO^{8}, 2xl0^{8}, 3xl0^{8}, 4xl0^{8}, 5xl0^{8}, 6xl0^{8}, 7xl0^{8}, 8xl0^{8}, 9xl0^{8}, lxlO^{9}, 2xl0^{9}, 3xl0^{9}, 4xl0^{9}, 5xl0^{9}, 6xl0^{9}, 7xl0^{9}, 8xl0^{9}, 9xl0^{9}, lxlO^{10}, 2xl0^{10}, 3xl0^{10}, 4xl0^{10}, 5xl0^{10}, 6xl0^{10}, 7xl0^{10}, 8xl0^{10}, 9xl0^{10}, lxlO^{11}, 2xlO^{11}, 3xl0^{11}, 4xlO^{11}, 5xl0^{11}, 6xlO^{11}, 7xlO^{11}, 8xl0^{11}, 9xlO^{11}, lxlO^{12}, 2xl0^{12}, 3xl0^{12}, 4xl0^{12}, 5xl0^{12}, 6xl0^{12}, 7xl0^{12}, 8xl0^{12}, 9xl0^{12} or more different sequences from at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, lxlO^{6}, 2xl0^{6}, 3xl0^{6}, 4xl0^{6}, 5xl0^{6},

$6xl0^{6}$, $7xl0^{6}$, $8xl0^{6}$, $9xl0^{6}$, $lxlO^{7}$, $2xl0^{7}$, $3xl0^{7}$, $4xl0^{7}$, $5xl0^{7}$, $6xl0^{7}$, $7xl0^{7}$, $8xl0^{7}$, $9xl0^{7}$, $lxlO^{8}$, $2xl0^{8}$, $3xl0^{8}$, $4xl0^{8}$, $5xl0^{8}$, $6xl0^{8}$, $7xl0^{8}$, $8xl0^{8}$, $9xl0^{8}$, $lxlO^{9}$, $2xl0^{9}$, $3xl0^{9}$, $4xl0^{9}$, $5xl0^{9}$, $6xl0^{9}$, $7xl0^{9}$, $8xl0^{9}$, $9xl0^{9}$, $lxlO^{10}$, $2xl0^{10}$, $3xl0^{10}$, $4xl0^{10}$, $5xl0^{10}$, $6xl0^{10}$, $7xl0^{10}$, $8xl0^{10}$, $9xl0^{10}$, $lxlO^{11}$, $2xlO^{11}$, $3xlO^{11}$, $4xlO^{11}$, $5xlO^{11}$, $6xlO^{11}$, $7xlO^{11}$, $8xlO^{11}$, $9xlO^{11}$, $lxlO^{12}$, $2xl0^{12}$, $3xl0^{12}$, $4xl0^{12}$, $5xl0^{12}$, $6xl0^{12}$, $7xl0^{12}$, $8xl0^{12}$, $9xl0^{12}$ or more target polynucleotides.

[00314] In some embodiments, one or more vessel barcodes are used to group or bin sequences. In some embodiments, one or more vessel barcodes are used to group or bin sequences, wherein the sequences in each bin contain the same vessel barcode. In some embodiments, one or more vessel barcodes are used to group or bin sequences, wherein the sequences in each bin comprise one or more amplicon sets. In some embodiments, one or more vessel barcodes are used to group or bin sequences, wherein the sequences in each bin comprise a plurality of sequences wherein the polynucleotides from which the plurality of sequences were generated were derived from the polynucleotides from a single vessel or single cell.

XII. Flow Cytometric Isolation of Conjugates

[00315] Specific cell surface markers on T cells and APCs can be used to selectively isolate APC:T cell conjugates by cell sorting, e.g., by using a flow cytometer. Once labeled, stable APC:T cell conjugates can be partitioned from unconjugated APCs and T cells. In some embodiments, APC:T cell conjugates can be stabilized prior to sorting. For example, the APC:T cell conjugates can be fixed prior to the cell sorting to stabilize APC:T cell conjugates.

[00316] In some embodiments, the cell sorting can be performed under conditions to prevent destabilization of APC:T cell conjugates. For example, the cell sorting can be performed at a flow rate that prevents destabilization of APC:T cell conjugates. For example, the cell sorting can be performed at a pressure that prevents destabilization of APC:T cell conjugates. For example, the cell sorting can be performed in the presence of a fixative that prevents destabilization of APC:T cell conjugates.

[00317] The method can comprise contacting an agent (e.g., and antibody) comprising a marker to a culture of T cells and APCs, wherein the agent is specific to a T cell; detecting the marker; and isolating T cells conjugated to APCs based on detecting the marker. For example, the method can comprise contacting a CD3 antibody to a culture of T cells and APCs and performing flow cytometry to isolate T cells in conjugation with APCs.

[00318] The method can comprise contacting an agent (e.g., and antibody) comprising a marker to a culture of T cells and APCs, wherein the agent is specific to T cell subset; detecting the marker; and isolating T cells of the T cell subset conjugated to APCs based on detecting the marker. For example, the method can comprise contacting a CD4 or CD8 antibody to a culture of T cells and APCs and performing flow cytometry to isolate T cells in conjugation with APCs.

[00319] The method can comprise identifying an immunogenic neoantigen sequence from an APC:T cell conjugate , wherein the T cell can be a T cell that is stimulated in an antigen-specific manner. In some embodiments, the method can comprise contacting an agent (e.g., and antibody) comprising a marker to a culture of T cells and APCs, wherein the agent is specific to an activated T cell; detecting the marker; and

isolating activated T cells based on detecting the marker. For example, the method can comprise contacting a CD40L or CD137 antibody conjugated to a magnetic-bead to a culture of T cells and APCs and to isolate T cells that are bound to the bead.

[00320] The method can comprise identifying an immunogenic neoantigen sequence from APC:T cell conjugates, wherein the APC:T cell conjugates are isolated from unconjugated APCs. The method can comprise identifying an immunogenic neoantigen sequence, wherein the APC:T cell conjugates are isolated from unconjugated T cells. The method can comprise identifying an immunogenic neoantigen sequence, wherein the APC:T cell conjugates are isolated from unconjugated APCs and unconjugated T cells.

[00321] In some embodiments, the method can comprise contacting a first agent (e.g., a first antibody) comprising a first marker to a culture of T cells and APCs, wherein the first agent is specific to a T cell or a T cell subset; contacting a second agent (e.g., a second antibody) comprising a second marker to the culture of T cells and APCs, wherein the second agent is specific to an APC; detecting the first marker and the second marker; and isolating T cells conjugated to APCs based on detecting the first marker and the second marker. For example, the method can comprise contacting a CD11c or CD80 or CD86 antibody to a culture of T cells and APCs; contacting a CD3 or CD4 or CD8 antibody to the culture of T cells and APCs; and performing flow cytometry to isolate T cells conjugated to APCs. For example, the method can comprise contacting a CD11c or CD80 or CD86 antibody to a culture of T cells; contacting a CD3 or CD4 or CD8 antibody to the culture of T cells and APCs; identifying unconjugated T cells when the first marker is detected; and identifying APC:T cell conjugates when the first marker is detected and the second marker is detected. For example, the method can comprise contacting a CD11c or CD80 or CD86 antibody to a culture of T cells; contacting a CD3 or CD4 or CD8 antibody to the culture of T cells and APCs; identifying unconjugated APCs when the second marker is detected; and identifying APC:T cell conjugates when the first marker is detected and the second marker is detected. For example, the method can comprise contacting a CD11c or CD80 or CD86 antibody to a culture of T cells; contacting a CD3 or CD4 or CD8 antibody to the culture of T cells and APCs; identifying unconjugated T cells when the first marker is detected; identifying unconjugated APCs when the second marker is detected; and identifying APC:T cell conjugates when the first marker is detected and the second marker is detected. For example, the method can comprise contacting a CD11c or CD80 or CD86 antibody to a culture of T cells; contacting a CD3 or CD4 or CD8 antibody to the culture of T cells and APCs; and performing flow cytometry to differentiate unconjugated T cells from APC:T cell conjugates. For example, the method can comprise contacting a CD11c or CD80 or CD86 antibody to a culture of T cells; contacting a CD3 or CD4 or CD8 antibody to the culture of T cells and APCs; and performing flow cytometry to differentiate unconjugated APCs from APC:T cell conjugates.

[00322] In some embodiments, the method can comprise contacting an first agent (e.g., a first antibody) comprising a first marker to a culture of T cells and APCs, wherein the agent is specific to an activated T cell; contacting a second agent (e.g., a second antibody) comprising a second marker to the culture of T cells and APCs, wherein the second agent is specific to an APC; detecting the first marker and the second marker; and isolating activated T cells conjugated to APCs based on detecting the first and the second marker. For

example, the method can comprise contacting a CD137 or CD40L antibody to a culture of T cells; contacting a CDllc or CD80 or CD86 antibody to the culture of T cells and APCs; and identifying APC:T cell conjugates when the first marker is detected and the second marker is detected. For example, the method can comprise contacting a CD137 or CD40L antibody to a culture of T cells; contacting a CDllc or CD80 or CD86 antibody to the culture of T cells and APCs; and performing flow cytometry to differentiate unconjugated or unactivated T cells from APC:T cell conjugates.

[00323] The method can further comprise analyzing APCs that are not conjugated to T cells. For example, the method can further comprise determining an antigen sequence of an APC that is not conjugated to a T cell. The method can further comprise comparing an antigen sequence of an APC that is not conjugated to a T cell to a neoantigen sequence of an APC:T cell conjugate. For example, the method can further comprise comparing an antigen sequence of an APC that is not conjugated to a T cell to a neoantigen sequence of an APC:T cell conjugate to confirm antigen-specific interactions are selected. For example, the method can further comprise comparing an antigen sequence of an APC that is not conjugated to a T cell to a neoantigen sequence of an APC:T cell conjugate to confirm antigens selected are not from non-specific APCs.

[00324] The method can comprise identifying an immunogenic neoantigen sequence from an APC:T cell conjugate, wherein the T cell of an APC:T cell conjugate is a T cell that is stimulated in an antigen-specific manner. The method can comprise identifying an immunogenic neoantigen sequence from an APC:T cell conjugate, wherein the T cell of an APC:T cell conjugate is an activated T cell expressing a protein indicating antigen-specific activation. In some embodiments, the method can comprise contacting an agent (e.g., an antibody) comprising a marker to a culture of T cells and APCs, wherein the agent is specific to an activated T cell; detecting the marker; and isolating T cells conjugated to APCs based on detecting the marker.

XIII. Magnetic Bead Based Isolation of Conjugates

[00325] Magnetic bead-based cell isolation can be very fast and can require limited equipment. An activated T cell marker can be used to select activated T cells. In some embodiments, activated T cells can be captured independent of whether or not they are conjugated to APCs. In some embodiments, activated T cells can be captured when they are conjugated to APCs.

[00326] The method can comprise identifying an immunogenic neoantigen sequence from an APC:T cell conjugate, wherein the T cell of an APC:T cell conjugate is a T cell that is stimulated in an antigen-specific manner and the APC:T cell conjugate is bound to a solid support. The method can comprise identifying an immunogenic neoantigen sequence from an APC:T cell conjugate, wherein the T cell of an APC:T cell conjugate is an activated T cell expressing a protein indicating antigen-specific activation and the APC:T cell conjugate is bound to a solid support. In some embodiments, the method can comprise contacting a first agent (e.g., an antibody) comprising a marker to a culture of T cells and APCs, wherein the first agent is specific to an activated T cell; contacting a solid support comprising a second agent to the culture of T cells and APCs, wherein the second agent binds to the first agent; and isolating the solid support, thereby isolating T cells conjugated to APCs. In some embodiments, the method can comprise contacting a first agent (e.g., an antibody) comprising a marker to a culture of T cells and APCs, wherein the first agent is specific to an

activated T cell; contacting a solid support comprising a second agent to the culture of T cells and APCs, wherein the second agent binds to the first agent; and isolating T cells conjugated to APCs bound to the solid support. For example, the method can comprise contacting a CD137 antibody conjugated to a magnetic-bead to a culture of T cells and APCs and to isolate T cells conjugated to APCs that are bound to the bead. For example, the method can comprise contacting a CD40L antibody conjugated to a magnetic-bead to a culture of T cells and APCs and to isolate T cells conjugated to APCs that are bound to the bead.

[00327] In some embodiments, the isolation can be performed under conditions to prevent destabilization of APC:T cell conjugates bound to the solid support. For example, the isolation can be performed under less stringent washing conditions to prevent destabilization of bound APC:T cell conjugates. For example, the cell sorting can be performed in the presence of a fixative, e.g., as paraformaldehyde (PFA) that prevents destabilization of APC:T cell conjugates.

XIV. Isolating mRNA from APC:T cell Conjugates

[00328] The method can comprise isolating mRNA from APC:T cell conjugates. mRNA can be isolated from isolated APC:T cell conjugates by any method known in the art. In some embodiments, isolating mRNA from APC:T cell conjugates can be performed after isolating APC:T cell conjugates. In some embodiments, isolating mRNA from APC:T cell conjugates can be performed before isolating APC:T cell conjugates. In some embodiments, isolating mRNA from APC:T cell conjugates can be performed inside the vessels or compartments after partitioning APC:T cell conjugates.

[00329] A variety of extraction methods are available to extract polynucleotides from cells in a sample. For example, nucleic acids can be purified by organic extraction with phenol, phenol/chloroform/isoamyl alcohol, or similar formulations, including TRIzol and TriReagent. Other non-limiting examples of extraction techniques include organic extraction followed by ethanol precipitation, e.g., using a phenol/chloroform organic reagent, with or without the use of an automated nucleic acid extractor; stationary phase adsorption methods; and salt-induced nucleic acid precipitation methods, such precipitation methods. Another example of nucleic acid isolation and/or purification includes the use of magnetic particles to which nucleic acids can specifically or non-specifically bind, followed by isolation of the beads using a magnet, and washing and eluting the nucleic acids from the beads (e.g., U.S. Pat. No. 5,705,628). In some embodiments, the above isolation methods may be preceded by an enzyme digestion step to help eliminate unwanted protein from the sample, e.g., digestion with proteinase K, or other like proteases (e.g., U.S. Pat. No. 7,001,724). RNase inhibitors may be added to the lysis buffer. For certain cell or sample types, it may be desirable to add a protein denaturation/digestion step to the protocol. Purification methods may be directed to isolate DNA, RNA, or both. When both DNA and RNA are isolated together during or subsequent to an extraction procedure, further steps may be employed to purify one or both separately from the other. Sub-fractions of extracted nucleic acids can also be generated, for example, purification by size, sequence, or other physical or chemical characteristic. In addition to an initial nucleic acid isolation step, purification of nucleic acids can be performed after any step in the disclosed methods, such as to remove excess or unwanted reagents, reactants, or products. A variety of methods for determining the amount and/or purity of nucleic acids in a sample are

available, such as by absorbance (e.g., absorbance of light at 260 nm, 280 nm, and a ratio of these) and detection of a label (e.g., fluorescent dyes and intercalating agents, such as SYBR green, SYBR blue, DAPI, propidium iodine, Hoechst stain, SYBR gold, ethidium bromide).

[00330] Nucleic acid extraction techniques can incorporate addition of a protease or proteinase K (e.g., QIAGEN Protease or QIAGEN Proteinase K) to the sample solution prior to lysis. Upon lysis of the sample cells, a centrifugation step can be used to separate supernatant from cell debris. Next, an alcohol (e.g., ethanol, isopropanol) precipitation step can follow to extract the nucleic acid from the supernatant.

[00331] Alternatively, nucleic acid isolation or extraction kits can be utilized for extraction of DNA and/or RNA from a sample. Exemplary list of nucleic acid isolation or extraction kits include: QIAGEN's QIAamp viralRNA mini kit, QIAamp UltraSens virus kit, QIAamp MinElute virus spin kit; Life Technologies' PureLink® Pro 96 viral RNA/DNA purification kit, PureLink® viral RNA/DNA mini kit, MagMAX™ pathogen RNA/DNA kit; GeneOn's viral nucleic acid extraction kit; Zymo Research's ZR Viral RNA Kit™; and Roche's High Pure viral nucleic acid kit.

[00332] Methods of DNA extraction are well-known in the art. A classical DNA isolation protocol is based on extraction using organic solvents such as a mixture of phenol and chloroform, followed by precipitation with ethanol. Other methods include: salting out DNA extraction, trimethylammonium bromide salts DNA extraction and guanidinium thiocyanate DNA extraction. A variety of kits are commercially available for extracting DNA from biological samples (e.g., BD Biosciences Clontech (Palo Alto, CA): Epicentre Technologies (Madison, WI); Gentra Systems, Inc. (Minneapolis, MN); MicroProbe Corp. (Bothell, WA); Organon Teknika (Durham, NC); and Qiagen Inc. (Valencia, CA)).

[00333] Methods of RNA extraction are also well known in the art and several kits for RNA extraction from bodily fluids are commercially available (e.g., Ambion, Inc. (Austin, TX); Amersham Biosciences (Piscataway, NJ); BD Biosciences Clontech (Palo Alto, CA); BioRad Laboratories (Hercules, CA); Dynal Biotech Inc. (Lake Success, NY); Epicentre Technologies (Madison, WI); Gentra Systems, Inc. (Minneapolis, MN); GIBCO BRL (Gaithersburg, MD); Invitrogen Life Technologies (Carlsbad, CA); MicroProbe Corp. (Bothell, WA); Organon Teknika (Durham, NC); Promega, Inc. (Madison, WI); and Qiagen Inc. (Valencia, CA)).

XV. Generating cDNA from mRNA

Reverse Transcription

[00334] The method can comprise generating cDNA from mRNA. RNA polynucleotides can be reverse transcribed using suitable reagents known in the art. RNA can comprise mRNA.

[00335] As used herein, mRNA or an mRNA transcript can comprise pre-mRNA transcript(s), transcript processing intermediates, mature mRNA(s), or transcripts of one or more genes. Transcript processing may include splicing, editing and degradation. As used herein, a cDNA can comprise a nucleic acid reverse transcribed from an mRNA transcript. Other classes of RNAs that can be target polynucleotides include ribosomal RNA, snRNA, miRNA, non-coding RNA, siRNA, and viral RNA. Methods of the invention disclosed herein can use targets including relatively short regulatory non-coding RNAs, such as micro RNAs

(miRNAs), Piwi-interacting RNAs (piRNAs), snoRNAs, snRNAs, moRNAs, PARs, sdRNAs, tel-sRNAs, crasiRNAs, small interfering RNAs (siRNAs), long non-coding RNAs (long ncRNAs), traditional non-coding tRNAs, and ribosomal RNA (rRNA).

[00336] In some embodiments, a method can comprise reverse transcribing a target RNA polynucleotide to form cDNA using one or more primers (RT primers). In some embodiments, an RT primer can comprise an oligo-dT primer or a sequence specific primer. In some embodiments, a plurality of RT primers can comprise one or more oligo-dT primers or one or more sequence specific primers.

[00337] For example, the method can comprise reverse transcribing mRNA from APC:T cell conjugates to form cDNA. The reverse transcription can be carried out by one or more reverse transcriptases. In some instances, the reverse transcriptase can be a HIV-1 reverse transcriptase, M-MLV reverse transcriptase, AMV reverse transcriptase, and telomerase reverse transcriptase.

[00338] The temperature to carry out the reverse transcription reaction can depend on the reverse transcriptase being used. In some instances, a thermostable reverse transcriptase can be used and the reverse transcription reaction can be carried out at about 37° C to about 75° C, at about 37° C to about 50° C, at about 37° C to about 55° C, at about 37° C to about 60° C, at about 55° C to about 75° C, at about 55° C to about 60° C, at about 37° C, or at about 60° C.

Amplification

[00339] The method can comprise amplifying a polynucleic acid, e.g., mRNA from APC:T cell conjugates. The method can comprise amplifying cDNA reverse transcribed from mRNA from APC:T cell conjugates to form cDNA. Amplification of nucleic acids can be performed by any means known in the art. Nucleic acids can be amplified by polymerase chain reaction (PCR) or isothermal DNA amplification. Examples of PCR techniques that can be used include, but are not limited to, quantitative PCR, quantitative fluorescent PCR (QF-PCR), multiplex fluorescent PCR (MF-PCR), real time PCR (reverse transcription-PCR), single cell PCR, restriction fragment length polymorphism PCR (PCR-RFLP), PCR-RFLP/reverse transcription-PCR-RFLP, hot start PCR, nested PCR, in situ polony PCR, in situ rolling circle amplification (RCA), digital PCR (dPCR), droplet digital PCR (ddPCR), bridge PCR, PicoTiter PCR and emulsion PCR. Other suitable amplification methods include the ligase chain reaction (LCR), transcription amplification, molecular inversion probe (MIP) PCR, self-sustained sequence replication (3SR), selective amplification of polynucleotide sequences, consensus sequence primed polymerase chain reaction (CP-PCR), arbitrarily primed polymerase chain reaction (AP-PCR), degenerate polynucleotide-primed PCR (DOP-PCR) and nucleic acid based sequence amplification (NABSA), loop mediated isothermal amplification (LAMP), strand displacement amplification (SDA), whole genome amplification, multiple displacement amplification, helicase dependent amplification, nicking enzyme amplification reaction, recombinant polymerase amplification, reverse transcription PCR, ligation mediated PCR, methylation specific PCR, isothermal amplification, linear amplification, or isothermal linear amplification. Other amplification methods that can be used herein include those described in U.S. Pat. Nos. 5,242,794; 5,494,810; 4,988,617; and 6,582,938, as well as include Q beta replicase

mediated RNA amplification. Amplification can be isothermal amplification, e.g., isothermal linear amplification.

[00340] Additional methods that can be used to obtain a nucleic acid sequence include, e.g., array-based comparative genomic hybridization, detecting single nucleotide polymorphisms (SNPs) with arrays, subtelomeric fluorescence in situ hybridization (ST-FISH) (e.g., to detect submicroscopic copy-number variants (CNVs)), DNA microarray, high-density oligonucleotide microarray, whole-genome RNA expression array, peptide microarray, enzyme-linked immunosorbent assay (ELISA), genome sequencing, de novo sequencing, Pacific Biosciences SMRT sequencing, Genia Technologies nanopore single-molecule DNA sequencing, Oxford Nanopore single-molecule DNA sequencing, polony sequencing, copy number variation (CNV) analysis sequencing, small nucleotide polymorphism (SNP) analysis, immunohistochemistry (IHC), immunocytochemistry (ICC), mass spectrometry, tandem mass spectrometry, matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), in-situ hybridization, fluorescent in-situ hybridization (FISH), chromogenic in-situ hybridization (CISH), silver in situ hybridization (SISH), nCounter Analysis (NanoString technology), Western blotting, Southern blotting, SDS-PAGE, gel electrophoresis, and Northern blotting.

[00341] Thermocycling reactions can be performed to amplify the cDNA. Any DNA polymerase that catalyzes primer extension can be used, including but not limited to *E. coli* DNA polymerase, Klenow fragment of *E. coli* DNA polymerase 1, T7 DNA polymerase, T4 DNA polymerase, Taq polymerase, Pfu DNA polymerase, Vent DNA polymerase, bacteriophage 29, REDTaq™, Genomic DNA polymerase, or sequenase. Examples of polymerases include, but are not limited to, DNA polymerases and RNA polymerases. In some instances, the DNA polymerase can be a DNA polymerase I, DNA polymerase II, DNA polymerase III holoenzyme, and DNA polymerase IV. Commercially available DNA polymerases include, but are not limited to, Bst 2.0 DNA Polymerase, Bst 2.0 WarmStart™ DNA Polymerase, Bst DNA Polymerase, Sulfolobus DNA Polymerase IV, Taq DNA Polymerase, 9°N™m DNA Polymerase, Deep VentR™ (exo-) DNA Polymerase, Deep VentR™ DNA Polymerase, Hemo KlenTaq™, LongAmp® Taq DNA Polymerase, OneTaq® DNA Polymerase, Phusion® DNA Polymerase, Q5™ High-Fidelity DNA Polymerase, Therminator™ yDNA Polymerase, Therminator™ DNA Polymerase, Therminator™ II DNA Polymerase, Therminator™ III DNA Polymerase, VentR® DNA Polymerase, VentR® (exo-) DNA Polymerase, Bsu DNA Polymerase, phi29 DNA Polymerase, T4 DNA Polymerase, T7 DNA Polymerase, Terminal Transferase, Titanium® Taq Polymerase, KAPA Taq DNA Polymerase and KAPA Taq Hot Start DNA Polymerase.

[00342] In some instances, the polymerase can be an RNA polymerases such as RNA polymerase I, RNA polymerase II, RNA polymerase III, *E. coli* Poly(A) polymerase, phi6 RNA polymerase (RdRP), Poly(U) polymerase, SP6 RNA polymerase, and T7 RNA polymerase.

[00343] In some instances, a thermostable DNA polymerase can be used. A hot start PCR can also be performed wherein the reaction is heated to 95° C for two minutes prior to addition of the polymerase or the polymerase can be kept inactive until the first heating step in cycle 1. Hot start PCR can be used to minimize nonspecific amplification. Any number of PCR cycles can be used to amplify the DNA, e.g., about, more than

about, or less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45 cycles. The number of amplification cycles can be about 1-45, 10-45, 20-45, 30-45, 35-45, 10-40, 10-30, 10-25, 10-20, 10-15, 20-35, 25-35, 30-35, or 35-40.

[00344] Neoantigen-encoding sequences from APC:T cell conjugates can be enriched using any suitable method known in the art. In some embodiments, the cDNA molecules thus-formed can be operably linked with a nucleotide sequence suitable for the initiation of transcription, i.e., *in vitro* transcription (IVT), using a DNA-dependent RNA-polymerase (e.g., T7 RNA polymerase). Thus, the cDNA pool can be used as template material in an IVT reaction to generate a pool of RNA encoding neoantigens.

[00345] IVT reactions are, in general, amplification reactions, as they produce large amounts of RNA from minimal starting quantities of a DNA template. The DNA template can be amplified up to 1000-fold in an IVT reaction. IVT reactions utilize a DNA template (e.g., a cDNA molecule or pool of cDNA molecules) having an operably linked promoter initiation sequence, a DNA-dependent RNA polymerase (e.g., T7, SP6 or T3 RNA polymerases) and free ribonucleotide triphosphates (rNTPs) to enzymatically produce RNA molecules complementary to one strand of the starting DNA template.

[00346] The double-stranded cDNA IVT template is generally a linear molecule. The cDNA molecule can consist primarily of a cDNA sequence operably linked to the transcription promoter, or alternatively, the cDNA can be subcloned into a suitable vector (e.g., a bacteriophage λ-based vector, e.g., λ-gtl l or μ¾ï 12, or a circularized expression vector). In some embodiments, the circularized vector containing the cDNA can be linearized prior to the IVT reaction.

[00347] In these methods, the DNA-dependent RNA-polymerase can be used to generate either an antisense transcript (i.e., complementary, or cRNA) or a "sense" RNA transcript. A sense RNA transcript is a transcript that is produced in the same orientation as its corresponding endogenous transcript. That is, the sense transcript has the same orientation and the same, or substantially the same, nucleotide sequence as the primary mRNA transcript. In contrast, a cRNA has a sequence that is complementary to the corresponding mRNA product. Whether a sense or antisense product is formed is dependent on the orientation of transcription.

[00348] A wide variety of reagents and reaction conditions for performing IVT are known in the art, and which find use with the present invention. It is not intended that the present invention be limited to the IVT reaction conditions and reagents specifically recited herein, as these conditions are only exemplary in nature. Methods and reagents for IVT are common in the art and are available from various manufacturers, and are described in many sources, for example, Ausubel et al. (eds.), Current Protocols in Molecular Biology, Vol. 1-4, John Wiley & Sons, Inc., New York (1994) and Sambrook et al. (eds.), Molecular Cloning: A Laboratory Manual, Second Edition, Vol. 1-3, Cold Spring Harbor Laboratory Press, NY, (1989).

[00349] The RNA products generated by the IVT reaction can be sequenced or introduced into APCs. For example, the RNA products can be delivered to APCs for another round of selection. In some aspects, the methods described herein comprise *ex vivo* selection of immunogenic neoantigen sequences. In some embodiments, the method can comprise 2 or more rounds of *ex vivo* selection of immunogenic neoantigen

sequences. For example, the method can comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more rounds of *ex vivo* selection of immunogenic neoantigen sequences.

[00350] Amplification of polynucleotides can be performed by any means known in the art. Polynucleotides can be amplified by polymerase chain reaction (PCR) or isothermal DNA amplification. Examples of PCR techniques that can be used include, but are not limited to, quantitative PCR, quantitative fluorescent PCR (QF-PCR), multiplex fluorescent PCR (MF-PCR), real time PCR (RT-PCR), single cell PCR, restriction fragment length polymorphism PCR (PCR-RFLP), PCR-RFLP/RT-PCR-RFLP, hot start PCR, nested PCR, *in situ* polony PCR, *in situ* rolling circle amplification (RCA), digital PCR (dPCR), droplet digital PCR (ddPCR), bridge PCR, picotiter PCR and emulsion PCR. Other suitable amplification methods include the ligase chain reaction (LCR), transcription amplification, molecular inversion probe (MIP) PCR, self-sustained sequence replication, selective amplification of polynucleotide sequences, consensus sequence primed polymerase chain reaction (CP-PCR), arbitrarily primed polymerase chain reaction (AP-PCR), degenerate oligonucleotide-primed PCR (DOP-PCR) and nucleic acid based sequence amplification (NABSA). Other amplification methods can be used herein (U.S. Patent Nos. 5,242,794, 5,494,810, 4,988,617, and 6,582,938) including Q beta replicase mediated RNA amplification. In some embodiments, amplification reactions include polymerase chain reactions (PCRs), linear polymerase reactions, nucleic acid sequence-based amplification (NASBAs), or rolling circle amplifications. In some embodiments, amplification can be isothermal, multiple displacement amplification (MDA), whole genome amplification, degenerate oligonucleotide-primed PCR, polony PCR, *in situ* amplification (e.g., amplification of a polynucleotide on a support), emulsion PCR (ePCR), anchor PCR, RACE PCR, ligation chain reaction (LCR), self-sustained sequence replication, transcriptional amplification system, recursive PCR, and other amplification methods (U.S. Patent Nos. 4,683,195, 4,965,188, 4,683,202, 4,800,159, 5,210,015, 6,174,670, 5,399,491, 5,854,033, 6,391,544, 6,365,375, 6,294,323, 6,261,797, 6,124,090, 6,432,360 and 5,612,199; U.S. Publication Nos. 2009/0226975; and PCT publication Nos. WO 98/44151, WO 00/18957, WO 02/46456, WO 06/064199, and WO 07/071025 1). Amplification of polynucleotides can occur on a solid support, such as a bead. In other cases, amplification may not occur on a solid support. In some embodiments, amplification of one or more polynucleotides can occur on a solid support and amplification of one or more other polynucleotides may not occur on a solid support. Any number of cycles can be used to amplify a polynucleotide, for example, about, more than about, or less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45 cycles. The number of amplification cycles can be from about 1-45, 10-45, 20-45, 30-45, 35-45, 10-40, 10-30, 10-25, 10-20, 10-15, 20-35, 25-35, 30-35, or 35-40 cycles. In some embodiments, a DNA polymerase that catalyzes primer extension on a DNA template can be used for amplification. In some embodiments, an RNA polymerase that catalyzes primer extension on a RNA template can be used for amplification.

[00351] In some embodiments, amplification reactions include linear amplification reactions. In some embodiments, amplification reactions include non-exponential amplification reactions. In some embodiments, amplification reactions include exponential amplification reactions. In some embodiments, amplification

reactions utilize a pair of primers. In some embodiments, amplification reactions comprise extension of two primers, each hybridized to a complimentary strand of a polynucleotide.

[00352] An amplification reaction can comprise one or more additives. In some embodiments, the one or more additives can be dimethyl sulfoxide (DMSO), glycerol, betaine (mono)hydrate (N,N,N-trimethylglycine = [caroxy-methyl] trimethylammonium), trehalose, BSA (bovine serum albumin), formamide (methanamide), tetramethylammonium chloride (TMAC), other tetraalkylammonium derivatives (e.g., tetraethyammonium chloride (TEA-C1) and tetrapropylammonium chloride (TPrA-Cl), non-ionic detergent (e.g., Triton X-100, Tween-20, Nonidet P-40 (NP-40)), or PREXCEL-Q. In some embodiments, an amplification reaction can comprise 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different additives. In other cases, an amplification reaction can comprise at least 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different additives.

[00353] A polymerase chain reaction (PCR) can comprise an *in vitro* amplification reaction of specific polynucleotide sequences by the simultaneous primer extension of complementary strands of a double stranded polynucleotide. PCR reactions can produce copies of a template polynucleotide flanked by primer binding sites. The result, with two primers, is an exponential increase in template polynucleotide copy number of both strands with each cycle, because with each cycle both strands are replicated. The polynucleotide duplex has termini corresponding to the ends of primers used. PCR can comprise one or more repetitions of denaturing a template polynucleotide, annealing primers to primer binding sites, and extending the primers by a DNA or RNA polymerase in the presence of nucleotides. Particular temperatures, durations at each step, and rates of change between steps depend on many factors well-known to those of ordinary skill in the art. (McPherson et al., IRL Press, Oxford (1991 and 1995)). For example, in a conventional PCR using Taq DNA polymerase, a double stranded template polynucleotide can be denatured at a temperature >90° C, primers can be annealed at a temperature in the range 50-75° C, and primers can be extended at a temperature in the range 72-78° C. In some embodiments, PCR comprises Reverse transcription PCR (RT-PCR), real-time PCR, nested PCR, quantitative PCR, multiplexed PCR, or the like. In some embodiments, PCR does not comprise RT-PCR. (U.S. Patent Nos. 5,168,038, 5,210,015, 6,174,670, 6,569,627, and 5,925,517; Mackay et al, Nucleic Acids Research, 30: 1292-1305 (2002)). RT-PCR comprises a PCR reaction preceded by a reverse transcription reaction and a resulting cDNA is amplified, Nested PCR comprises a two-stage PCR wherein an amplicon of a first PCR reaction using a first set of primers becomes the sample for a second PCR reaction using a second primer set, at least one of which binds to an interior location of an amplicon of a first PCR reaction. Multiplexed PCR can comprise a PCR reaction, wherein a plurality of polynucleotide sequences are carried out in the same reaction mixture simultaneously. PCR reaction volumes can be anywhere from 0.2 nL-1000 μL. Quantitative PCR comprises a PCR reaction designed to measure an absolute or relative amount, abundance, or concentration of one or more sequences in a sample. Quantitative measurements can include comparing one or more reference sequences or standards to a polynucleotide sequence of interest. (Freeman et al., Biotechniques, 26: 112-126 (1999); Becker-Andre et al., Nucleic Acids Research, 17: 9437-9447 (1989); Zimmerman et al., Biotechniques, 21: 268-279 (1996); Diviacco et al., Gene, 122: 3013-3020 (1992); Becker-Andre et al., Nucleic Acids Research, 17: 9437-9446 (1989)).

[00354] In some embodiments, the methods, kits, and compositions disclosed herein may comprise a support. As used herein, a solid support can comprise one or more materials comprising one or more rigid or semi-rigid surfaces. In some embodiments, the support can be a non-solid support. The support or substrate may comprise a membrane, paper, plastic, coated surface, flat surface, glass, slide, chip, or any combination thereof. In some embodiments, one or more surfaces of a support are substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different compounds with, for example, wells, raised regions, pins, etched trenches, or the like. In some embodiments, solid supports can comprise beads, resins, gels, microspheres, or other geometric configurations. Alternatively, solid supports can comprise silica chips, microparticles, nanoparticles, plates, and arrays. The solid support can comprise the use of beads that self-assemble in microwells. For example, the solid support can comprise Illumina's BeadArray Technology. Alternatively, the solid support can comprise Abbott Molecular's Bead Array technology, and Applied Microarray's FlexiPlex™ system. In other instances, the solid support can be a plate. Examples of plates include, but are not limited to, MSD multi-array plates, MSD Multi-Spot® plates, microplate, ProteOn microplate, AlphaPlate, DELFIA plate, IsoPlate, and LumaPlate. In some embodiments, a support can comprise a plurality of beads. In some embodiments, a support can comprise an array. In some embodiments, a support can comprise a glass slide. Methods, substrates, and techniques applicable to polymers (U.S. Patent Nos. 5,744,305, 5,143,854, 5,242,974, 5,252,743, 5,324,633, 5,384,261, 5,405,783, 5,424,186, 5,451,683, 5,482,867, 5,491,074, 5,527,681, 5,550,215, 5,571,639, 5,578,832, 5,593,839, 5,599,695, 5,624,711, 5,631,734, 5,795,716, 5,831,070, 5,837,832, 5,856,101, 5,858,659, 5,936,324, 5,968,740, 5,974,164, 5,981,185, 5,981,956, 6,025,601, 6,033,860, 6,040,193, 6,090,555, 6,136,269, 6,269,846 and 6,428,752; US Patent Pub. Nos. 20090149340, 20080038559, 20050074787; and in PCT Publication Nos. WO 00/58516, WO 99/36760, and WO 01/58593). The attachment of the polynucleotides to a support may comprise amine-thiol crosslinking, maleimide crosslinking, N-hydroxysuccinimide or N-hydroxysulfosuccinimide, Zenon or SiteClick. Attaching the labeled nucleic acids to the support may comprise attaching biotin to the plurality of polynucleotides and coating the one or more beads with streptavidin. In some embodiments, the solid support is a bead. Examples of beads include, but are not limited to, streptavidin beads, agarose beads, magnetic beads, Dynabeads®, MACS® microbeads, antibody conjugated beads (e.g., anti-immunoglobulin microbead), protein A conjugated beads, protein G conjugated beads, protein A/G conjugated beads, protein L conjugated beads, oligodT conjugated beads, silica beads, silica-like beads, anti-biotin microbead, anti-fluorochrome microbead, and BcMag™ Carboxy-Terminated Magnetic Beads. The diameter of the beads may be about 5 μm, 10 μm, 20 μm, 25 μm, 30 μm, 35 μm, 40 μm, 45 μm or 50 μm. The solid support may be an array or microarray. The solid support may comprise discrete regions. The solid support may be an array, such as an addressable array.

XVI. Identification of Immunogenic Neoantigens

[00355] The method can comprise sequencing a polynucleic acid, e.g., mRNA from an APC:T cell conjugates. The method can comprise sequencing a cDNA reverse transcribed from mRNA from an APC:T cell conjugate. The method can comprise sequencing an amplicon of a polynucleic acid, e.g., mRNA from an APC:T cell

conjugate. The method can comprise sequencing an amplicon of a cDNA reverse transcribed from mRNA from an APC:T cell conjugate.

[00356] Sequencing can be performed by any sequencing method known in the art. The sequencing method employed can be a high-throughput sequencing method. The sequencing method employed can be a next-generation sequencing method. In some embodiments, sequencing can be performed in high throughput. Suitable next generation sequencing technologies include the 454 Life Sciences platform (Roche, Branford, CT) (Margulies et al, Nature, 437, 376-380 (2005)); lllumina's Genome Analyzer, GoldenGate Methylation Assay, or Infinium Methylation Assays, i.e., Infinium HumanMethylation 27K BeadArray or VeraCode GoldenGate methylation array (Illumina, San Diego, CA; Bibkova et al., Genome Res. 16, 383-393 (2006); and U.S. Patent Nos. 6,306,597, 7,598,035, 7,232,656), or DNA Sequencing by Ligation, SOLiD System (Applied Biosystems/Life Technologies; U.S. Patent Nos. 6,797,470, 7,083,917, 7,166,434, 7,320,865, 7,332,285, 7,364,858, and 7,429,453); or the Helicos True Single Molecule DNA sequencing technology (Harris et al., Science, 320, 106-109 (2008); and U.S. Patent Nos. 7,037,687, 7,645,596, 7,169,560, and7,769,400), the single molecule, real-time (SMRTTm) technology of Pacific Biosciences, and sequencing (Soni et al., Clin. Chem. 53, 1996-2001 (2007)). These systems allow multiplexed parallel sequencing of many polynucleotides isolated from a sample (Dear, Brief Funct. Genomic Proteomic, 1(4), 397-416 (2003) and McCaughan et al, J. Pathol., 220, 297-306 (2010)). In some embodiments, polynucleotides are sequenced by sequencing by ligation of dye-modified probes, pyrosequencing, or single-molecule sequencing. Determining the sequence of a polynucleotide may be performed by sequencing methods such as Helioscope™ single molecule sequencing, Nanopore DNA sequencing, Lynx Therapeutics' Massively Parallel Signature Sequencing (MPSS), 454 pyrosequencing, Single Molecule real time (RNAP) sequencing, Illumina (Solexa) sequencing, SOLiD sequencing, Ion Torrent™, Ion semiconductor sequencing, Single Molecule SMRT(™) sequencing, Polony sequencing, DNA nanoball sequencing, and VisiGen Biotechnologies approach. Alternatively, determining the sequence of polynucleotides may use sequencing platforms, including, but not limited to, Genome Analyzer IIx, HiSeq, and MiSeq offered by Illumina, Single Molecule Real Time (SMRT™) technology, such as the PacBio RS system offered by Pacific Biosciences (California) and the Solexa Sequencer, True Single Molecule Sequencing (tSMS™) technology such as the HeliScope™ Sequencer offered by Helicos Inc. (Cambridge, MA). Sequencing can comprise MiSeq sequencing. Sequencing can comprise HiSeq sequencing. In some embodiments, determining the sequence of a polynucleotide comprises paired-end sequencing, nanopore sequencing, high-throughput sequencing, shotgun sequencing, dye-terminator sequencing, multiple-primer DNA sequencing, primer walking, Sanger dideoxy sequencing, Maxim-Gilbert sequencing, pyrosequencing, true single molecule sequencing, or any combination thereof. Alternatively, the sequence of a polynucleotide can be determined by electron microscopy or a chemical-sensitive field effect transistor (chemFET) array.

[00357] A sequence of a polynucleotide from an APC of an APC:T cell conjugate can be identified as an immunogenic neoantigen. In some embodiments, an immunogenic neoantigen identified according to the methods described herein is a differentiation antigen expressed in a tumor and cells of the type of tissue from

which they are generated. In some embodiments, an immunogenic neoantigen identified according to the methods described herein is a cancer/germ line antigens not expressed in another differentiated tissue. In some embodiments, an immunogenic neoantigen identified according to the methods described herein is a mutated antigen. For example, an immunogenic neoantigen identified according to the methods described herein can comprise a missense-point mutation or a neoantigen of a fusion protein generated through tumor specific translocation of a gene segment. In some embodiments, an immunogenic neoantigen identified according to the methods described herein is an over-expressed antigen. In some embodiments, an immunogenic neoantigen is found in tumors. For example, an immunogenic neoantigen identified according to the methods described herein includes a protein whose expression is strictly regulated in cells of differentiated normal tissue.

[00358] In some embodiments, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen comprises determining the neoantigen encoded by the sequenced polynucleotide; and selecting the neoantigen as an immunogenic neoantigen.

[00359] In some embodiments, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen comprises comparing barcode sequences. In some embodiments, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen comprises comparing neoantigen sequences. In some embodiments, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen comprises comparing neoantigen sequences and barcode sequences. For example, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen can comprise comparing a neoantigen sequence and a barcode sequence from a first sequenced polynucleotide to a neoantigen sequence and a barcode sequence from a second sequenced polynucleotide. For example, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen can comprise comparing a neoantigen sequence and a barcode sequence from a first sequenced polynucleotide to a neoantigen sequence and a barcode sequence from a second sequenced polynucleotide; and selecting the neoantigen as an immunogenic neoantigen when the neoantigen sequence of the first and second sequenced polynucleotides are the same and the barcode sequences of the of the first and second sequenced polynucleotides are different.

[00360] In some embodiments, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen comprises comparing a barcode sequence of a first sequenced polynucleotide to a barcode sequence of a second sequenced polynucleotide. For example, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen can comprise comparing a barcode sequence of a first sequenced polynucleotide to a barcode sequence of one or more other sequenced polynucleotides.

[00361] In some embodiments, identifying a sequence of a polynucleotide from a first APC of a first APC:T cell conjugate as an immunogenic neoantigen comprises comparing a barcode sequence of a first sequenced polynucleotide from the first APC of a first APC:T cell conjugate to a barcode sequence of a second

sequenced polynucleotide from a second APC of a second APC:T cell conjugate. For example, the first neoantigen sequence can be selected as an immunogenic neoantigen when the barcode of the first sequenced polynucleotide is different than the barcode of the second sequenced polynucleotide.

[00362] In some embodiments, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen comprises comparing a barcode sequence of a first sequenced polynucleotide encoding a first neoantigen to a barcode sequence of a second sequenced polynucleotide encoding the first neoantigen. For example, recovering multiple barcodes associated with a given neoantigen sequence can provide confidence that the interaction is functionally significant. In some embodiments, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen comprises comparing a barcode sequence of a first sequenced polynucleotide encoding a first neoantigen to a barcode sequence of a second sequenced polynucleotide encoding the first neoantigen; and selecting the first neoantigen as an immunogenic neoantigen when the barcode of the first sequenced polynucleotide is different than the barcode of the second sequenced polynucleotide.

[00363] In some embodiments, identifying a sequence of a polynucleotide from an APC of an APC:T cell conjugate as an immunogenic neoantigen comprises comparing a barcode sequence of a first sequenced polynucleotide from an APC of an APC:T cell conjugate encoding a first neoantigen to a barcode sequence of a second sequenced polynucleotide encoding the first neoantigen from an APC that is not conjugated to a T cell. For example, the first neoantigen sequence can be not selected as an immunogenic neoantigen when the barcode of the first sequenced polynucleotide is different than the barcode of the second sequenced polynucleotide.

XVII. Therapeutic Compositions and Methods of Treatment

[00364] The present disclosure provides methods of treatment comprising an immunogenic therapy. Methods of treatment for a disease (such as cancer or a viral infection) are provided. A method can comprise administering to a subject an effective amount of a composition comprising an immunogenic antigen identified according to the methods provided herein. In some embodiments, the antigen comprises a viral antigen. In some embodiments, the antigen comprises a tumor antigen.

[00365] Non-limiting examples of therapeutics that can be prepared include a peptide-based therapy, a nucleic acid-based therapy, an antibody based therapy, a T cell based therapy, and an antigen-presenting cell based therapy.

[00366] Therapeutic compositions can be formulated using one or more physiologically acceptable carriers including excipients and auxiliaries which facilitate processing of the active agents into preparations which can be used pharmaceutically. Proper formulation can be dependent upon the route of administration chosen. Any of the well-known techniques, carriers, and excipients can be used as suitable and as understood in the art.

[00367] In some cases, the therapeutic composition is formulated as a peptide-based therapy, a nucleic acid-based therapy, an antibody based therapy, or a cell based therapy. For example, a therapeutic composition can include naked cDNA in cationic lipid formulations; lipopeptides (e.g., Vitiello, A. et al, J. Clin. Invest. 95:341, 1995), naked cDNA or peptides, encapsulated e.g., in poly(DL-lactide-co-glycolide) ("PLG")

microspheres (see, e.g., Eldridge, et al., Molec. Immunol. 28:287-294, 1991: Alonso et al, Vaccine 12:299-306, 1994; Jones et al, Vaccine 13:675-681, 1995); peptide composition contained in immune stimulating complexes (ISCOMS) (e.g., Takahashi et al., Nature 344: 873-875, 1990; Hu et al, Clin Exp Immunol. 113:235-243, 1998); or multiple antigen peptide systems (MAPs) (see e.g., Tarn, J. P., Proc. Natl Acad. Sci. U.S.A. 85:5409-5413, 1988; Tarn, J.P., J. Immunol. Methods 196: 17-32, 1996). Sometimes, a therapeutic is formulated as a peptide-based therapeutic, or nucleic acid based therapuetic in which the nucleic acid encodes the polypeptides. Sometimes, a therapuetic is formulated as an antibody based therapuetic. Sometimes, a therapuetic is formulated as a cell based therapuetic.

[00368] The amino acid sequence of an identified disease-specific immunogenic neoantigen peptide can be used develop a pharmaceutically acceptable composition. The source of antigen can be, but is not limited to, natural or synthetic proteins, including glycoproteins, peptides, and superantigens; antibody/antigen complexes; lipoproteins; RNA or a translation product thereof; and DNA or a polypeptide encoded by the DNA. The source of antigen may also comprise non-transformed, transformed, transfected, or transduced cells or cell lines. Cells may be transformed, transfected, or transduced using any of a variety of expression or retroviral vectors known to those of ordinary skill in the art that may be employed to express recombinant antigens. Expression may also be achieved in any appropriate host cell that has been transformed, transfected, or transduced with an expression or retroviral vector containing a DNA molecule encoding recombinant antigen(s). Any number of transfection, transformation, and transduction protocols known to those in the art may be used. Recombinant vaccinia vectors and cells infected with the vaccina vector, may be used as a source of antigen.

[00369] A composition can comprise a synthetic disease-specific immunogenic neoantigen peptide. A composition can comprise two or more disease-specific immunogenic neoantigen peptides. A composition may comprise a precursor to a disease-specific immunogenic peptide (such as a protein, peptide, DNA and RNA). A precursor to a disease-specific immunogenic peptide can generate or be generated to the identified disease-specific immunogenic neoantigen peptide. In some embodiments, a therapeutic composition comprises a precursor of an immunogenic peptide. The precursor to a disease-specific immunogenic peptide can be a pro-drug. In some embodiments, the composition comprising a disease-specific immunogenic neoantigen peptide may further comprise an adjuvant. For example, the neoantigen peptide can be utilized as a vaccine. In some embodiments, an immunogenic therapuetic may comprise a pharmaceutically acceptable immunogenic neoantigen peptide identified according to the methods described herein. In some embodiments, an immunogenic therapuetic may comprise a pharmaceutically acceptable precursor to an immunogenic neoantigen peptide identified according to the methods described herein (such as a protein, peptide, DNA and RNA). In some embodiments, a method of treatment comprises administering to a subject an effective amount of an antibody specifically recognizing an immunogenic neoantigen peptide identified according to the methods described herein. In some embodiments, a method of treatment comprises administering to a subject an effective amount of a soluble TCR or TCR analog specifically recognizing an immunogenic neoantigen peptide identified according to the methods described herein.

[00370] In some embodiments, the cancer is selected from the group consisting of carcinoma, lymphoma, blastoma, sarcoma, leukemia, squamous cell cancer, lung cancer (including small cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, head and neck cancer, colorectal cancer, rectal cancer, soft-tissue sarcoma, Kaposi's sarcoma, B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL), small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's macroglobulinemia), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), myeloma, Hairy cell leukemia, chronic myeloblasts leukemia, and post-transplant lymphoproliferative disorder (PTLD), abnormal vascular proliferation associated with phakomatoses, edema, Meigs' syndrome, and combinations thereof.

[00371] The methods described herein are particularly useful in the personalized medicine context, where immunogenic neoantigen peptides identified according to the methods described herein are used to develop therapeutics (such as vaccines or therapeutic antibodies) for the same individual. Thus, a method of treating a disease in a subject can comprise identifying an immunogenic neoantigen peptide in a subject according to the methods described herein; and synthesizing the peptide (or a precursor thereof); and administering the peptide or an antibody specifically recognizing the peptide to the subject. In some embodiments, an expression pattern of an immunogenic neoantigen identified according to the methods described herein can serve as the essential basis for the generation of patient specific therapuetics. In some embodiments, an expression pattern of an immunogenic neoantigen identified according to the methods described herein can serve as the essential basis for the generation of a therapuetic for a group of patients with a particular disease. Thus, particular diseases, e.g., particular types of tumors, can be selectively treated in a patient group.

[00372] In some embodiments, the methods described herein can be used to identify structurally normal ("shared") antigens that can be recognized by autologous anti-disease T cells in a large patient group. In some embodiments, the methods described herein can be used to identify an antigen-expression pattern of a group of diseased subjects whose disease expresses structurally normal ("shared") neoantigens.

[00373] There are a variety of ways in which to produce immunogenic neoantigens. Proteins or peptides may be made by any technique known to those of skill in the art, including the expression of proteins, polypeptides or peptides through standard molecular biological techniques, the isolation of proteins or peptides from natural sources, in vitro translation, or the chemical synthesis of proteins or peptides. In general, such disease specific neoantigens may be produced either in vitro or in vivo. Immunogenic neoantigens may be produced in vitro as peptides or polypeptides, which may then be formulated into a personalized therapuetic or immunogenic composition and administered to a subject. In vitro production of immunogenic neoantigens can comprise

peptide synthesis or expression of a peptide/polypeptide from a DNA or RNA molecule in any of a variety of bacterial, eukaryotic, or viral recombinant expression systems, followed by purification of the expressed peptide/polypeptide. Alternatively, immunogenic neoantigens can be produced in vivo by introducing molecules (e.g., DNA, RNA, and viral expression systems) that encode an immunogenic neoantigen into a subject, whereupon the encoded immunogenic neoantigens are expressed. In some embodiments, a polynucleotide encoding an immunogenic neoantigen peptide identified according to the methods described herein can be used to produce the neoantigen peptide in vitro.

[00374] In some embodiments, a polynucleotide comprises a sequence with at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a polynucleotide encoding an immunogenic neoantigen identified according to the methods described herein.

[00375] The polynucleotide may be, e.g., DNA, cDNA, PNA, CNA, RNA, single- and/or double-stranded, native or stabilized forms of polynucleotides, or combinations thereof. A nucleic acid encoding an immunogenic neoantigen peptide may or may not contain introns so long as it codes for the peptide. In one embodiment in vitro translation is used to produce the peptide.

[00376] Expression vectors comprising sequences encoding the neoantigen, as well as host cells containing the expression vectors, are also contemplated. Expression vectors suitable for use in the present invention can comprise at least one expression control element operationally linked to the nucleic acid sequence. The expression control elements are inserted in the vector to control and regulate the expression of the nucleic acid sequence. Examples of expression control elements are well known in the art and include, for example, the lac system, operator and promoter regions of phage lambda, yeast promoters and promoters derived from polyoma, adenovirus, retrovirus or SV40. Additional operational elements include, but are not limited to, leader sequences, termination codons, polyadenylation signals and any other sequences necessary or preferred for the appropriate transcription and subsequent translation of the nucleic acid sequence in the host system. It will be understood by one skilled in the art the correct combination of expression control elements will depend on the host system chosen. It will further be understood that the expression vector should contain additional elements necessary for the transfer and subsequent replication of the expression vector containing the nucleic acid sequence in the host system. Examples of such elements include, but are not limited to, origins of replication and selectable markers.

[00377] The neoantigen peptides may be provided in the form of RNA or cDNA molecules encoding the desired neoantigen peptides. One or more neoantigen peptides of the invention may be encoded by a single expression vector. Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression, if necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognized by the desired host (e.g., bacteria), although such controls are generally available in the expression vector. The vector is then introduced into the host bacteria for cloning using standard techniques. Useful expression vectors for eukaryotic hosts, especially mammals or humans include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for

bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single-stranded DNA phages.

[00378] In embodiments, a DNA sequence encoding a polypeptide of interest can be constructed by chemical synthesis using an oligonucleotide synthesizer. Such oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest is produced. Standard methods can be applied to synthesize an isolated polynucleotide sequence encoding an isolated polypeptide of interest.

[00379] Suitable host cells for expression of a polypeptide include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin. Cell-free translation systems can also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are well known in the art. Various mammalian or insect cell culture systems can be employed to express recombinant protein. Exemplary mammalian host cell lines include, but are not limited to COS-7, L cells, C127, 3T3, Chinese hamster ovary (CHO), 293, HeLa and BHK cell lines. Mammalian expression vectors can comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

[00380] The proteins produced by a transformed host can be purified according to any suitable method. Such standard methods include chromatography (e.g., ion exchange, affinity and sizing column chromatography, and the like), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexahistidine, maltose binding domain, influenza coat sequence, glutathione-S-transferase, and the like can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, nuclear magnetic resonance and x-ray crystallography.

[00381] A therapy can comprise an entity that binds a polypeptide sequence described herein. The entity can be an antibody. Antibody-based therapies can be formulated using any of the well-known techniques, carriers, and excipients as suitable and as understood in the art. In some embodiments, the peptides described herein can be used for making neoantigen specific therapeutics such as antibody therapeutics. For example, neoantigens can be used to raise and/or identify antibodies specifically recognizing the neoantigens. These antibodies can be used as therapeutics. The antibody can be a natural antibody, a chimeric antibody, a humanized antibody, or can be an antibody fragment. The antibody may recognize one or more of the polypeptides described herein. The antibody can recognize a polypeptide that has a sequence that is at most 40%, 50%, 60%, 70%, 80%, 90%, 95%, or less in sequence homology to a polypeptide described herein. The antibody can recognize a polypeptide that has a sequence that is at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more in sequence homology to a polypeptide described herein. The antibody can also recognize a

polypeptide with sequence length is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more in sequence length compared to a polypeptide described herein. The antibody can recognize a polypeptide with a sequence length at most 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or less in sequence length compared to a polypeptide described herein.

[00382] The present invention also contemplates the use of nucleic acid molecules as vehicles for delivering neoantigen peptides/polypeptides to the subject in need thereof, *in vivo,* in the form of, e.g., DNA/RNA therapuetics.

[00383] In some embodiments, the therapy can be a nucleic acid therapy. In one embodiment, neoantigens can be administered to a subject by use of a plasmid. Plasmids may be introduced into animal tissues by a number of different methods, e.g., injection or aerosol instillation of naked DNA on mucosal surfaces, such as the nasal and lung mucosa. In some embodiments, physical delivery, such as with a "gene-gun" may be used. The exact choice of expression vectors can depend upon the peptide/polypeptides to be expressed, and is well within the skill of the ordinary artisan.

[00384] In some embodiments, the nucleic acid can encode an immunogenic peptide or peptide precursor. In some embodiments, the nucleic acid therapy can comprise sequences flanking the sequence coding the immunogenic peptide or peptide precursor. In some embodiments, the nucleic acid therapycan comprise more than one immunogenic epitope. In some embodiments, the nucleic acid therapycan be a DNA-based therapy. In some embodiments, the nucleic acid therapycan be a RNA-based therapy. In some embodiments, the RNA-based therapycan comprise mRNA. In some embodiments, the RNA-based therapy can comprise naked mPvNA. In some embodiments, the RNA-based therapy can comprise modified mRNA (e.g., mRNA protected from degradation using protamine. mRNA containing modified 5' CAP structure or mRNA containing modified nucleotides). In some embodiments, the RNA-based therapy can comprise single-stranded mRNA.

[00385] The polynucleotide may be substantially pure, or contained in a suitable vector or delivery system. Suitable vectors and delivery systems include viral, such as systems based on adenovirus, vaccinia virus, retroviruses, herpes virus, adeno-associated virus or hybrids containing elements of more than one virus. Non-viral delivery systems include cationic lipids and cationic polymers (e.g., cationic liposomes).

[00386] One or more neoantigen peptides can be encoded and expressed *in vivo* using a viral based system. Viral vectors may be used as recombinant vectors in the present invention, wherein a portion of the viral genome is deleted to introduce new genes without destroying infectivity of the virus. The viral vector of the present invention is a nonpathogenic virus. In one embodiment the viral vector has a tropism for a specific cell type in the mammal. In another embodiment, the viral vector of the present invention is able to infect professional antigen presenting cells such as dendritic cells and macrophages. In yet another embodiment of the present invention, the viral vector is able to infect any cell in the mammal. The viral vector may also infect tumor cells. Viral vectors used in the present invention include but is not limited to Poxvirus such as vaccinia virus, *avipox* virus, fowlpox virus and a highly attenuated vaccinia virus (Ankara or MVA), retrovirus, adenovirus, baculovirus and the like.

[00387] The agents and compositions provided herein may be used alone or in combination with conventional therapeutic regimens such as surgery, irradiation, chemotherapy and/or bone marrow transplantation (autologous, syngeneic, allogeneic or unrelated). A set of tumor antigens can be identified using the methods described herein and are useful, e.g., in a large fraction of cancer patients.

[00388] In some embodiments, at least one or more chemotherapeutic agents may be administered in addition to the composition comprising an immunogenic therapy. In some embodiments, the one or more chemotherapeutic agents may belong to different classes of chemotherapeutic agents.

XVIII. Pharmaceutical Compositions

[00389] Identified neoantigens that induce an immune response can be used as a composition when combined with an acceptable carrier or excipient. Such compositions are useful for *in vitro* or *in vivo* analysis or for administration to a subject *in vivo* or *ex vivo* for treating a subject with a disease.

[00390] Thus pharmaceutical compositions can include, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration.

[00391] Pharmaceutical formulations comprising a protein of interest, e.g., an immunogenic neoantigen identified by the methods described herein can be prepared for storage by mixing the neoantigen having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Oslo, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are those that are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS® or polyethylene glycol (PEG).

[00392] Acceptable carriers are physiologically acceptable to the administered patient and retain the therapeutic properties of the compounds with/in which it is administered. Acceptable carriers and their formulations are generally described in, for example, Remington' pharmaceutical Sciences (18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA 1990). One exemplary carrier is physiological saline. A pharmaceutically acceptable carrier is a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or

transporting the subject compounds from the administration site of one organ, or portion of the body, to another organ, or portion of the body, or in an *in vitro* assay system. Acceptable carriers are compatible with the other ingredients of the formulation and not injurious to a subject to whom it is administered. Nor should an acceptable carrier alter the specific activity of the neoantigens.

[00393] In one aspect, provided herein are pharmaceutically acceptable or physiologically acceptable compositions including solvents (aqueous or non-aqueous), solutions, emulsions, dispersion media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration. Pharmaceutical compositions or pharmaceutical formulations therefore refer to a composition suitable for pharmaceutical use in a subject. The pharmaceutical compositions and formulations include an amount of a neoantigen (or polynucleotide encoding a neoantigen) and a pharmaceutically or physiologically acceptable carrier. Compositions can be formulated to be compatible with a particular route of administration (i.e., systemic or local). Thus, compositions include carriers, diluents, or excipients suitable for administration by various routes.

[00394] In some embodiments, a composition can further comprise an acceptable additive in order to improve the stability of the neoantigen in the composition and/or to control the release rate of the composition. Acceptable additives may not alter the specific activity of the neoantigens. Exemplary acceptable additives include, but are not limited to, a sugar such as mannitol, sorbitol, glucose, xylitol, trehalose, sorbose, sucrose, galactose, dextran, dextrose, fructose, lactose and mixtures thereof. Acceptable additives can be combined with acceptable carriers and/or excipients such as dextrose. Alternatively, exemplary acceptable additives include, but are not limited to, a surfactant such as polysorbate 20 or polysorbate 80 to increase stability of the peptide and decrease gelling of the solution. The surfactant can be added to the composition in an amount of 0.01% to 5% of the solution. Addition of such acceptable additives increases the stability and half-life of the composition in storage.

[00395] The pharmaceutical composition can be administered, for example, by injection. Compositions for injection include aqueous solutions (e.g., water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Antibacterial and antifungal agents include, for example, parabens, chlorobutanol, phenol, ascorbic acid and thimerosal. Isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride can be included in the composition. The resulting solutions can be packaged for use as is, or lyophilized; the lyophilized preparation can later be combined with a sterile solution prior to administration. For intravenous, injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well

able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as needed. Sterile injectable solutions can be prepared by incorporating an active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active ingredient into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation can be vacuum drying and freeze drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00396] Compositions can be conventionally administered intravenously, such as by injection of a unit dose, for example. For injection, an active ingredient can be in the form of a parenterally acceptable aqueous solution which is substantially pyrogen-free and has suitable pH, isotonicity and stability. One can prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as required. Additionally, compositions can be administered via aerosolization.

[00397] In one instance, the composition is lyophilized, for example, to increase shelf-life in storage. When the compositions are considered for use in medicaments or any of the methods provided herein, it is contemplated that the composition can be substantially free of pyrogens such that the composition will not cause an inflammatory reaction or an unsafe allergic reaction when administered to a human patient. Testing compositions for pyrogens and preparing compositions substantially free of pyrogens are well understood to one or ordinary skill of the art and can be accomplished using commercially available kits.

[00398] Acceptable carriers can contain a compound that stabilizes, increases or delays absorption, or increases or delays clearance. Such compounds include, for example, carbohydrates, such as glucose, sucrose, or dextrans; low molecular weight proteins; compositions that reduce the clearance or hydrolysis of peptides; or excipients or other stabilizers and/or buffers. Agents that delay absorption include, for example, aluminum monostearate and gelatin. Detergents can also be used to stabilize or to increase or decrease the absorption of the pharmaceutical composition, including liposomal carriers. To protect from digestion the compound can be complexed with a composition to render it resistant to acidic and enzymatic hydrolysis, or the compound can be complexed in an appropriately resistant carrier such as a liposome. Means of protecting compounds from digestion are known in the art (e.g., Fix (1996) Pharm Res. 13: 1760 1764; Samanen (1996) J. Pharm. Pharmacol. 48: 119 135; and U.S. Pat. No. 5,39 1,377).

[00399] The compositions can be administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of binding capacity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. Suitable regimes for initial administration and booster shots

are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusions sufficient to maintain concentrations in the blood are contemplated.

[00400] In some embodiments the present invention is directed to an immunogenic composition, e.g., a therapeutic composition capable of raising a neoantigen-specific response (e.g., a humoral or cell-mediated immune response). In some embodiments, the immunogenic composition comprises neoantigen therapeutics (e.g., peptides, polynucleotides, TCR, CAR, cells containing TCR or CAR, dendritic cell containing polypeptide, dendritic cell containing polynucleotide, antibody, etc.) described herein corresponding to tumor specific neoantigen identified herein.

[00401] In some embodiments, immunogenic peptides are identified from one or more subjects with a disease or condition. In some embodiments, immunogenic peptides are specific to one or more subjects with a disease or condition. In some embodiments, immunogenic peptides can bind to an HLA that is matched to an HLA haplotype of one or more subjects with a disease or condition.

[00402] A person skilled in the art will be able to select neoantigenic therapeutics by testing, for example, the generation of T cells in vitro as well as their efficiency and overall presence, the proliferation, affinity and expansion of certain T cells for certain peptides, and the functionality of the T cells, e.g. by analyzing the IFN-γ production or tumor killing by T cells. The most efficient peptides can then be combined as an immunogenic composition.

[00403] In some embodiments of the present invention the different neoantigenic peptides and/or polypeptides are selected so that one immunogenic composition comprises neoantigenic peptides and/or polypeptides capable of associating with different MHC molecules, such as different MHC class I molecule. In some embodiments, an immunogenic composition comprises neoantigenic peptides and/or polypeptides capable of associating with the most frequently occurring MHC class I molecules. Hence immunogenic compositions described herein comprise different peptides capable of associating with at least 2, at least 3, or at least 4 MHC class I or class II molecules.

[00404] In some embodiments, an immunogenic composition described herein is capable of raising a specific cytotoxic T cells response, specific helper T cell response, or a B cell response.

[00405] In some embodiments, an immunogenic composition described herein can further comprise an adjuvant and/or a carrier. Examples of useful adjuvants and carriers are given herein below. Polypeptides and/or polynucleotides in the composition can be associated with a carrier such as e.g. a protein or an antigen-presenting cell such as e.g. a dendritic cell (DC) capable of presenting the peptide to a T cell or a B cell. In further embodiments, DC-binding peptides are used as carriers to target the neoantigenic peptides and polynucleotides encoding the neoantigen peptides to dendritic cells (Sioud et al. FASEB J 27: 3272-3283 (20 13)).

[00406] In embodiments, the neoantigenic polypeptides or polynucleotides can be provided as antigen presenting cells (e.g., dendritic cells) containing such polypeptides or polynucleotides. In other embodiments, such antigen presenting cells are used to stimulate T cells for use in patients.

[00407] In some embodiments, the antigen presenting cells are dendritic cells. In related embodiments, the dendritic cells are autologous dendritic cells that are pulsed with the neoantigenic peptide or nucleic acid. The neoantigenic peptide can be any suitable peptide that gives rise to an appropriate T cell response. T cell therapy using autologous dendritic cells pulsed with peptides from a tumor associated antigen is disclosed in Murphy et al. (1996) The Prostate 29, 371-380 and Tjua et al. (1997) The Prostate 32, 272-278. In some embodiments, the T cell is a CTL. In some embodiments, the T cell is a HTL.

[00408] Thus, one embodiment of the present invention is an immunogenic composition containing at least one antigen presenting cell (e.g., a dendritic cell) that is pulsed or loaded with one or more neoantigenic polypeptides or polynucleotides described herein. In embodiments, such APCs are autologous (e.g., autologous dendritic cells). Alternatively, peripheral blood mononuclear cells (PBMCs) isolated from a patient can be loaded with neoantigenic peptides or polynucleotides *ex vivo*. In related embodiments, such APCs or PBMCs are injected back into the patient.

[00409] The polynucleotide can be any suitable polynucleotide that is capable of transducing the dendritic cell, thus resulting in the presentation of a neoantigenic peptide and induction of immunity. In some embodiments, the polynucleotide can be naked DNA that is taken up by the cells by passive loading. In another embodiment, the polynucleotide is part of a delivery vehicle, for example, a liposome, virus like particle, plasmid, or expression vector. In another embodiment, the polynucleotide is delivered by a vector-free delivery system, for example, high performance electroporation and high-speed cell deformation). In embodiments, such antigen presenting cells (APCs) (e.g., dendritic cells) or peripheral blood mononuclear cells (PBMCs) are used to stimulate a T cell (e.g., an autologous T cell). In related embodiments, the T cell is a CTL. In other related embodiments, the T cell is an HTL. Such T cells are then injected into the patient. In some embodiments, CTL is injected into the patient. In some embodiments, HTL is injected into the patient. In some embodiments, both CTL and HTL are injected into the patient. Administration of either therapeutic can be performed simultaneously or sequentially and in any order.

[00410] The pharmaceutical compositions (e.g., immunogenic compositions) described herein for therapeutic treatment are intended for parenteral, topical, nasal, oral or local administration. In some embodiments, the pharmaceutical compositions described herein are administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. In embodiments, the composition can be administered intratumorally. The compositions can be administered at the site of surgical excision to induce a local immune response to the tumor. In some embodiments, described herein are compositions for parenteral administration which comprise a solution of the neoantigenic peptides and immunogenic compositions are dissolved or suspended in an acceptable carrier, for example, an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.9% saline, 0.3% glycine, hyaluronic acid and the like. These compositions can be sterilized by conventional, well known sterilization techniques, or can be sterile filtered. The resulting aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering

agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

[00411] The concentration of neoantigenic peptides and polynucleotides described herein in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected by fluid volumes, viscosities, etc., according to the particular mode of administration selected.

[00412] The neoantigenic peptides and polynucleotides described herein can also be administered via liposomes, which target the peptides to a particular cells tissue, such as lymphoid tissue. Liposomes are also useful in increasing the half-life of the peptides. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, e.g., a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the DEC205 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes filled with a desired peptide or polynucleotide described herein can be directed to the site of lymphoid cells, where the liposomes then deliver the selected therapeutic/immunogenic polypeptide/polynucleotide compositions. Liposomes can be formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, for example, cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al, Ann. Rev. Biophys. Bioeng. 9; 467 (1980), U.S. Pat. Nos. 4,235,871, 4,50 1,728, 4,501,728, 4,837,028, and 5,019,369.

[00413] For targeting to the immune cells, a neoantigen polypeptides or polynucleotides to be incorporated into the liposome for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide can be administered intravenously, locally, topically, etc. in a dose which varies according to, inter alia, the manner of administration, the polypeptide or polynucleotide being delivered, and the stage of the disease being treated.

[00414] In some embodiments, neoantigen polypeptides and polynucleotides are targeted to dendritic cells. In some embodiments, the neoantigen polypeptides and polynucleotides are target to dendritic cells using the markers DEC205, XCR1, CD 197, CD80, CD86, CD 123, CD209, CD273, CD283, CD289, CD 184, CD85h, CD85j, CD85k, CD85d, CD85g, CD85a, TSLP receptor, Clec9a or CD la.

[00415] For solid compositions, conventional or nanoparticle nontoxic solid carriers can be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more neoantigenic polypeptides or polynucleotides described herein at a concentration of 25%-75%.

[00416] For aerosol administration, the neoantigenic polypeptides or polynucleotides can be supplied in finely divided form along with a surfactant and propellant. Representative of such agents are the esters or partial

esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides can be employed. The surfactant can constitute 0.1%-20% by weight of the composition, or 0.25-5%. The balance of the composition can be propellant. A carrier can also be included as desired, as with, e.g., lecithin for intranasal delivery.

[00417] Additional methods for delivering the neoantigenic polynucleotides described herein are also known in the art. For instance, the nucleic acid can be delivered directly, as "naked DNA". This approach is described, for instance, in Wolff et al., Science 247: 1465-1468 (1990) as well as U.S. Pat. Nos. 5,580,859 and 5,589,466. The nucleic acids can also be administered using ballistic delivery as described, for instance, in U.S. Pat. No. 5,204,253 . Particles comprised solely of DNA can be administered. Alternatively, DNA can be adhered to particles, such as gold particles.

[00418] For therapeutic or immunization purposes, mRNA encoding the neoantigenic peptides, or peptide binding agents can also be administered to the patient. In some embodiments, the mRNA is self-amplifying RNA. In a further embodiment, the self-amplifying RNA is a part of a synthetic lipid nanoparticle formulation (Geall et al., Proc Natl Acad Sci U S A . 109: 14604-14609 (20 12)).

[00419] The nucleic acids can also be delivered complexed to cationic compounds, such as cationic lipids. Lipid-mediated gene delivery methods are described, for instance, in WO 96/1 8372, WO 93/24640; Mannino & Gould-Fogerite, BioTechniques 6(7): 682-69 1 (1988); U.S. Pat. No. 5,279,833; WO 9 1/06309; and Feigner et al., Proc. Natl. Acad. Sci. USA 84: 74 13-7414 (1987).

[00420] The neoantigenic peptides and polypeptides described herein can also be expressed by attenuated viruses, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptide described herein. Upon introduction into an acutely or chronically infected host or into a noninfected host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Pat. No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al. (Nature 35 1:456-460 (199 1)). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides described herein will be apparent to those skilled in the art from the description herein.

[00421] Adjuvants are any substance whose admixture into the immunogenic composition increases or otherwise modifies the immune response to the therapeutic agent. Carriers are scaffold structures, for example a polypeptide or a polysaccharide, to which a neoantigenic polypeptide or polynucleotide, is capable of being associated. Optionally, adjuvants are conjugated covalently or non-covalently to the polypeptides or polynucleotides described herein.

[00422] The ability of an adjuvant to increase the immune response to an antigen is typically manifested by a significant increase in immune-mediated reaction, or reduction in disease symptoms. For example, an increase in humoral immunity can be manifested by a significant increase in the titer of antibodies raised to the antigen, and an increase in T cell activity can be manifested in increased cell proliferation, or cellular cytotoxicity, or

cytokine secretion. An adjuvant can also alter an immune response, for example, by changing a primarily humoral or T helper 2 response into a primarily cellular, or T helper 1 response.

[00423] Suitable adjuvants are known in the art (see, WO 2015/095811) and include, but are not limited to poly(I:C), poly-ICLC, *STING* agonist, 1018 ISS, aluminium salts, Amplivax, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, GM-CSF, IC30, IC31, Imiquimod, ImuFact IMP321, IS Patch, ISS, ISCOMATRIX, Juvlmmune, LipoVac, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, OK-432, OM-174, OM-197-MP-EC, ONTAK, PepTel®. vector system, PLG microparticles, resiquimod, SRL172, virosomes and other virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Pam3CSK4, Aquila's QS21 stimulon (Aquila Biotech, Worcester, Mass., USA) which is derived from saponin, mycobacterial extracts and synthetic bacterial cell wall mimics, and other proprietary adjuvants such as Ribi's Detox. Quil or Superfos. Adjuvants also include incomplete Freund's or GM-CSF. Several immunological adjuvants (e.g., MF59) specific for dendritic cells and their preparation have been described previously (Dupuis M, et al., Cell Immunol. 1998; 186(1): 18-27; Allison A C; Dev Biol Stand. 1998; 92:3-11) (Mosca et al. Frontiers in Bioscience, 2007; 12:4050-4060) (Gamvrellis et al. Immunol & Cell Biol. 2004; 82: 506-516). Also cytokines can be used. Several cytokines have been directly linked to influencing dendritic cell migration to lymphoid tissues (e.g., TNF-alpha), accelerating the maturation of dendritic cells into efficient antigen-presenting cells for T-lymphocytes (e.g., GM-CSF, PGE1, PGE2, IL-1, IL-lb, IL-4, IL-6 and CD40L) (U.S. Pat. No. 5,849,589 incorporated herein by reference in its entirety) and acting as immunoadjuvants (e.g., IL-12) (Gabrilovich D I, et al., J Immunother Emphasis Tumor Immunol. 1996 (6):414-418).

[00424] CpG immunostimulatory oligonucleotides have also been reported to enhance the effects of adjuvants in a therapuetic setting. Without being bound by theory, CpG oligonucleotides act by activating the innate (non-adaptive) immune system via Toll-like receptors (TLR), mainly TLR9. CpG triggered TLR9 activation enhances antigen-specific humoral and cellular responses to a wide variety of antigens, including peptide or protein antigens, live or killed viruses, dendritic cell immunogenic pharmaceutical compositions, autologous cellular immunogenic pharmaceutical compositions and polysaccharide conjugates in both prophylactic and therapeutic immunogenic pharmaceutical compositions. Importantly, it enhances dendritic cell maturation and differentiation, resulting in enhanced activation of TH1 cells and strong cytotoxic T-lymphocyte (CTL) generation, even in the absence of CD4 T cell help. The TH1 bias induced by TLR9 stimulation is maintained even in the presence of adjuvants such as alum or incomplete Freund's adjuvant (IFA) that normally promote a TH2 bias. CpG oligonucleotides show even greater adjuvant activity when formulated or co-administered with other adjuvants or in formulations such as microparticles, nano particles, lipid emulsions or similar formulations, which are especially necessary for inducing a strong response when the antigen is relatively weak. They also accelerate the immune response and enabled the antigen doses to be reduced with comparable antibody responses to the full-dose immunogenic pharmaceutical composition without CpG in some experiments (Arthur M. Krieg, Nature Reviews, Drug Discovery, 5, June 2006, 471-484). U.S. Pat. No. 6,406,705 B1 describes the combined use of CpG oligonucleotides, non-nucleic acid adjuvants and an antigen

to induce an antigen-specific immune response. A commercially available CpG TLR9 antagonist is dSLIM (double Stem Loop Immunomodulator) by Mologen (Berlin, GERMANY), which is a component of the pharmaceutical composition described herein. Other TLR binding molecules such as RNA binding TLR 7, TLR 8 and/or TLR 9 can also be used.

[00425] Other examples of useful adjuvants include, but are not limited to, chemically modified CpGs (e.g. CpR, Idera), Poly(I:C)(e.g. polyi:CI2U), non-CpG bacterial DNA or RNA, ssRNA40 for TLR8, as well as immunoactive small molecules and antibodies such as cyclophosphamide, sunitinib, bevacizumab, celebrex, NCX-40 16, sildenafil, tadalafil, vardenafil, sorafinib, XL-999, CP-547632, pazopanib, ZD2 17 1, AZD2 17 1, ipilimumab, tremelimumab, and SC58 175, which can act therapeutically and/or as an adjuvant. The amounts and concentrations of adjuvants and additives useful in the context of the present invention can readily be determined by the skilled artisan without undue experimentation. Additional adjuvants include colony-stimulating factors, such as Granulocyte Macrophage Colony Stimulating Factor (GM-CSF, sargramostim).

[00426] In some embodiments, an immunogenic composition according to the present invention can comprise more than one different adjuvants. Furthermore, the invention encompasses a therapeutic composition comprising any adjuvant substance including any of the above or combinations thereof. It is also contemplated that the neoantigenic therapeutic (e.g., a humoral or cell-mediated immune response). In some embodiments, the immunogenic composition comprises neoantigen therapeutics (e.g., peptides, polynucleotides, TCR, CAR, cells containing TCR or CAR, dendritic cell containing polypeptide, dendritic cell containing polynucleotide, antibody, etc.) and the adjuvant can be administered separately in any appropriate sequence.

[00427] A carrier can be present independently of an adjuvant. The function of a carrier can for example be to increase the molecular weight of in particular mutant in order to increase their activity or immunogenicity, to confer stability, to increase the biological activity, or to increase serum half-life. Furthermore, a carrier can aid presenting peptides to T cells. The carrier can be any suitable carrier known to the person skilled in the art, for example a protein or an antigen presenting cell. A carrier protein could be but is not limited to keyhole limpet hemocyanin, serum proteins such as transferrin, bovine serum albumin, human serum albumin, thyroglobulin or ovalbumin, immunoglobulins, or hormones, such as insulin or palmitic acid. In some embodiments, the carrier comprises a human fibronection type III domain (Koide et al. Methods Enzymol. 20 12;503 :135-56). For immunization of humans, the carrier must be a physiologically acceptable carrier acceptable to humans and safe. However, tetanus toxoid and/or diptheria toxoid are suitable carriers In some embodiments of the invention. Alternatively, the carrier can be dextrans for example sepharose.

[00428] In some embodiments, the polypeptides can be synthesized as multiply linked peptides as an alternative to coupling a polypeptide to a carrier to increase immunogenicity. Such molecules are also known as multiple antigenic peptides (MAPS).

[00429] Neoantigens that are immungenic can be used as a composition when combined with an acceptable carrier or excipient. Such compositions are useful for in vitro or in vivo analysis or for administration to a subject in vivo or ex vivo for treating a subject with a disease.

[00430] Thus pharmaceutical compositions can include, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration.

[00431] Pharmaceutical formulations comprising a protein of interest, e.g., a neoantigen described herein, can be prepared for storage by mixing the neoantigen having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Oslo, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are those that are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS® or polyethylene glycol (PEG).

[00432] Acceptable carriers are physiologically acceptable to the administered patient and retain the therapeutic properties of the compounds with/in which it is administered. Acceptable carriers and their formulations are generally described in, for example, Remington' pharmaceutical Sciences (18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA 1990). One exemplary carrier is physiological saline. A pharmaceutically acceptable carrier is a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject compounds from the administration site of one organ, or portion of the body, to another organ, or portion of the body, or in an in vitro assay system. Acceptable carriers are compatible with the other ingredients of the formulation and not injurious to a subject to whom it is administered. Nor should an acceptable carrier alter the specific activity of the neoantigens.

[00433] In one aspect, provided herein are pharmaceutically acceptable or physiologically acceptable compositions including solvents (aqueous or non-aqueous), solutions, emulsions, dispersion media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration. Pharmaceutical compositions or pharmaceutical formulations therefore refer to a composition suitable for pharmaceutical use in a subject. The pharmaceutical compositions and formulations include an amount of a neoantigen (or polynucleotide encoding a neoantigen) and a pharmaceutically or physiologically acceptable carrier. Compositions can be formulated to be compatible with a particular route of administration (i.e.,

systemic or local). Thus, compositions include carriers, diluents, or excipients suitable for administration by various routes.

[00434] In some embodiments, a composition further comprises an acceptable additive in order to improve the stability of the neoantigen in the composition and/or to control the release rate of the composition. Acceptable additives do not alter the specific activity of the neoantigens. Exemplary acceptable additives include, but are not limited to, a sugar such as mannitol, sorbitol, glucose, xylitol, trehalose, sorbose, sucrose, galactose, dextran, dextrose, fructose, lactose and mixtures thereof. Acceptable additives can be combined with acceptable carriers and/or excipients such as dextrose. Alternatively, exemplary acceptable additives include, but are not limited to, a surfactant such as polysorbate 20 or polysorbate 80 to increase stability of the peptide and decrease gelling of the solution. The surfactant can be added to the composition in an amount of 0.01% to 5% of the solution. Addition of such acceptable additives increases the stability and half-life of the composition in storage.

[00435] The pharmaceutical composition can be administered, for example, by injection. Compositions for injection include aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. Fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Antibacterial and antifungal agents include, for example, parabens, chlorobutanol, phenol, ascorbic acid and thimerosal. Isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride can be included in the composition. The resulting solutions can be packaged for use as is, or lyophilized; the lyophilized preparation can later be combined with a sterile solution prior to administration. For intravenous, injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as needed. Sterile injectable solutions can be prepared by incorporating an active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active ingredient into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00436] Compositions can be conventionally administered intravenously, such as by injection of a unit dose, for example. For injection, an active ingredient can be in the form of a parenterally acceptable aqueous solution which is substantially pyrogen-free and has suitable pH, isotonicity and stability. One can prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as required. Additionally, compositions can be administered via aerosolization.

[00437] In some embodiments, the composition is lyophilized, for example, to increase shelf-life in storage. When the compositions are considered for use in medicaments or any of the methods provided herein, it is contemplated that the composition can be substantially free of pyrogens such that the composition will not cause an inflammatory reaction or an unsafe allergic reaction when administered to a human patient. Testing compositions for pyrogens and preparing compositions substantially free of pyrogens are well understood to one or ordinary skill of the art and can be accomplished using commercially available kits.

[00438] Acceptable carriers can contain a compound that stabilizes, increases or delays absorption, or increases or delays clearance. Such compounds include, for example, carbohydrates, such as glucose, sucrose, or dextrans; low molecular weight proteins; compositions that reduce the clearance or hydrolysis of peptides; or excipients or other stabilizers and/or buffers. Agents that delay absorption include, for example, aluminum monostearate and gelatin. Detergents can also be used to stabilize or to increase or decrease the absorption of the pharmaceutical composition, including liposomal carriers. To protect from digestion the compound can be complexed with a composition to render it resistant to acidic and enzymatic hydrolysis, or the compound can be complexed in an appropriately resistant carrier such as a liposome.

[00439] The compositions can be administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of binding capacity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusions sufficient to maintain concentrations in the blood are contemplated.

[00440] Peptide-based immunogenic pharmaceutical compositions can be formulated using any of the well-known techniques, carriers, and excipients as suitable and as understood in the art. The polypeptides can be a cocktail of multiple polypeptides containing the same sequence, or a cocktail of multiple copies of different polypeptides. The peptides can be modified, such as for example by lipidation, or attachment to a carrier protein. Lipidation can be the covalent attachment of a lipid group to a polypeptide. Lipidated peptides, or lipidated polypeptides, can stabilize structures and can enhance efficacy of the treatment.

[00441] Lipidation can be classified into several different types, such as N-myristoylation, palmitoylation, GPI-anchor addition, prenylation, and several additional types of modifications. N-myristoylation is the covalent attachment of myristate, a C14 saturated acid, to a glycine residue. Palmitoylation is thioester linkage

of long-chain fatty acids (C16) to cysteine residues. GPI-anchor addition is glycosyl-phosphatidylinositol (GPI) linkage via amide bond. Prenylation is the thioether linkage of an isoprenoid lipid (e.g. farnesyl (C-15), geranylgeranyl (C-20)) to cysteine residues. Additional types of modifications can include attachment of S-diacylglycerol by a sulfur atom of cysteines, O-octanoyl conjugation via serine or threonine residues, S-archaeol conjugation to cysteine residues, and cholesterol attachment.

[00442] Fatty acids for generating a lipidated peptides can include C2 to C30 saturated, monounsaturated, or polyunsaturated fatty acyl groups. Exemplary fatty acids can include palmitoyl, myristoyl, stearoyl and decanoyl groups. In some instances, a lipid moiety that has adjuvant property is attached to a polypeptide of interest to elicit or enhance immunogenicity in the absence of an extrinsic adjuvant. A lipidated peptide or lipopeptide can be referred to as a self-adjuvant lipopeptide. Any of the fatty acids described above and elsewhere herein can elicit or enhance immunogenicity of a polypeptide of interest. A fatty acid that can elicit or enhance immunogenicity can include palmitoyl, myristoyl, stearoyl, lauroyl, octanoyl, and decanoyl groups.

[00443] Polypeptides such as naked peptides or lipidated peptides can be incorporated into a liposome. Sometimes, lipidated peptides can be incorporated into a liposome. For example, the lipid portion of the lipidated peptide can spontaneously integrate into the lipid bilayer of a liposome. Thus, a lipopeptide can be presented on the "surface" of a liposome.

[00444] Exemplary liposomes suitable for incorporation in the formulations include, and are not limited to, multilamellar vesicles (MLV), oligolamellar vesicles (OLV), unilamellar vesicles (UV), small unilamellar vesicles (SUV), medium-sized unilamellar vesicles (MUV), large unilamellar vesicles (LUV), giant unilamellar vesicles (GUV), multivesicular vesicles (MVV), single or oligolamellar vesicles made by reverse-phase evaporation method (REV), multilamellar vesicles made by the reverse-phase evaporation method (MLV-REV), stable plurilamellar vesicles (SPLV), frozen and thawed MLV (FATMLV), vesicles prepared by extrusion methods (VET), vesicles prepared by French press (FPV), vesicles prepared by fusion (FUV), dehydration-rehydration vesicles (DRV), and bubblesomes (BSV).

[00445] Depending on the method of preparation, liposomes can be unilamellar or multilamellar, and can vary in size with diameters ranging from about 0.02 μm to greater than about 10 μm. Liposomes can adsorb many types of cells and then release an incorporated agent (e.g., a peptide described herein). In some cases, the liposomes fuse with the target cell, whereby the contents of the liposome then empty into the target cell. A liposome can be endocytosed by cells that are phagocytic. Endocytosis can be followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents.

[00446] The liposomes provided herein can also comprise carrier lipids. In some embodiments the carrier lipids are phospholipids. Carrier lipids capable of forming liposomes include, but are not limited to dipalmitoylphosphatidylcholine (DPPC), phosphatidylcholine (PC; lecithin), phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS). Other suitable phospholipids further include distearoylphosphatidylcholine (DSPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidyglycerol (DPPG), distearoylphosphatidyglycerol (DSPG), dimyristoylphosphatidylglycerol (DMPG), dipalmitoylphosphatidic acid (DPPA); dimyristoylphosphatidic

acid (DMPA), distearoylphosphatidic acid (DSPA), dipalmitoylphosphatidylserine (DPPS), dimyristoylphosphatidylserine (DMPS), distearoylphosphatidylserine (DSPS), dipalmitoylphosphatidyethanolamine (DPPE), dimyristoylphosphatidylethanolamine (DMPE), distearoylphosphatidylethanolamine (DSPE) and the like, or combinations thereof. In some embodiments, the liposomes further comprise a sterol (e.g., cholesterol) which modulates liposome formation. The carrier lipids can be any known non-phosphate polar lipids.

[00447] A pharmaceutical composition can be encapsulated within liposomes using well-known technology. Biodegradable microspheres can also be employed as carriers for the pharmaceutical compositions of this invention.

[00448] The pharmaceutical composition can be administered in liposomes or microspheres (or microparticles). Methods for preparing liposomes and microspheres for administration to a patient are well known to those of skill in the art. Essentially, material is dissolved in an aqueous solution, the appropriate phospholipids and lipids added, along with surfactants if required, and the material dialyzed or sonicated, as necessary.

[00449] Microspheres formed of polymers or proteins are well known to those skilled in the art, and can be tailored for passage through the gastrointestinal tract directly into the blood stream. Alternatively, the compound can be incorporated and the microspheres, or composite of microspheres, implanted for slow release over a period of time ranging from days to months.

[00450] A polypeptide can also be attached to a carrier protein for delivery. The carrier protein can be an immunogenic carrier element and can be attached by any recombinant technology. Exemplary carrier proteins include Mariculture keyhole limpet hemocyanin (mcKLH), PEGylated mcKLH, Blue Carrier* Proteins, bovine serum albumin (BSA), cationized BSA, ovalbumin, and bacterial proteins such as tetanus toxoid (TT).

[00451] A polypeptide can also be prepared as multiple antigenic peptides (MAPs). Peptides may be attached at the N-terminus or the C-terminus to small non-immunogenic cores. Peptides built upon this core can offer highly localized peptide density. The core can be a dendritic core residue or matrix composed of bifunctional units. Suitable core molecules for constructing MAPs can include ammonia, ethylenediamine, aspartic acid, glutamic acid, and lysine. For example, a lysine core molecule can be attached via peptide bonds through each of its amino groups to two additional lysines.

[00452] A polypeptide can be chemically synthesized, or recombinantly expressed in a cell system or a cell-free system. A peptide can be synthesized, such as by a liquid-phase synthesis, a solid-phase synthesis, or by microwave assisted peptide synthesis. A polypeptide can be modified, such as for example, by acylation, alkylation, amidation, arginylation, polyglutamylation, polyglycylation, butyrylation, gamma-carboxylation, glycosylation, malonylation, hydroxylation, iodination, nucleotide addition (e.g. ADP-ribosylation), oxidation, phosphorylation, adenylylation, propionylation, S-glutathionylation, S-nitrosylation, succinylation, sulfation, glycation, palmitoylation, myristoylation, isoprenylation or prenylation (e.g. farnesylation or geranylgeranylation), glypiation, lipoylation, attachement of flavin moiety (e.g. FMN or FAD), attachment of heme C, phosphopantetheinylation, retinylidene Schiff base formation, diphthamide formation, ethanolamine

phosphoglycerol attachment, hypusine formuation, biotinylation, pegylation, ISGylation, SUMUylation, ubiquitination, Neddylation, Pupylation, citrullination, deamidation, eliminylation, carbamylation, or a combination thereof.

[00453] After generation of a polypeptide, the polypeptide can be subjected to one or more rounds of purification steps to remove impurities. The purification step can be a chromatographic step utilizing separation methods such as affinity-based, size-exclusion based, ion-exchange based, or the like. In some cases, the polypeptide is at most 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or 100% pure or without the presence of impurities. In some cases, the polypeptide is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or 100% pure or without the presence of impurities.

[00454] A polypeptide can include natural amino acids, unnatural amino acids, or a combination thereof. An amino acid residue can refer to a molecule containing both an amino group and a carboxyl group. Suitable amino acids include, without limitation, both the **D**- and L-isomers of the naturally-occurring amino acids, as well as non-naturally occurring amino acids prepared by organic synthesis or other metabolic routes.

[00455] "Hydrophobic amino acids" include small hydrophobic amino acids and large hydrophobic amino acids. "Small hydrophobic amino acid" can be glycine, alanine, proline, and analogs thereof. "Large hydrophobic amino acids" can be valine, leucine, isoleucine, phenylalanine, methionine, tryptophan, and analogs thereof. "Polar amino acids" can be serine, threonine, asparagine, glutamine, cysteine, tyrosine, and analogs thereof. "Charged amino acids" can be lysine, arginine, histidine, aspartate, glutamate, and analogs thereof. A peptide provided herein can comprise one or more hydrophobic, polar, or charged amino acids.

[00456] A peptide provided herein can comprise one or more amino acid analogs. An "amino acid analog" can be a molecule which is structurally similar to an amino acid and which can be substituted for an amino acid in the formation of a peptidomimetic macrocycle Amino acid analogs include, without limitation, β-amino acids and amino acids where the amino or carboxy group is substituted by a similarly reactive group (e.g., substitution of the primary amine with a secondary or tertiary amine, or substitution of the carboxy group with an ester).

[00457] A peptide provided herein can comprise one or more non-natural amino acids. A "non-natural amino acid" can be an amino acid which is not one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, **D,** Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V. Non-natural amino acids or amino acid analogs include structures disclosed, e.g., in U.S. Patent Application Publication No. 20130123169, which is herein incorporated by reference.

[00458] Amino acid analogs can include β-amino acid analogs. Examples of β-amino acid analogs and analogs of alanine, valine, glycine, leucine, arginine, lysine, aspartic acids, glutamic acids, cysteine, methionine, phenylalanine, tyrosine, proline, serine, threonine, and tryptophan can include structures disclosed, e.g., in U.S. Patent Application Publication No. 20130123169, which is herein incorporated by reference.

[00459] Amino acid analogs can be racemic. In some instances, the **D** isomer of the amino acid analog is used. In some cases, the L isomer of the amino acid analog is used. In some instances, the amino acid analog comprises chiral centers that are in the R or S configuration. Sometimes, the amino group(s) of a β-amino acid

analog is substituted with a protecting group, e.g., tert-butyloxycarbonyl (BOC group), 9-fluorenylmethyloxycarbonyl (FMOC), tosyl, and the like. Sometimes, the carboxylic acid functional group of a β-amino acid analog is protected, e.g., as its ester derivative. In some cases, the salt of the amino acid analog is used.

[00460] A "non-essential" amino acid residue can be a residue that can be altered from the wild-type sequence of a polypeptide without abolishing or substantially altering its essential biological or biochemical activity (e.g., receptor binding or activation). An "essential" amino acid residue can be a residue that, when altered from the wild-type sequence of the polypeptide, results in abolishing or substantially abolishing the polypeptide's essential biological or biochemical activity.

[00461] A "conservative amino acid substitution" can be one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families can include amino acids with basic side chains (e.g., K, R, H), acidic side chains (e.g., D, E), uncharged polar side chains (e.g., G, N, Q, S, T, Y, C), nonpolar side chains (e.g., A, V, L, I, P, F, M, W), beta-branched side chains (e.g., T, V, I) and aromatic side chains (e.g., Y, F, W, H). Thus, a predicted nonessential amino acid residue in a polypeptide, for example, can be replaced with another amino acid residue from the same side chain family. Other examples of acceptable substitutions can be substitutions based on isosteric considerations (e.g. norleucine for methionine) or other properties (e.g. 2-thienylalanine for phenylalanine, or 6-Cl-tryptophan for tryptophan).

[00462] Nucleic acid -based immunogenic pharmaceutical compositions can also be administered to a subject. Nucleic acid -based immunogenic pharmaceutical compositionscan be formulated using any of the well-known techniques, carriers, and excipients as suitable and as understood in the art. The nucleic acid can be DNA, genomic DNA or cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine.

[00463] Nucleic acids can be obtained by chemical synthesis methods or by recombinant methods. The immunogenic pharmaceutical composition can be a DNA-based immunogenic pharmaceutical composition, an RNA-based immunogenic pharmaceutical composition, a hybrid DNA/RNA based immunogenic pharmaceutical composition, or a hybrid nucleic acid/peptide based immunogenic pharmaceutical composition. The peptide can be a peptide derived from a peptide in Tables 2-4, a peptide that has a sequence that is at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more in sequence homology to a peptide in Tables 2-4, or a peptide that has a sequence that is at most 40%, 50%, 60%, 70%, 80%, 90%, 95%, or less in sequence homology to a peptide in Tables 2-4.

[00464] A nucleic acid described herein can contain phosphodiester bonds, although in some cases, as outlined below (for example in the construction of primers and probes such as label probes), nucleic acid analogs are included that can have alternate backbones, comprising, for example, phosphoramide, phosphorothioate, O-methylphosphoroamidite linkages, and peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with bicyclic structures including locked nucleic acids, positive backbones and non-ribose

backbones. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids. Locked nucleic acids (LNAs) are also included within the definition of nucleic acid analogs. LNAs are a class of nucleic acid analogues in which the ribose ring is "locked" by a methylene bridge connecting the 2'-0 atom with the 4'-C atom. These modifications of the ribose-phosphate backbone can be done to increase the stability and half-life of such molecules in physiological environments. For example, PNA:DNA and LNA-DNA hybrids can exhibit higher stability and thus can be used in some embodiments. The nucleic acids can be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. Depending on the application, the nucleic acids can be DNA (including, e.g., genomic DNA, mitochondrial DNA, and cDNA), RNA (including, e.g., mRNA and rRNA) or a hybrid, where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xathanine hypoxathanine, isocytosine, isoguanine, etc.

[00465] A nucleic acid -based immunogenic pharmaceutical compositions can be in the form of a vector. A vector can be a circular plasmid or a linear nucleic acid. A circular plasmid or linear nucleic acid can be capable of directing expression of a particular nucleotide sequence in an appropriate subject cell.

[00466] A vector can have a promoter operably linked to the polypeptide-encoding nucleotide sequence, which can be operably linked to termination signals. A vector can contain sequences required for proper translation of the nucleotide sequence. The vector comprising the nucleotide sequence of interest can be chimeric, meaning that at least one of its components can be heterologous with respect to at least one of its other components. The expression of the nucleotide sequence in an expression cassette can be under the control of a constitutive promoter or of an inducible promoter, which can initiate transcription only when the host cell is exposed to some particular internal or external stimulus.

[00467] The vector can be a plasmid. A plasmid can be useful for transfecting cells with nucleic acid encoding the polypeptide, and the transformed host cells can be cultured and maintained under conditions wherein expression of the polypeptide takes place.

[00468] A plasmid can comprise a nucleic acid sequence that encodes one or more of the various polypeptides disclosed herein. A single plasmid can contain coding sequence for a single polypeptide, or coding sequence for more than one polypeptide. Sometimes, the plasmid can further comprise coding sequence that encodes an adjuvant, such as an immune stimulating molecule, such as a cytokine.

[00469] A plasmid can further comprise an initiation codon, which can be upstream of the coding sequence, and a stop codon, which can be downstream of the coding sequence. The initiation and termination codon can be in frame with the coding sequence. A plasmid can also comprise a promoter that is operably linked to the coding sequence, and an enhancer upstream of the coding sequence. The enhancer can be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, FMDV, RSV or EBV.

[00470] A plasmid can also comprise a mammalian origin of replication in order to maintain the plasmid extrachromosomally and produce multiple copies of the plasmid in a cell. A plasmid can also comprise a

regulatory sequence, which can be well suited for gene expression in a cell into which the plasmid is administered. The coding sequence can comprise a codon that can allow more efficient transcription of the coding sequence in the host cell.

[00471] The nucleic acid based immunogenic pharmaceutical compositions can also be a linear nucleic acid immunogenic pharmaceutical composition, or linear expression cassette, that is capable of being efficiently delivered to a subject via electroporation and expressing one or more polypeptides disclosed herein.

[00472] Cell-based immunogenic pharmaceutical compositions can also be administered to a subject. For example, an antigen presenting cell (APC) based immunogenic pharmaceutical composition can be formulated using any of the well-known techniques, carriers, and excipients as suitable and as understood in the art. APCs include monocytes, monocyte-derived cells, macrophages, and dendritic cells. Sometimes, an APC based immunogenic pharmaceutical composition can be a dendritic cell-based immunogenic pharmaceutical composition.

[00473] A dendritic cell-based immunogenic pharmaceutical composition can be prepared by any methods well known in the art. In some cases, dendritic cell-based immunogenic pharmaceutical compositions can be prepared through an ex vivo or in vivo method. The ex vivo method can comprise the use of autologous DCs pulsed ex vivo with the polypeptides described herein, to activate or load the DCs prior to administration into the patient. The in vivo method can comprise targeting specific DC receptors using antibodies coupled with the polypeptides described herein. The DC-based immunogenic pharmaceutical composition can further comprise DC activators such as TLR3, TLR-7-8, and CD40 agonists. The DC-based immunogenic pharmaceutical composition can further comprise adjuvants, and a pharmaceutically acceptable carrier.

[00474] An adjuvant can be used to enhance the immune response (humoral and/or cellular) elicited in a patient receiving the immunogenic pharmaceutical composition. Sometimes, adjuvants can elicit a Thl-type response. Other times, adjuvants can elicit a Th2-type response. A Thl-type response can be characterized by the production of cytokines such as IFN-γ as opposed to a Th2-type response which can be characterized by the production of cytokines such as IL-4, IL-5 and IL-10.

[00475] In some aspects, lipid-based adjuvants, such as MPLA and MDP, can be used with the immunogenic pharmaceutical compositions disclosed herein. Monophosphoryl lipid A (MPLA), for example, is an adjuvant that causes increased presentation of liposomal antigen to specific T Lymphocytes. In addition, a muramyl dipeptide (MDP) can also be used as a suitable adjuvant in conjunction with the immunogenic pharmaceutical formulations described herein.

[00476] Adjuvant can also comprise stimulatory molecules such as cytokines. Non-limiting examples of cytokines include: CCL20, a-interferon(IFN- a), β-interferon (IFN-β), γ- interferon, platelet derived growth factor (PDGF), TNFa, TNFp, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15, IL-28, MHC, CD80, CD86, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-la, MIP-1-, IL-8, L- selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18,

CD40, CD40L, vascular growth factor, fibroblast growth factor, **IL-7,** nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Fit, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DRS, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-I, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAPI, and TAP2.

[00477] Additional adjuvants include: MCP-1, MIP-la, MIP-lp, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, **IL-7,** IL-22, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Fit, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP I, TAP2 and functional fragments thereof.

[00478] In some aspects, an adjuvant can be a modulator of a toll like receptor. Examples of modulators of toll-like receptors include TLR-9 agonists and are not limited to small molecule modulators of toll-like receptors such as Imiquimod. Other examples of adjuvants that are used in combination with an immunogenic pharmaceutical composition described herein can include and are not limited to saponin, CpG ODN and the like. Sometimes, an adjuvant is selected from bacteria toxoids, polyoxypropylene-polyoxyethylene block polymers, aluminum salts, liposomes, CpG polymers, oil-in-water emulsions, or a combination thereof. Sometimes, an adjuvant is an oil-in-water emulsion. The oil-in-water emulsion can include at least one oil and at least one surfactant, with the oil(s) and surfactant(s) being biodegradable (metabolisable) and biocompatible. The oil droplets in the emulsion can be less than 5 μm in diameter, and can even have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with a size less than 220 nm can be subjected to filter sterilization.

[00479] In some instances, an immunogenic pharmaceutical composition can include carriers and excipients (including but not limited to buffers, carbohydrates, mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents, suspending agents, thickening agents and/or preservatives), water, oils including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like, saline solutions, aqueous dextrose and glycerol solutions, flavoring agents, coloring agents, detackifiers and other acceptable additives, adjuvants, or binders, other pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents, tonicity adjusting agents, emulsifying agents, wetting agents and the like. Examples of excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. In another instances, the pharmaceutical preparation is substantially free of preservatives. In other

instances, the pharmaceutical preparation can contain at least one preservative. It will be recognized that, while any suitable carrier known to those of ordinary skill in the art can be employed to administer the pharmaceutical compositions described herein, the type of carrier will vary depending on the mode of administration.

[00480] An immunogenic pharmaceutical composition can include preservatives such as thiomersal or 2-phenoxyethanol. In some instances, the immunogenic pharmaceutical composition is substantially free from (e.g. <10 μg/ml) mercurial material e.g. thiomersal-free. a-Tocopherol succinate may be used as an alternative to mercurial compounds.

[00481] For controlling the tonicity, a physiological salt such as sodium salt can be included in the immunogenic pharmaceutical composition. Other salts can include potassium chloride, potassium dihydrogen phosphate, disodium phosphate, and/or magnesium chloride, or the like.

[00482] An immunogenic pharmaceutical composition can have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, between 240-360 mOsm/kg, or within the range of 290-310 mOsm/kg.

[00483] An immunogenic pharmaceutical composition can comprise one or more buffers, such as a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer (particularly with an aluminum hydroxide adjuvant); or a citrate buffer. Buffers, in some cases, are included in the 5-20 mM range.

[00484] The pH of the immunogenic pharmaceutical composition can be between about 5.0 and about 8.5, between about 6.0 and about 8.0, between about 6.5 and about **7**.5, or between about **7**.**0** and about **7**.8.

[00485] An immunogenic pharmaceutical composition can be sterile. The immunogenic pharmaceutical composition can be non-pyrogenic e.g. containing < 1 EU (endotoxin unit, a standard measure) per dose, and can be <0. 1 EU per dose. The composition can be gluten free.

[00486] An immunogenic pharmaceutical composition can include detergent e.g. a polyoxyethylene sorbitan ester surfactant (known as 'Tweens'), or an octoxynol (such as octoxynol-9 (Triton X-100) or t-octylphenoxypolyethoxyethanol). The detergent can be present only at trace amounts. The immunogenic pharmaceutical composition can include less than 1 mg/ml of each of octoxynol-10 and polysorbate 80. Other residual components in trace amounts can be antibiotics (e.g. neomycin, kanamycin, polymyxin B).

[00487] An immunogenic pharmaceutical composition can be formulated as a sterile solution or suspension, in suitable vehicles, well known in the art. The pharmaceutical compositions can be sterilized by conventional, well-known sterilization techniques, or can be sterile filtered. The resulting aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration.

[00488] An immunogenic pharmaceutical composition can be formulated with one or more pharmaceutically acceptable salts. Pharmaceutically acceptable salts can include those of the inorganic ions, such as, for example, sodium, potassium, calcium, magnesium ions, and the like. Such salts can include salts with inorganic or organic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, nitric acid, sulfuric acid, methanesulfonic acid, p-toluenesulfonic acid, acetic acid, fumaric acid, succinic acid, lactic acid, mandelic acid, malic acid, citric acid, tartaric acid or maleic acid. In addition, if the agent(s) contain a carboxy

group or other acidic group, it can be converted into a pharmaceutically acceptable addition salt with inorganic or organic bases. Examples of suitable bases include sodium hydroxide, potassium hydroxide, ammonia, cyclohexylamine, dicyclohexyl-amine, ethanolamine, diethanolamine, triethanolamine, and the like.

[00489] Pharmaceutical compositions comprising, for example, an active agent such as a peptide, a nucleic acid, an antibody or fragments thereof, and/or an APC described herein, in combination with one or more adjuvants can be formulated to comprise certain molar ratios. For example, molar ratios of about 99: 1 to about 1:99 of an active agent such as a peptide, a nucleic acid, an antibody or fragments thereof, and/or an APC described herein, in combination with one or more adjuvants can be used. In some instances, the range of molar ratios of an active agent such as a peptide, a nucleic acid, an antibody or fragments thereof, and/or an APC described herein, in combination with one or more adjuvants can be selected from about 80:20 to about 20: 80; about 75:25 to about 25:75, about 70:30 to about 30:70, about 66:33 to about 33:66, about 60:40 to about 40:60; about 50:50; and about 90: 10 to about 10:90. The molar ratio of an active agent such as a peptide, a nucleic acid, an antibody or fragments thereof, and/or an APC described herein, in combination with one or more adjuvants can be about 1:9, and in some cases can be about 1:1. The active agent such as a peptide, a nucleic acid, an antibody or fragments thereof, and/or an APC described herein, in combination with one or more adjuvants can be formulated together, in the same dosage unit e.g., in one vial, suppository, tablet, capsule, an aerosol spray; or each agent, form, and/or compound can be formulated in separate units, e.g., two vials, suppositories, tablets, two capsules, a tablet and a vial, an aerosol spray, and the like.

[00490] In some instances, an immunogenic pharmaceutical composition can be administered with an additional agent. The choice of the additional agent can depend, at least in part, on the condition being treated. The additional agent can include, for example, any agents having a therapeutic effect for a pathogen infection (e.g. viral infection), including, e.g., drugs used to treat inflammatory conditions such as an NSAID, e.g., ibuprofen, naproxen, acetaminophen, ketoprofen, or aspirin. As another example, formulations can additionally contain one or more supplements, such as vitamin C, E or other anti-oxidants.

[00491] A pharmaceutical composition comprising an active agent such as a peptide, a nucleic acid, an antibody or fragments thereof, and/or an APC described herein, in combination with one or more adjuvants can be formulated in conventional manner using one or more physiologically acceptable carriers, comprising excipients, diluents, and/or auxiliaries, e.g., which facilitate processing of the active agents into preparations that can be administered. Proper formulation can depend at least in part upon the route of administration chosen. The agent(s) described herein can be delivered to a patient using a number of routes or modes of administration, including oral, buccal, topical, rectal, transdermal, transmucosal, subcutaneous, intravenous, and intramuscular applications, as well as by inhalation.

[00492] The active agents can be formulated for parenteral administration (e.g., by injection, for example bolus injection or continuous infusion) and can be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol.

[00493] For injectable formulations, the vehicle can be chosen from those known in art to be suitable, including aqueous solutions or oil suspensions, or emulsions, with sesame oil, corn oil, cottonseed oil, or peanut oil, as well as elixirs, mannitol, dextrose, or a sterile aqueous solution, and similar pharmaceutical vehicles. The formulation can also comprise polymer compositions which are biocompatible, biodegradable, such as poly(lactic-co-glycolic)acid. These materials can be made into micro or nanospheres, loaded with drug and further coated or derivatized to provide superior sustained release performance. Vehicles suitable for periocular or intraocular injection include, for example, suspensions of therapeutic agent in injection grade water, liposomes and vehicles suitable for lipophilic substances. Other vehicles for periocular or intraocular injection are well known in the art.

[00494] In some instances, pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition can also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

[00495] When administration is by injection, the active agent can be formulated in aqueous solutions, specifically in physiologically compatible buffers such as Hanks solution, Ringer's solution, or physiological saline buffer. The solution can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active compound can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. In another embodiment, the pharmaceutical composition does not comprise an adjuvant or any other substance added to enhance the immune response stimulated by the peptide. In another embodiment, the pharmaceutical composition comprises a substance that inhibits an immune response to the peptide.

[00496] In addition to the formulations described previously, the active agents can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation or transcutaneous delivery (for example subcutaneously or intramuscularly), intramuscular injection or use of a transdermal patch. Thus, for example, the agents 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 a sparingly soluble salt.

[00497] In cases, pharmaceutical compositions comprising one or more agents exert local and regional effects when administered topically or injected at or near particular sites of infection. Direct topical application, e.g., of a viscous liquid, solution, suspension, dimethylsulfoxide (DMSO)-based solutions, liposomal formulations, gel, jelly, cream, lotion, ointment, suppository, foam, or aerosol spray, can be used for local administration, to

produce for example local and/or regional effects. Pharmaceutically appropriate vehicles for such formulation include, for example, lower aliphatic alcohols, polyglycols (e.g., glycerol or polyethylene glycol), esters of fatty acids, oils, fats, silicones, and the like. Such preparations can also include preservatives (e.g., p-hydroxybenzoic acid esters) and/or antioxidants (e.g., ascorbic acid and tocopherol). See also Dermatological Formulations: Percutaneous absorption, Barry (Ed.), Marcel Dekker Incl, 1983. In another embodiment, local/topical formulations comprising a transporter, carrier, or ion channel inhibitor are used to treat epidermal or mucosal viral infections.

[00498] Pharmaceutical compositions can contain a cosmetically or dermatologically acceptable carrier. Such carriers are compatible with skin, nails, mucous membranes, tissues and/or hair, and can include any conventionally used cosmetic or dermatological carrier meeting these requirements. Such carriers can be readily selected by one of ordinary skill in the art. In formulating skin ointments, an agent or combination of agents can be formulated in an oleaginous hydrocarbon base, an anhydrous absorption base, a water-in-oil absorption base, an oil-in-water water-removable base and/or a water-soluble base. Examples of such carriers and excipients include, but are not limited to, humectants (e.g., urea), glycols (e.g., propylene glycol), alcohols (e.g., ethanol), fatty acids (e.g., oleic acid), surfactants (e.g., isopropyl myristate and sodium lauryl sulfate), pyrrolidones, glycerol monolaurate, sulfoxides, terpenes (e.g., menthol), amines, amides, alkanes, alkanols, water, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[00499] Ointments and creams can, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions can be formulated with an aqueous or oily base and will in general also containing one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. Such patches can be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

[00500] Lubricants which can be used to form pharmaceutical compositions and dosage forms can include, but are not limited to, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, or mixtures thereof. Additional lubricants include, for example, a syloid silica gel, a coagulated aerosol of synthetic silica, or mixtures thereof. A lubricant can optionally be added, in an amount of less than about 1 weight percent of the pharmaceutical composition.

[00501] The pharmaceutical compositions can be in any form suitable for topical application, including aqueous, aqueous-alcoholic or oily solutions, lotion or serum dispersions, aqueous, anhydrous or oily gels, emulsions obtained by dispersion of a fatty phase in an aqueous phase (O/W or oil in water) or, conversely, (W/O or water in oil), microemulsions or alternatively microcapsules, microparticles or lipid vesicle dispersions of ionic and/or nonionic type. These compositions can be prepared according to conventional methods. The amounts of the various constituents of the compositions are those conventionally used in the art.

These compositions in particular constitute protection, treatment or care creams, milks, lotions, gels or foams for the face, for the hands, for the body and/or for the mucous membranes, or for cleansing the skin. The compositions can also consist of solid preparations constituting soaps or cleansing bars.

[00502] Pharmaceutical compositions can contain adjuvants such as hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active agents, preserving agents, antioxidants, solvents, fragrances, fillers, sunscreens, odor-absorbers and dyestuffs. The amounts of these various adjuvants are those conventionally used in the fields considered and, for example, are from about 0.01% to about 20% of the total weight of the composition. Depending on their nature, these adjuvants can be introduced into the fatty phase, into the aqueous phase and/or into the lipid vesicles.

[00503] In instances relating to topical/local application, the pharmaceutical compositions can include one or more penetration enhancers. For example, the formulations can comprise suitable solid or gel phase carriers or excipients that increase penetration or help delivery of agents or combinations of agents of the invention across a permeability barrier, e.g., the skin. Many of these penetration-enhancing compounds are known in the art of topical formulation, and include, e.g., water, alcohols (e.g., terpenes like methanol, ethanol, 2-propanol), sulfoxides (e.g., dimethyl sulfoxide, decylmethyl sulfoxide, tetradecylmethyl sulfoxide), pyrrolidones (e.g., 2-pyrrolidone, N-methyl-2-pyrrolidone, N-(2-hydroxyethyl)pyrrolidone), laurocapram, acetone, dimethylacetamide, dimethylformamide, tetrahydrofurfuryl alcohol, L-a-amino acids, anionic, cationic, amphoteric or nonionic surfactants (e.g., isopropyl myristate and sodium lauryl sulfate), fatty acids, fatty alcohols (e.g., oleic acid), amines, amides, clofibric acid amides, hexamethylene lauramide, proteolytic enzymes, a-bisabolol, d-limonene, urea and N,N-diethyl-m-toluamide, and the like. Additional examples include humectants (e.g., urea), glycols (e.g., propylene glycol and polyethylene glycol), glycerol monolaurate, alkanes, alkanols, ORGELASE, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and/or other polymers. In another embodiment, the pharmaceutical compositions will include one or more such penetration enhancers.

[00504] The pharmaceutical compositions for local/topical application can include one or more antimicrobial preservatives such as quaternary ammonium compounds, organic mercurials, p-hydroxy benzoates, aromatic alcohols, chlorobutanol, and the like.

[00505] The pharmaceutical compositions can be formulated into aerosol solutions, suspensions or dry powders. The aerosol can be administered through the respiratory system or nasal passages. For example, one skilled in the art will recognize that a composition of the present invention can be suspended or dissolved in an appropriate carrier, e.g., a pharmaceutically acceptable propellant, and administered directly into the lungs using a nasal spray or inhalant. For example, an aerosol formulation comprising a transporter, carrier, or ion channel inhibitor can be dissolved, suspended or emulsified in a propellant or a mixture of solvent and propellant, e.g., for administration as a nasal spray or inhalant. Aerosol formulations can contain any acceptable propellant under pressure, such as a cosmetically or dermatologically or pharmaceutically acceptable propellant, as conventionally used in the art.

[00506] An aerosol formulation for nasal administration is generally an aqueous solution designed to be administered to the nasal passages in drops or sprays. Nasal solutions can be similar to nasal secretions in that they are generally isotonic and slightly buffered to maintain a pH of about 5.5 to about 6.5, although pH values outside of this range can additionally be used. Antimicrobial agents or preservatives can also be included in the formulation.

[00507] An aerosol formulation for inhalations and inhalants can be designed so that the agent or combination of agents is carried into the respiratory tree of the subject when administered by the nasal or oral respiratory route. Inhalation solutions can be administered, for example, by a nebulizer. Inhalations or insufflations, comprising finely powdered or liquid drugs, can be delivered to the respiratory system as a pharmaceutical aerosol of a solution or suspension of the agent or combination of agents in a propellant, e.g., to aid in disbursement. Propellants can be liquefied gases, including halocarbons, for example, fluorocarbons such as fluorinated chlorinated hydrocarbons, hydrochlorofluorocarbons, and hydrochlorocarbons, as well as hydrocarbons and hydrocarbon ethers.

[00508] Halocarbon propellants can include fluorocarbon propellants in which all hydrogens are replaced with fluorine, chlorofluorocarbon propellants in which all hydrogens are replaced with chlorine and at least one fluorine, hydrogen-containing fluorocarbon propellants, and hydrogen-containing chlorofluorocarbon propellants. Hydrocarbon propellants useful in the invention include, for example, propane, isobutane, n-butane, pentane, isopentane and neopentane. A blend of hydrocarbons can also be used as a propellant. Ether propellants include, for example, dimethyl ether as well as the ethers. An aerosol formulation of the invention can also comprise more than one propellant. For example, the aerosol formulation can comprise more than one propellant from the same class, such as two or more fluorocarbons; or more than one, more than two, more than three propellants from different classes, such as a fluorohydrocarbon and a hydrocarbon. Pharmaceutical compositions of the present invention can also be dispensed with a compressed gas, e.g., an inert gas such as carbon dioxide, nitrous oxide or nitrogen.

[00509] Aerosol formulations can also include other components, for example, ethanol, isopropanol, propylene glycol, as well as surfactants or other components such as oils and detergents. These components can serve to stabilize the formulation and/or lubricate valve components.

[00510] The aerosol formulation can be packaged under pressure and can be formulated as an aerosol using solutions, suspensions, emulsions, powders and semisolid preparations. For example, a solution aerosol formulation can comprise a solution of an agent of the invention such as a transporter, carrier, or ion channel inhibitor in (substantially) pure propellant or as a mixture of propellant and solvent. The solvent can be used to dissolve the agent and/or retard the evaporation of the propellant. Solvents can include, for example, water, ethanol and glycols. Any combination of suitable solvents can be use, optionally combined with preservatives, antioxidants, and/or other aerosol components.

[00511] An aerosol formulation can be a dispersion or suspension. A suspension aerosol formulation can comprise a suspension of an agent or combination of agents of the instant invention, e.g., a transporter, carrier, or ion channel inhibitor, and a dispersing agent. Dispersing agents can include, for example, sorbitan trioleate,

oleyl alcohol, oleic acid, lecithin and corn oil. A suspension aerosol formulation can also include lubricants, preservatives, antioxidant, and/or other aerosol components.

[00512] An aerosol formulation can similarly be formulated as an emulsion. An emulsion aerosol formulation can include, for example, an alcohol such as ethanol, a surfactant, water and a propellant, as well as an agent or combination of agents of the invention, e.g., a transporter, carrier, or ion channel. The surfactant used can be nonionic, anionic or cationic. One example of an emulsion aerosol formulation comprises, for example, ethanol, surfactant, water and propellant. Another example of an emulsion aerosol formulation comprises, for example, vegetable oil, glyceryl monostearate and propane.

[00513] The pharmaceutical compounds can be formulated for administration as suppositories. A low melting wax, such as a mixture of triglycerides, fatty acid glycerides, Witepsol S55 (trademark of Dynamite Nobel Chemical, Germany), or cocoa butter is first melted and the active component is dispersed homogeneously, for example, by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and to solidify.

[00514] The pharmaceutical compositions can be formulated for vaginal administration. Pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

[00515] The pharmaceutical compositions can be attached releasably to biocompatible polymers for use in sustained release formulations on, in or attached to inserts for topical, intraocular, periocular, or systemic administration. The controlled release from a biocompatible polymer can be utilized with a water soluble polymer to form a instillable formulation, as well. The controlled release from a biocompatible polymer, such as for example, PLGA microspheres or nanospheres, can be utilized in a formulation suitable for intra ocular implantation or injection for sustained release administration, as well. Any suitable biodegradable and biocompatible polymer can be used.

Combinations of CTL peptides and HTL peptides

[00516] Immunogenic or therapeutic compositions comprising the neoantigenic polypeptides and polynucleotides described herein, or analogs thereof, which have immunostimulatory activity can be modified to provide desired attributes, such as improved serum half-life, or to enhance immunogenicity.

[00517] For instance, the ability of the neoantigenic peptides to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. In some embodiments, CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, e.g., Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus can be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL peptide can be linked to the T helper peptide without a spacer.

[00518] Although the CTL peptide epitope can be linked directly to the T helper peptide epitope, CTL epitope/HTL epitope conjugates can be linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, e.g., Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus can be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. The CTL peptide epitope can be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide can be acylated.

[00519] HTL peptide epitopes can also be modified to alter their biological properties. For example, peptides comprising HTL epitopes can contain D-amino acids to increase their resistance to proteases and thus extend their serum half-life. Also, the epitope peptides can be conjugated to other molecules such as lipids, proteins or sugars, or any other synthetic compounds, to increase their biological activity. For example, the T helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

[00520] In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences. Examples of amino acid sequences that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), Plasmodium falciparum CS protein at positions 378-398 (DIEKKIAKMEKASSVFNVVNS), and Streptococcus 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

[00521] Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (see, e.g., PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (e.g., PADRE, Epimmune, Inc., San Diego, CA) are designed to bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVWANTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and a is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type. An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

[00522] In some embodiments it can be desirable to include in a neoantigen therapeutic (e.g., peptides, polynucleotides, TCR, CAR, cells containing TCR or CAR, dendritic cell containing polypeptide, dendritic cell containing polynucleotide, antibody, etc.) in pharmaceutical compositions (e.g., immunogenic compositions) at least one component of which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL in vivo against viral antigens. For example, palmitic acid residues can be

attached to the ε-and a- amino groups of a lysine residue and then linked, e.g., via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic neoantigenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant. In some embodiments, a particularly effective immunogenic construct comprises palmitic acid attached to ε- and a- amino groups of Lys, which is attached via linkage, e.g., Ser-Ser, to the amino terminus of the immunogenic peptide.

[00523] As another example of lipid priming of CTL responses, E. coli lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P3CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide. (See, e.g., Deres, et al, Nature 342:561, 1989). Neoantigenic peptides described herein can be coupled to P3CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P3CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses to infection.

[00524] As noted herein, additional amino acids can be added to the termini of a neoantigenic peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide. However, it is to be noted that modification at the carboxyl terminus of a T cell epitope can, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH2 acylation, e.g., by alkanoyl (C1-C20) or thioglycolyl acetylation, terminal-carboxyl amidation, e.g., ammonia, methylamine, etc. In some instances these modifications can provide sites for linking to a support or other molecule.

[00525] An embodiment of an immunogenic composition described herein comprises ex vivo administration of a cocktail of epitope-bearing neoantigenic polypeptide or polynucleotides to PBMC, or isolated DC therefrom, from the patient's blood. A pharmaceutical to facilitate harvesting of dendritic cells (DCs) can be used, including GM-CSF, IL-4, IL-6, IL-Iβ, and TNFa. After pulsing the DCs with peptides or polynucleotides encoding the peptides, and prior to reinfusion into patients, the DC are washed to remove unbound peptides. In this embodiment, a therapeutic or immunogenic composition comprises peptide-pulsed DCs which present the pulsed peptide epitopes complexed with HLA molecules on their surfaces. The composition is then administered to the patient. In other embodiments, such pulsed DCs are used to stimulate T cells suitable for use in T cell therapy.

Multi-epitope immunogenic compositions

[00526] A number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the neoantigenic peptides described herein are a particularly useful embodiment of the invention. In some embodiments, the nucleic acid is RNA. In some embodiments, minigene constructs encoding a neoantigenic peptide comprising one or multiple epitopes described herein are used to administer nucleic acids encoding the neoantigenic peptides described herein uses.

[00527] The use of multi-epitope minigenes is described An, L. and Whitton, J. L., J. Virol. 71:2292, 1997; Thomson, S. A. et al., J. Immunol. 157:822, 1996; Whitton, J. L. et al., J. Virol. 67:348, 1993; Hanke, R. et al., Vaccine 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing neoantigen peptides, a universal helper T cell epitope (or multiple tumor associated antigen HTL epitopes), and an endoplasmic reticulum-translocating signal sequence can be engineered.

[00528] The immunogenicity of a multi-epitopic minigene can be tested in transgenic mice to evaluate the magnitude of immune response induced against the epitopes tested. Further, the immunogenicity of DNA-encoded epitopes in vivo can be correlated with the in vitro responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these experiments can show that the minigene serves to both: 1.) generate a cell mediated and/or humoral response and 2.) that the induced immune cells recognized cells expressing the encoded epitopes.

[00529] For example, to create a DNA sequence encoding the selected neoepitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes can be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These neoepitope-encoding DNA sequences can be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes can be improved by including synthetic (e.g. poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

[00530] The minigene sequence can be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) can be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

[00531] Standard regulatory sequences well known to those of skill in the art can be included in the vector to ensure expression in the target cells. For example, a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an E. coli origin of replication; and an E. coli selectable marker (e.g. ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, e.g., the human cytomegalovirus (hCMV) promoter. See, e.g., U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

[00532] Additional vector modifications can be used to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells can also be considered for increasing minigene expression.

[00533] Once an expression vector is selected, the minigene can be cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate E. coli strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, can be confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

[00534] In addition, immunomodulatory sequences appear to play a role in the immunogenicity of DNA therapuetics. These sequences can be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity. In some embodiments, the sequences are immunostimulatory. In another embodiment, the sequences are ISSs or CpGs.

[00535] In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (e.g., IL-2, IL-12, GM-CSF), cytokine-inducing molecules (e.g., LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins. Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (e.g. TGF-β) can be beneficial in certain diseases.

[00536] Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in E. coli, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor according to well-known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

[00537] Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA therapuetics, an alternative method for formulating purified plasmid DNA can be used. A variety of methods have been described, and new techniques can become available. Cationic lipids can also be used in the formulation (see, e.g., as described by WO 93/24640; Mannino & Gould-Fogerite, BioTechniques 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Feigner, et al, Proc. Nat'l Acad. Sci. USA 84:7413 (1987). In addition, glycolipids, fusogenic liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

[00538] In another embodiment, the nucleic acid is introduced into cells by use of high-speed cell deformation. During high-speed deformation, cells are squeezed such that temporary disruptions occur in the cell membrane, thus allowing the nucleic acid to enter the cell. Alternatively, protein can be produced from expression vectors - in a bacterial expression vector, for example, and the proteins can then be delivered to the cell.

[00539] Target cell sensitization can be used as a functional assay for expression and HLA class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct in vitro transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (51Cr) labeled and used as target cells for epitope-specific CTL lines; cytolysis, detected by 51Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes can be evaluated in an analogous manner using assays to assess HTL activity.

[00540] In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (e.g., IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA). An exemplary protocol is twenty-one days after immunization, splenocytes are harvested and restimulated for 1 week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, 51Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccines function for in vivo induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

[00541] Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

XIX. Cells for Use in the Invention

[00542] In one aspect, the present disclosure also provides cells expressing a neoantigen-recognizing receptor that activates an immunoresponsive cell (e.g., T cell receptor (TCR) or chimeric antigen receptor (CAR)), and methods of using such cells for the treatment of a disease that requires an enhanced immune response.

[00543] Such cells include genetically modified immunoresponsive cells (e.g., T cells, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTL) cells, helper T lymphocyte (HTL) cells) expressing an antigen-recognizing receptor (e.g., TCR or CAR) that binds one of the neoantigenic peptides described herein, and methods of use therefore for the treatment of neoplasia and other pathologies where an increase in an antigen-specific immune response is desired. T cell activation is mediated by a TCR or a CAR targeted to an antigen.

[00544] The present disclosure provides cells expressing a combination of an antigen-recognizing receptor that activates an immunoresponsive cell (e.g., TCR, CAR) and a chimeric co-stimulating receptor (CCR), and

methods of using such cells for the treatment of a disease that requires an enhanced immune response. In some embodiments, tumor antigen-specific T cells, NK cells, CTL cells or other immunoresponsive cells are used as shuttles for the selective enrichment of one or more co-stimulatory ligands for the treatment or prevention of neoplasia. Such cells are administered to a human subject in need thereof for the treatment or prevention of a particular cancer.

[00545] In some embodiments,the tumor antigen-specific human lymphocytes that can be used in the methods of the invention include, without limitation, peripheral donor lymphocytes genetically modified to express chimeric antigen receptors (CARs) (Sadelain, M., et al. 2003 Nat Rev Cancer 3:35-45), peripheral donor lymphocytes genetically modified to express a full-length tumor antigen-recognizing T cell receptor complex comprising the a and p heterodimer (Morgan, R. A., et al. 2006 Science 314:126-129), lymphocyte cultures derived from tumor infiltrating lymphocytes (TILs) in tumor biopsies (Panelli, M. C , et al. 2000 J Immunol 164:495-504; Panelli, M. C , et al. 2000 J Immunol 164:4382-4392), and selectively in vitro-expanded antigen-specific peripheral bloodleukocytes employing artificial antigen-presenting cells (AAPCs) or pulsed dendritic cells (Dupont, J., et al. 2005 Cancer Res 65:5417-5427; Papanicolaou, G. A., et al. 2003 Blood 102:2498-2505). The T cells may be autologous, allogeneic, or derived in vitro from engineered progenitor or stem cells.

[00546] In some embodiments, the immunotherapeutic is an engineered receptor. In some embodiments, the engineered receptor is a chimeric antigen receptor (CAR), a T cell receptor (TCR), or a B-cell receptor (BCR), an adoptive T cell therapy (ACT), or a derivative thereof. In other aspects, the engineered receptor is a chimeric antigen receptor (CAR). In some aspects, the CAR is a first generation CAR. In other aspects, the CAR is a second generation CAR. In still other aspects, the CAR is a third generation CAR.

[00547] In some aspects, the CAR comprises an extracellular portion, a transmembrane portion, and an intracellular portion. In some aspects, the intracellular portion comprises at least one T cell co-stimulatory domain. In some aspects, the T cell co-stimulatory domain is selected from the group consisting of CD27, CD28, TNFRS9 (4-1BB), TNFRSF4 (OX40), TNFRSF8 (CD30), CD40LG (CD40L), ICOS, ITGB2 (LFA-1), CD2, CD7, KLRC2 (NKG2C), TNFRS18 (GITR), TNFRSF14 (HVEM), or any combination thereof.

[00548] In some aspects, the engineered receptor binds a target. In some aspects, the binding is specific to a peptide specific to one or more subjects suffering from a disease or condition.

[00549] In some aspects, the immunotherapeutic is a cell as described in detail herein. In some aspects, the immunotherapeutic is a cell comprising a receptor that specifically binds a peptide. In some aspects, the immunotherapeutic is a cell used in combination with the peptides/nucleic acids of this invention. In some embodiments, the cell is a patient cell. In some embodiments, the cell is a T cell. In some embodiments, the cell is tumor infiltrating lymphocyte.

[00550] In some aspects, a subject with a condition or disease is treated based on a T cell receptor repertoire of the subject. In some embodiments, an antigen therapy is selected based on a T cell receptor repertoire of the subject. In some embodiments, a subject is treated with T cells expressing TCRs specific to an antigen or peptide. In some embodiments, a subject is treated with an antigen or peptide specific to TCRs, e.g., subject

specific TCRs. In some embodiments, a subject is treated with an antigen or peptide specific to T cells expressing TCRs, e.g., subject specific TCRs. In some embodiments, a subject is treated with an antigen or peptide specific to subject specific TCRs.

[00551] In some embodiments, an immunogenic antigen composition or therapy is selected based on TCRs identified in one or more subjects. In some embodiments identification of a T cell repertoire and testing in functional assays is used to determine an immunogenic composition or therapy to be administered to one or more subjects with a condition or disease. In some embodiments, the immunogenic composition is an antigen therapy. In some embodiments, the antigen therapy comprises subject specific antigen peptides. In some embodiments, antigen peptides to be included in an antigen therapy are selected based on a quantification of subject specific TCRs that bind to the antigens. In some embodiments, antigen peptides are selected based on a binding affinity of the peptide to a TCR. In some embodiments, the selecting is based on a combination of both the quantity and the binding affinity. For example, a TCR that binds strongly to an antigen in a functional assay, but that is not highly represented in a TCR repertoire may be a good candidate for an antigen therapy because T cells expressing the TCR would be advantageously amplified.

[00552] In some embodiments, antigens are selected for administering to one or more subjects based on binding to TCRs. In some embodiments, T cells, such as T cells from a subject with a disease or condition, can be expanded. Expanded T cells that express TCRs specific to an immunogenic antigen peptide, can be administered back to a subject. In some embodiments, suitable cells, e.g., PBMCs, are transduced or transfected with polynucleotides for expression of TCRs specific to an immunogenic antigen peptide and administered to a subject. T cells expressing TCRs specific to an immunogenic antigen peptide can be expanded and administered back to a subject. In some embodiments, T cells that express TCRs specific to an immunogenic antigen peptide that result in cytolytic activity when incubated with autologous diseased tissue can be expanded and administered to a subject. In some embodiments, T cells used in functional assays result in binding to an immunogenic antigen peptide can be expanded and administered to a subject. In some embodiments, TCRs that have been determined to bind to subject specific immunogenic antigen peptides can be expressed in T cells and administered to a subject.

XX. Methods of Use

[00553] The neoantigen therapeutics (e.g., peptides, polynucleotides, TCR, CAR, cells containing TCR or CAR, dendritic cell containing polypeptide, dendritic cell containing polynucleotide, antibody, etc.) described herein are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as the treatment of cancer. In some embodiments, the therapeutic treatment methods comprise immunotherapy. In certain embodiments, a neoantigen peptide is useful for activating, promoting, increasing, and/or enhancing an immune response, redirecting an existing immune response to a new target, increasing the immunogenicity of a tumor, inhibiting tumor growth, reducing tumor volume, increasing tumor cell apoptosis, and/or reducing the tumorigenicity of a tumor. The methods of use can be in vitro, ex vivo, or in vivo methods.

[00554] In some aspects, the present disclosure provides methods for activating an immune response in a subject using a neoantigen therapeutic described herein. In some embodiments, the invention provides

methods for promoting an immune response in a subject using a neoantigen therapeutic described herein. In some embodiments, the invention provides methods for increasing an immune response in a subject using a neoantigen peptide described herein. In some embodiments, the invention provides methods for enhancing an immune response using a neoantigen peptide. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing cell-mediated immunity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing T cell activity or humoral immunity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing CTL or HTL activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing T cell activity and increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises increasing CTL activity and increasing NK cell activity. In some embodiments, the activating, promoting, increasing, and/or enhancing of an immune response comprises inhibiting or decreasing the suppressive activity of Tregs. In some embodiments, the immune response is a result of antigenic stimulation. In some embodiments, the antigenic stimulation is a tumor cell. In some embodiments, the antigenic stimulation is cancer.

[00555] In some embodiments, the present disclosure provides methods of activating, promoting, increasing, and/or enhancing of an immune response using a neoantigen therapeutic described herein. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a neoantigen therapeutic that delivers a neoantigen polypeptide or polynucleotide to a tumor cell. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a neoantigen therapeutic that binds the tumor associated antigen and is internalized by the tumor cell. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a neoantigen polypeptide that is internalized by a tumor cell, and the neoantigen peptide is processed by the cell. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a neoantigen polypeptide that is internalized by a tumor cell, and an antigenic peptide is presented on the surface of the tumor cell. In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a neoantigen polypeptide that is internalized by the tumor cell, is processed by the cell, and an antigenic peptide is presented on the surface of the tumor cell.

[00556] In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a neoantigen polypeptide or polynucleotide described herein that delivers an exogenous polypeptide comprising at least one antigenic peptide to a tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell. In some embodiments, the antigenic peptide is presented on the surface of the tumor cell in complex with a MHC class I molecule. In some embodiments, the antigenic peptide is presented on the surface of the tumor cell in complex with a MHC class II molecule.

[00557] In some embodiments, a method comprises contacting a tumor cell with a neoantigen polypeptide or polynucleotide described herein that delivers an exogenous polypeptide comprising at least one antigenic peptide to the tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell. In some embodiments, the antigenic peptide is presented on the surface of the tumor cell in complex with a MHC class I molecule. In some embodiments, the antigenic peptide is presented on the surface of the tumor cell in complex with a MHC class II molecule.

[00558] In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a neoantigen polypeptide or polynucleotide described herein that delivers an exogenous polypeptide comprising at least one antigenic peptide to a tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell, and an immune response against the tumor cell is induced. In some embodiments, the immune response against the tumor cell is increased. In some embodiments, the neoantigen polypeptide or polynucleotide delivers an exogenous polypeptide comprising at least one antigenic peptide to a tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell, and tumor growth is inhibited.

[00559] In some embodiments, a method comprises administering to a subject in need thereof a therapeutically effective amount of a neoantigen polypeptide or polynucleotide described herein that delivers an exogenous polypeptide comprising at least one antigenic peptide to a tumor cell, wherein the antigenic peptide is presented on the surface of the tumor cell, and T cell killing directed against the tumor cell is induced. In some embodiments, T cell killing directed against the tumor cell is enhanced. In some embodiments, T cell killing directed against the tumor cell is increased.

[00560] In some embodiments, a method of increasing an immune response in a subject comprises administering to the subject a therapeutically effective amount of a neoantigen therapeutic described herein, wherein the agent is an antibody that specifically binds the neoantigen described herein. In some embodiments, a method of increasing an immune response in a subject comprises administering to the subject a therapeutically effective amount of the antibody.

[00561] The present disclosure provides methods of redirecting an existing immune response to a tumor. In some embodiments, a method of redirecting an existing immune response to a tumor comprises administering to a subject a therapeutically effective amount of a neoantigen therapeutic described herein. In some embodiments, the existing immune response is against a virus. In some embodiments, the virus is selected from the group consisting of: measles virus, varicella-zoster virus (VZV; chickenpox virus), influenza virus, mumps virus, poliovirus, rubella virus, rotavirus, hepatitis A virus (HAV), hepatitis B virus (HBV), Epstein Barr virus (EBV), and cytomegalovirus (CMV). In some embodiments, the virus is varicella-zoster virus. In some embodiments, the virus is cytomegalovirus. In some embodiments, the virus is measles virus. In some embodiments, the existing immune response has been acquired after a natural viral infection. In some embodiments, the existing immune response has been acquired after vaccination against a virus. In some embodiments, the existing immune response is a cell-mediated response. In some embodiments, the existing immune response comprises cytotoxic T cells (CTLs) or HTLs.

[00562] In some embodiments, a method of redirecting an existing immune response to a tumor in a subject comprises administering a fusion protein comprising (i) an antibody that specifically binds a neoantigen and (ii) at least one neoantigenic peptide described herein, wherein (a) the fusion protein is internalized by a tumor cell after binding to the tumor-associated antigen; (b) the neoantigenic peptide is processed and presented on the surface of the tumor cell associated with a MHC class I molecule; and (c) the neoantigenic peptide/MHC Class I complex is recognized by cytotoxic T cells. In some embodiments, the cytotoxic T cells are memory T cells. In some embodiments, the memory T cells are the result of a vaccination with the neoantigenic peptide.

[00563] The present disclosure provides methods of increasing the immunogenicity of a tumor. In some embodiments, a method of increasing the immunogenicity of a tumor comprises contacting a tumor or tumor cells with an effective amount of a neoantigen therapeutic described herein. In some embodiments, a method of increasing the immunogenicity of a tumor comprises administering to a subject a therapeutically effective amount of a neoantigen therapeutic described herein.

[00564] The present disclosure also provides methods for inhibiting growth of a tumor using a neoantigen therapeutic described herein. In certain embodiments, a method of inhibiting growth of a tumor comprises contacting a cell mixture with a neoantigen therapeutic in vitro. For example, an immortalized cell line or a cancer cell line mixed with immune cells (e.g., T cells) is cultured in medium to which a neoantigenic peptide is added. In some embodiments, tumor cells are isolated from a patient sample, for example, a tissue biopsy, pleural effusion, or blood sample, mixed with immune cells (e.g., T cells), and cultured in medium to which a neoantigen therapeutic is added. In some embodiments, a neoantigen therapeutic increases, promotes, and/or enhances the activity of the immune cells. In some embodiments, a neoantigen therapeutic inhibits tumor cell growth. In some embodiments, a neoantigen therapeutic activates killing of the tumor cells.

[00565] In certain embodiments, the subject is a human. In certain embodiments, the subject has a tumor or the subject had a tumor which was at least partially removed.

[00566] In some embodiments, a method of inhibiting growth of a tumor comprises redirecting an existing immune response to a new target, comprising administering to a subject a therapeutically effective amount of a neoantigen therapeutic, wherein the existing immune response is against an antigenic peptide delivered to the tumor cell by the neoantigenic peptide.

[00567] In certain embodiments, the tumor comprises cancer stem cells. In certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of the neoantigen therapeutic. In some embodiments, a method of reducing the frequency of cancer stem cells in a tumor in a subject, comprising administering to the subject a therapeutically effective amount of a neoantigen therapeutic is provided.

[00568] In addition, in some aspects the present disclosure provides a method of reducing the tumorigenicity of a tumor in a subject, comprising administering to the subject a therapeutically effective amount of a neoantigen therapeutic described herein. In certain embodiments, the tumor comprises cancer stem cells. In some embodiments, the tumorigenicity of a tumor is reduced by reducing the frequency of cancer stem cells in the tumor. In some embodiments, the methods comprise using the neoantigen therapeutic described herein. In

certain embodiments, the frequency of cancer stem cells in the tumor is reduced by administration of a neoantigen therapeutic described herein.

[00569] In some embodiments, the tumor is a solid tumor. In certain embodiments, the tumor is a tumor selected from the group consisting of: colorectal tumor, pancreatic tumor, lung tumor, ovarian tumor, liver tumor, breast tumor, kidney tumor, prostate tumor, neuroendocrine tumor, gastrointestinal tumor, melanoma, cervical tumor, bladder tumor, glioblastoma, and head and neck tumor. In certain embodiments, the tumor is a colorectal tumor. In certain embodiments, the tumor is an ovarian tumor. In some embodiments, the tumor is a breast tumor. In some embodiments, the tumor is a lung tumor. In certain embodiments, the tumor is a pancreatic tumor. In certain embodiments, the tumor is a melanoma tumor. In some embodiments, the tumor is a solid tumor.

[00570] The present disclosure further provides methods for treating cancer in a subject comprising administering to the subject a therapeutically effective amount of a neoantigen therapeutic described herein.

[00571] In some embodiments, a method of treating cancer comprises redirecting an existing immune response to a new target, the method comprising administering to a subject a therapeutically effective amount of neoantigen therapeutic, wherein the existing immune response is against an antigenic peptide delivered to the cancer cell by the neoantigenic peptide.

[00572] The present disclosure provides for methods of treating cancer comprising administering to a subject a therapeutically effective amount of a neoantigen therapeutic described herein (e.g., a subject in need of treatment). In certain embodiments, the subject is a human. In certain embodiments, the subject has a cancerous tumor. In certain embodiments, the subject has had a tumor at least partially removed.

[00573] In certain embodiments, the cancer is a cancer selected from the group consisting of colorectal cancer, pancreatic cancer, lung cancer, ovarian cancer, liver cancer, breast cancer, kidney cancer, prostate cancer, gastrointestinal cancer, melanoma, cervical cancer, neuroendocrine cancer, bladder cancer, glioblastoma, and head and neck cancer. In certain embodiments, the cancer is pancreatic cancer. In certain embodiments, the cancer is ovarian cancer. In certain embodiments, the cancer is colorectal cancer. In certain embodiments, the cancer is breast cancer. In certain embodiments, the cancer is prostate cancer. In certain embodiments, the cancer is lung cancer. In certain embodiments, the cancer is melanoma. In some embodiments, the cancer is a solid cancer. In some embodiments, the cancer comprises a solid tumor.

[00574] In some embodiments, the cancer is a hematologic cancer. In some embodiment, the cancer is selected from the group consisting of: acute myelogenous leukemia (AML), Hodgkin lymphoma, multiple myeloma, T cell acute lymphoblastic leukemia (T-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia, chronic myelogenous leukemia (CML), non-Hodgkin lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), and cutaneous T cell lymphoma (CTCL).

[00575] In some embodiements, the neoantigen therapeutic is administered as a combination therapy. Combination therapy with two or more therapeutic agents uses agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action can result in additive or synergetic effects. Combination therapy can allow for a lower dose of each agent than

is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of the agent(s). Combination therapy can decrease the likelihood that resistant cancer cells will develop. In some embodiments, combination therapy comprises a therapeutic agent that affects the immune response (e.g., enhances or activates the response) and a therapeutic agent that affects (e.g., inhibits or kills) the tumor/cancer cells.

[00576] Cancers include, but are not limited to, B cell cancer, e.g., multiple myeloma, Waldenstrom's macroglobulinemia, the heavy chain diseases, such as, for example, alpha chain disease, gamma chain disease, and mu chain disease, benign monoclonal gammopathy, and immunocytic amyloidosis, melanomas, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer (e.g., metastatic, hormone refractory prostate cancer), pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like. Other non-limiting examples of types of cancers applicable to the methods encompassed by the present invention include human sarcomas and carcinomas, Examples of cancers include, without limitation, leukemia (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblasts leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (e.g., Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, triple negative breast cancer (TNBC), ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease. In some embodiments, the cancer whose phenotype is determined by the method of the invention is an epithelial cancer such as, but not limited to, bladder cancer, breast cancer, cervical cancer, colon cancer, gynecologic cancers, renal cancer,

laryngeal cancer, lung cancer, oral cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, or skin cancer. In other embodiments, the cancer is breast cancer, prostate cancer, lung cancer, or colon cancer. In still other embodiments, the epithelial cancer is non-small-cell lung cancer, nonpapillary renal cell carcinoma, cervical carcinoma, ovarian carcinoma (e.g., serous ovarian carcinoma), or breast carcinoma. The epithelial cancers may be characterized in various other ways including, but not limited to, serous, endometrioid, mucinous, clear cell, brenner, or undifferentiated. In some embodiments, the present invention is used in the treatment, diagnosis, and/or prognosis of lymphoma or its subtypes, including, but not limited to, mantle cell lymphoma.oligodenroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma). Lymphoproliferative disorders are also considered to be proliferative diseases.

[00577] In some embodiments, specific examples of cancers that may be prevented or treated in accordance with the compositions include, but are not limited to: renal cancer, kidney cancer, glioblastoma multiforme, metastatic breast cancer; breast carcinoma; breast sarcoma; neurofibroma; neurofibromatosis; pediatric tumors; neuroblastoma; malignant melanoma; carcinomas of the epidermis; leukemias such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myclodysplastic syndrome, chronic leukemias such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenstrom's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone cancer and connective tissue sarcomas such as but not limited to bone sarcoma, myeloma bone disease, multiple myeloma, cholesteatoma-induced bone osteosarcoma, Paget's disease of bone, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, and synovial sarcoma; brain tumors such as but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, and primary brain lymphoma; breast cancer including but not limited to adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease (including juvenile Paget's disease) and inflammatory breast cancer; adrenal cancer such as but not limited to pheochromocytom and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer such as but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius; eye cancers such as but not limited to ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma;

vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers such as but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited to endometrial carcinoma and uterine sarcoma; ovarian cancers such as but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; cervical carcinoma; esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid cyctic carcinoma, mucoepi dermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers such as but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; KRAS- mutated colorectal cancer; colon carcinoma; rectal cancers; liver cancers such as but not limited to hepatocellular carcinoma and hepatoblastoma, gallbladder cancers such as adenocarcinoma; cholangiocarcinomas such as but not limited to papillary, nodular, and diffuse; lung cancers such as KRAS-mutated non-small cell lung cancer, non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; lung carcinoma; testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, androgen-independent prostate cancer, androgen-dependent prostate cancer, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal cancers; oral cancers such as but not limited to squamous cell carcinoma; basal cancers; salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and verrucous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers such as but not limited to renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/or uterer); renal carcinoma; Wilms' tumor; bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas.

[00578] In some embodiments, the combination of an agent described herein and at least one additional therapeutic agent results in additive or synergistic results. In some embodiments, the combination therapy results in an increase in the therapeutic index of the agent. In some embodiments, the combination therapy results in an increase in the therapeutic index of the additional therapeutic agent(s). In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the agent. In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the additional therapeutic agent(s).

[00579] In certain embodiments, in addition to administering a neoantigen therapeutic described herein, the method or treatment further comprises administering at least one additional therapeutic agent. An additional therapeutic agent can be administered prior to, concurrently with, and/or subsequently to, administration of the agent. In some embodiments, the at least one additional therapeutic agent comprises 1, 2, 3, or more additional therapeutic agents.

[00580] Therapeutic agents that can be administered in combination with the neoantigen therapeutic described herein include chemotherapeutic agents. Thus, in some embodiments, the method or treatment involves the administration of an agent described herein in combination with a chemotherapeutic agent or in combination with a cocktail of chemotherapeutic agents. Treatment with an agent can occur prior to, concurrently with, or subsequent to administration of chemotherapies. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. Preparation and dosing schedules for such chemotherapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in The Chemotherapy Source Book, 4th Edition, 2008, M. C. Perry, Editor, Lippincott, Williams & Wilkins, Philadelphia, PA.

[00581] Useful classes of chemotherapeutic agents include, for example, anti-tubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cisplatin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, anti-folates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like. In certain embodiments, the second therapeutic agent is an alkylating agent, an antimetabolite, an antimitotic, a topoisomerase inhibitor, or an angiogenesis inhibitor.

[00582] Chemotherapeutic agents useful in the instant disclosure include, but are not limited to, alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamime; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-

fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytosine arabinoside, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as folinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); taxoids, e.g. paclitaxel (TAXOL) and docetaxel (TAXOTERE); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; ibandronate; CPT1 1; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine (XELODA); and pharmaceutically acceptable salts, acids or derivatives of any of the above. Chemotherapeutic agents also include anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4 hydroxytamoxifen, trioxifene, keoxifene, LY 1170 18, onapristone, and toremifene (FARESTON); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In certain embodiments, the additional therapeutic agent is cisplatin. In certain embodiments, the additional therapeutic agent is carboplatin.

[00583] In certain embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerase inhibitors are chemotherapy agents that interfere with the action of a topoisomerase enzyme (e.g., topoisomerase I or II). Topoisomerase inhibitors include, but are not limited to, doxorubicin HC1, daunorubicin citrate, mitoxantrone HC1, actinomycin D, etoposide, topotecan HC1, teniposide (VM-26), and irinotecan, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In some embodiments, the additional therapeutic agent is irinotecan.

[00584] In certain embodiments, the chemotherapeutic agent is an anti-metabolite. An anti-metabolite is a chemical with a structure that is similar to a metabolite required for normal biochemical reactions, yet different enough to interfere with one or more normal functions of cells, such as cell division. Anti-metabolites include, but are not limited to, gemcitabine, fluorouracil, capecitabine, methotrexate sodium, ralitrexed, pemetrexed, tegafur, cytosine arabinoside, thioguanine, 5-azacytidine, 6 mercaptopurine, azathioprine, 6-thioguanine, pentostatin, fludarabine phosphate, and cladribine, as well as pharmaceutically acceptable salts, acids, or derivatives of any of these. In certain embodiments, the additional therapeutic agent is gemcitabine.

[00585] In certain embodiments, the chemotherapeutic agent is an antimitotic agent, including, but not limited to, agents that bind tubulin. In some embodiments, the agent is a taxane. In certain embodiments, the agent is paclitaxel or docetaxel, or a pharmaceutically acceptable salt, acid, or derivative of paclitaxel or docetaxel. In certain embodiments, the agent is paclitaxel (TAXOL), docetaxel (TAXOTERE), albumin-bound paclitaxel (ABRAXANE), DHA-paclitaxel, or PG-paclitaxel. In certain alternative embodiments, the antimitotic agent comprises a vinca alkaloid, such as vincristine, vinblastine, vinorelbine, or vindesine, or pharmaceutically acceptable salts, acids, or derivatives thereof. In some embodiments, the antimitotic agent is an inhibitor of kinesin Eg5 or an inhibitor of a mitotic kinase such as Aurora A or Plkl. In certain embodiments, the additional therapeutic agent is paclitaxel. In some embodiments, the additional therapeutic agent is albumin-bound paclitaxel.

[00586] In some embodiments, an additional therapeutic agent comprises an agent such as a small molecule. For example, treatment can involve the combined administration of an agent of the present invention with a small molecule that acts as an inhibitor against tumor-associated antigens including, but not limited to, EGFR, HER2 (ErbB2), and/or VEGF. In some embodiments, an agent of the present invention is administered in combination with a protein kinase inhibitor selected from the group consisting of: gefitinib (IRESSA), erlotinib (TARCEVA), sunitinib (SUTENT), lapatanib, vandetanib (ZACTIMA), AEE788, CI-1033, cediranib (RECENTIN), sorafenib (NEXAVAR), and pazopanib (GW786034B). In some embodiments, an additional therapeutic agent comprises an mTOR inhibitor. In another embodiment, the additional therapeutic agent is chemotherapy or other inhibitors that reduce the number of Treg cells. In certain embodiments, the therapeutic agent is cyclophosphamide or an anti-CTLA4 antibody. In another embodiment, the additional therapeutic reduces the presence of myeloid-derived suppressor cells. In a further embodiment, the additional therapeutic is carbotaxol. In another embodiment, the additional therapeutic agent shifts cells to a T helper 1 response. In a further embodiment, the additional therapeutic agent is ibrutinib.

[00587] In some embodiments, an additional therapeutic agent comprises a biological molecule, such as an antibody. For example, treatment can involve the combined administration of an agent of the present invention with antibodies against tumor-associated antigens including, but not limited to, antibodies that bind EGFR, HER2/ErbB2, and/or VEGF. In certain embodiments, the additional therapeutic agent is an antibody specific for a cancer stem cell marker. In certain embodiments, the additional therapeutic agent is an antibody that is an angiogenesis inhibitor (e.g., an anti-VEGF or VEGF receptor antibody). In certain embodiments, the additional therapeutic agent is bevacizumab (AVASTIN), ramucirumab, trastuzumab (HERCEPTIN), pertuzumab (OMNITARG), panitumumab (VECTIBIX), nimotuzumab, zalutumumab, or cetuximab (ERBITUX).

[00588] The agents and compositions provided herein may be used alone or in combination with conventional therapeutic regimens such as surgery, irradiation, chemotherapy and/or bone marrow transplantation (autologous, syngeneic, allogeneic or unrelated). A set of tumor antigens can be useful, e.g., in a large fraction of cancer patients.

[00589] In some embodiments, at least one or more chemotherapeutic agents may be administered in addition to the composition comprising an immunogenic therapy. In some embodiments, the one or more chemotherapeutic agents may belong to different classes of chemotherapeutic agents.

[00590] Examples of chemotherapy agents include, but are not limited to, alkylating agents such as nitrogen mustards (e.g. mechlorethamine (nitrogen mustard), chlorambucil, cyclophosphamide (Cytoxan®), ifosfamide, and melphalan); nitrosoureas (e.g. N-Nitroso-N-methylurea, streptozocin, carmustine (BCNU), lomustine, and semustine); alkyl sulfonates (e.g. busulfan); tetrazines (e.g. dacarbazine (DTIC), mitozolomide and temozolomide (Temodar®)); aziridines (e.g. thiotepa, mytomycin and diaziquone); and platinum drugs (e.g. cisplatin, carboplatin, and oxaliplatin); non-classical alkylating agents such as procarbazine and altretamine (hexamethylmelamine); anti-metabolite agents such as 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), capecitabine (Xeloda®), cladribine, clofarabine, cytarabine (Ara-C®), decitabine, floxuridine, fludarabine, nelarabine, gemcitabine (Gemzar®), hydroxyurea, methotrexate, pemetrexed (Alimta®), pentostatin, thioguanine, Vidaza; anti-microtubule agents such as vinca alkaloids (e.g. vincristine, vinblastine, vinorelbine, vindesine and vinflunine); taxanes (e.g. paclitaxel (Taxol®), docetaxel (Taxotere®)); podophyllotoxin (e.g. etoposide and teniposide); epothilones (e.g. ixabepilone (Ixempra®)); estramustine (Emcyt®); anti-tumor antibiotics such as anthracyclines (e.g. daunorubicin, doxorubicin (Adriamycin®, epirubicin, idarubicin); actinomycin-D; and bleomycin; topoisomerase I inhibitors such as topotecan and irinotecan (CPT-11); topoisomerase II inhibitors such as etoposide (VP-16), teniposide, mitoxantrone, novobiocin, merbarone and aclarubicin; corticosteroids such as prednisone, methylprednisolone (Solumedrol®), and dexamethasone (Decadron®); L-asparaginase; bortezomib (Velcade®); immunotherapeutic agents such as rituximab (Rituxan®), alemtuzumab (Campath®), thalidomide, lenalidomide (Revlimid®), BCG, interleukin-2, interferon-alfa and cancer therapies such as Provenge®; hormone therapeutic agents such as fulvestrant (Faslodex®), tamoxifen, toremifene (Fareston®), anastrozole (Arimidex®), exemestan (Aromasin®), letrozole (Femara®), megestrol acetate (Megace®), estrogens, bicalutamide (Casodex®), flutamide (Eulexin®), nilutamide (Nilandron®), leuprolide (Lupron®) and goserelin (Zoladex®); differentiating agents such as retinoids, tretinoin (ATRA or Atralin®), bexarotene (Targretin®) and arsenic trioxide (Arsenox®); and targeted therapeutic agents such as imatinib (Gleevec®), gefitinib (Iressa®) and sunitinib (Sutent®). In some embodiments, the chemotherapy is a cocktail therapy. Examples of a cocktail therapy includes, but is not limited to, CHOP/R-CHOP (rituxan, cyclophosphamide, hydroxydoxorubicin, vincristine, and prednisone), EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, hydroxydoxorubicin), Hyper-CVAD (cyclophosphamide, vincristine, hydroxydoxorubicin, dexamethasone), FOLFOX (fluorouracil (5-FU), leucovorin, oxaliplatin), ICE (ifosfamide, carboplatin, etoposide), DHAP (high-dose cytarabine [ara-C], dexamethasone, cisplatin), ESHAP (etoposide, methylprednisolone, cytarabine [ara-C], cisplatin) and CMF (cyclophosphamide, methotrexate, fluouracil).

[00591] In certain embodiments, an additional therapeutic agent comprises a second immunotherapeutic agent. In some embodiments, the additional immunotherapeutic agent includes, but is not limited to, a colony stimulating factor, an interleukin, an antibody that blocks immunosuppressive functions (e.g., an anti-CTLA-4

antibody, anti-CD28 antibody, anti-CD3 antibody, anti-PD-1 antibody, anti-PD-Ll antibody, anti-TIGIT antibody), an antibody that enhances immune cell functions (e.g., an anti-GITR antibody, an anti-OX-40 antibody, an anti-CD40 antibody, or an anti-4-lBB antibody), a toll-like receptor (e.g., TLR4, TLR7, TLR9), a soluble ligand (e.g., GITRL, GITRL-Fc, OX-40L, OX-40L-Fc, CD40L, CD40L-Fc, 4-1BB ligand, or 4-1BB ligand-Fc), or a member of the B7 family (e.g., CD80, CD86). In some embodiments, the additional immunotherapeutic agent targets CTLA-4, CD28, CD3, PD-1, PD-L1, TIGIT, GITR, OX-40, CD-40, or 4-1BB.

[00592] In some embodiments, the additional therapeutic agent is an immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-Ll antibody, an anti-CTLA-4 antibody, an anti-CD28 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, an anti-TIM3 antibody, an anti-GITR antibody, an anti-4-lBB antibody, or an anti-OX-40 antibody. In some embodiments, the additional therapeutic agent is an anti-TIGIT antibody. In some embodiments, the additional therapeutic agent is an anti-PD-1 antibody selected from the group consisting of: nivolumab (OPDIVO), pembrolizumab (KEYTRUDA), pidilzumab, MEDI0680, REGN2810, BGB-A317, and PDR001. In some embodiments, the additional therapeutic agent is an anti-PD-Ll antibody selected from the group consisting of: BMS935559 (MDX-1105), atexolizumab (MPDL3280A), durvalumab (MEDI4736), and avelumab (MSB0010718C). In some embodiments, the additional therapeutic agent is an anti-CTLA-4 antibody selected from the group consisting of: ipilimumab (YERVOY) and tremelimumab. In some embodiments, the additional therapeutic agent is an anti-LAG-3 antibody selected from the group consisting of: BMS-986016 and LAG525. In some embodiments, the additional therapeutic agent is an anti-OX-40 antibody selected from the group consisting of: MEDI6469, MEDI0562, and MOXR0916. In some embodiments, the additional therapeutic agent is an anti-4-1BB antibody selected from the group consisting of: PF-05082566.

[00593] In some embodiments, the neoantigen therapeutic can be administered in combination with a biologic molecule selected from the group consisting of: adrenomedullin (AM), angiopoietin (Ang), BMPs, BDNF, EGF, erythropoietin (EPO), FGF, GDNF, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor (SCF), GDF9, HGF, HDGF, IGF, migration-stimulating factor, myostatin (GDF-8), NGF, neurotrophins, PDGF, thrombopoietin, TGF-a, TGF-β, TNF-a, VEGF, P1GF, gamma-IFN, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, and IL-18.

[00594] In some embodiments, treatment with a neoantigen therapeutic described herein can be accompanied by surgical removal of tumors, removal of cancer cells, or any other surgical therapy deemed necessary by a treating physician.

[00595] In certain embodiments, treatment involves the administration of a neoantigen therapeutic described herein in combination with radiation therapy. Treatment with an agent can occur prior to, concurrently with, or subsequent to administration of radiation therapy. Dosing schedules for such radiation therapy can be determined by the skilled medical practitioner.

[00596] Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously.

[00597] It will be appreciated that the combination of a neoantigen therapeutic described herein and at least one additional therapeutic agent can be administered in any order or concurrently. In some embodiments, the agent will be administered to patients that have previously undergone treatment with a second therapeutic agent. In certain other embodiments, the neoantigen therapeutic and a second therapeutic agent will be administered substantially simultaneously or concurrently. For example, a subject can be given an agent while undergoing a course of treatment with a second therapeutic agent (e.g., chemotherapy). In certain embodiments, a neoantigen therapeutic will be administered within 1 year of the treatment with a second therapeutic agent. It will further be appreciated that the two (or more) agents or treatments can be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[00598] For the treatment of a disease, the appropriate dosage of a neoantigen therapeutic described herein depends on the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, whether the agent is administered for therapeutic or preventative purposes, previous therapy, the patient's clinical history, and so on, all at the discretion of the treating physician. The neoantigen therapeutic can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (e.g., reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual agent. The administering physician can determine optimum dosages, dosing methodologies, and repetition rates.

[00599] In some embodiments, a neoantigen therapeutic can be administered at an initial higher "loading" dose, followed by one or more lower doses. In some embodiments, the frequency of administration can also change. In some embodiments, a dosing regimen can comprise administering an initial dose, followed by additional doses (or "maintenance" doses) once a week, once every two weeks, once every three weeks, or once every month. For example, a dosing regimen can comprise administering an initial loading dose, followed by a weekly maintenance dose of, for example, one-half of the initial dose. Or a dosing regimen can comprise administering an initial loading dose, followed by maintenance doses of, for example one-half of the initial dose every other week. Or a dosing regimen can comprise administering three initial doses for 3 weeks, followed by maintenance doses of, for example, the same amount every other week.

[00600] As is known to those of skill in the art, administration of any therapeutic agent can lead to side effects and/or toxicities. In some cases, the side effects and/or toxicities are so severe as to preclude administration of the particular agent at a therapeutically effective dose. In some cases, therapy must be discontinued, and other agents can be tried. However, many agents in the same therapeutic class display similar side effects and/or toxicities, meaning that the patient either has to stop therapy, or if possible, suffer from the unpleasant side effects associated with the therapeutic agent.

[00601] In some embodiments, the dosing schedule can be limited to a specific number of administrations or "cycles". In some embodiments, the agent is administered for 3, 4, 5, 6, 7, 8, or more cycles. For example, the agent is administered every 2 weeks for 6 cycles, the agent is administered every 3 weeks for 6 cycles, the agent is administered every 2 weeks for 4 cycles, the agent is administered every 3 weeks for 4 cycles, etc. Dosing schedules can be decided upon and subsequently modified by those skilled in the art.

[00602] The present invention provides methods of administering to a subject a neoantigen therapeutic described herein comprising using an intermittent dosing strategy for administering one or more agents, which can reduce side effects and/or toxicities associated with administration of an agent, chemotherapeutic agent, etc. In some embodiments, a method for treating cancer in a human subject comprises administering to the subject a therapeutically effective dose of a neoantigen therapeutic in combination with a therapeutically effective dose of a chemotherapeutic agent, wherein one or both of the agents are administered according to an intermittent dosing strategy. In some embodiments, a method for treating cancer in a human subject comprises administering to the subject a therapeutically effective dose of a neoantigen therapeutic in combination with a therapeutically effective dose of a second immunotherapeutic agent, wherein one or both of the agents are administered according to an intermittent dosing strategy. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a neoantigen therapeutic to the subject, and administering subsequent doses of the agent about once every 2 weeks. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a neoantigen therapeutic to the subject, and administering subsequent doses of the agent about once every 3 weeks. In some embodiments, the intermittent dosing strategy comprises administering an initial dose of a neoantigen therapeutic to the subject, and administering subsequent doses of the agent about once every 4 weeks. In some embodiments, the agent is administered using an intermittent dosing strategy and the additional therapeutic agent is administered weekly.

[00603] The present disclosure provides compositions comprising the neoantigen therapeutic described herein. The present invention also provides pharmaceutical compositions comprising a neoantigen therapeutic described herein and a pharmaceutically acceptable vehicle. In some embodiments, the pharmaceutical compositions find use in immunotherapy. In some embodiments, the compositions find use in inhibiting tumor growth. In some embodiments, the pharmaceutical compositions find use in inhibiting tumor growth in a subject (e.g., a human patient). In some embodiments, the compositions find use in treating cancer. In some embodiments, the pharmaceutical compositions find use in treating cancer in a subject (e.g., a human patient).

[00604] Formulations are prepared for storage and use by combining a neoantigen therapeutic of the present invention with a pharmaceutically acceptable vehicle (e.g., a carrier or excipient). Those of skill in the art generally consider pharmaceutically acceptable carriers, excipients, and/or stabilizers to be inactive ingredients of a formulation or pharmaceutical composition. Exemplary formulations are listed in WO 2015/095811.

[00605] Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride,

benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (e.g., less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes; and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG). (Remington: The Science and Practice of Pharmacy, 22st Edition, 2012, Pharmaceutical Press, London.). In some embodiments, the vehicle is 5% dextrose in water.

[00606] The pharmaceutical compositions described herein can be administered in any number of ways for either local or systemic treatment. Administration can be topical by epidermal or transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, and intranasal; oral; or parenteral including intravenous, intraarterial, intratumoral, subcutaneous, intraperitoneal, intramuscular (e.g., injection or infusion), or intracranial (e.g., intrathecal or intraventricular).

[00607] The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories.

[00608] The neoantigenic peptides described herein can also be entrapped in microcapsules. Such microcapsules are prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions.

[00609] In certain embodiments, pharmaceutical formulations include a neoantigen therapeutic described herein complexed with liposomes. Methods to produce liposomes are known to those of skill in the art. For example, some liposomes can be generated by reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes can be extruded through filters of defined pore size to yield liposomes with the desired diameter.

[00610] In certain embodiments, sustained-release preparations comprising the neoantigenic peptides described herein can be produced. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing an agent, where the matrices are in the form of shaped articles (e.g., films or microcapsules). Examples of sustained-release matrices include polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(vinyl alcohol), polylactides, copolymers of L-glutamic acid and **7** ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[00611] The present disclosure provides methods of treatment comprising an immunogenic therapy. Methods of treatment for a disease (such as cancer or a viral infection) are provided. A method can comprise administering to a subject an effective amount of a composition comprising an immunogenic antigen. In some embodiments, the antigen comprises a viral antigen. In some embodiments, the antigen comprises a tumor antigen.

[00612] Non-limiting examples of therapeutics that can be prepared include a peptide-based therapeutics, a nucleic acid-based therapeutic, an antibody based therapeutic, a T cell based therapeutic, and an antigen-presenting cell based therapeutic.

[00613] Therapeutic compositions can be formulated using one or more physiologically acceptable carriers including excipients and auxiliaries which facilitate processing of the active agents into preparations which can be used pharmaceutically. Proper formulation can be dependent upon the route of administration chosen. Any of the well-known techniques, carriers, and excipients can be used as suitable and as understood in the art.

[00614] In some cases, the therapuetic composition is formulated as a peptide-based therapuetic, a nucleic acid-based therapuetic, an antibody based therapuetic, or a cell based therapuetic. For example, a therapuetic composition can include naked cDNA in cationic lipid formulations; lipopeptides, naked cDNA or peptides, encapsulated e.g., in poly(DL-lactide-co-glycolide) ("PLG") microspheres; peptide composition contained in immune stimulating complexes (ISCOMS); or multiple antigen peptide systems (MAPs). Sometimes, a therapuetic is formulated as a peptide-based therapuetic, or nucleic acid based therapuetic in which the nucleic acid encodes the polypeptides. Sometimes, a therapuetic is formulated as an antibody based therapuetic. Sometimes, a therapuetic is formulated as a cell based therapuetic.

[00615] The amino acid sequence of an identified disease-specific immunogenic neoantigen peptide can be used develop a pharmaceutically acceptable composition. The source of antigen can be, but is not limited to, natural or synthetic proteins, including glycoproteins, peptides, and superantigens; antibody/antigen complexes; lipoproteins; RNA or a translation product thereof; and DNA or a polypeptide encoded by the DNA. The source of antigen may also comprise non-transformed, transformed, transfected, or transduced cells or cell lines. Cells may be transformed, transfected, or transduced using any of a variety of expression or retroviral vectors known to those of ordinary skill in the art that may be employed to express recombinant antigens. Expression may also be achieved in any appropriate host cell that has been transformed, transfected, or transduced with an expression or retroviral vector containing a DNA molecule encoding recombinant antigen(s). Any number of transfection, transformation, and transduction protocols known to those in the art may be used. Recombinant vaccinia vectors and cells infected with the vaccina vector may be used as a source of antigen.

[00616] A composition can comprise a disease-specific immunogenic neoantigen peptide. A composition can comprise two or more disease-specific immunogenic neoantigen peptides. A composition may comprise a precursor to a disease-specific immunogenic peptide (such as a protein, peptide, DNA and RNA). A precursor to a disease-specific immunogenic peptide can generate or be generated to the identified disease-specific immunogenic neoantigen peptide. In some embodiments, a therapeutic composition comprises a precursor of

an immunogenic peptide. The precursor to a disease-specific immunogenic peptide can be a pro-drug. In some embodiments, the composition comprising a disease-specific immunogenic neoantigen peptide may further comprise an adjuvant. For example, the neoantigen peptide can be utilized as a vaccine. In some embodiments, an immunogenic therapuetic may comprise a pharmaceutically acceptable immunogenic neoantigen peptide. In some embodiments, an immunogenic therapuetic may comprise a pharmaceutically acceptable precursor to an immunogenic neoantigen peptide (such as a protein, peptide, DNA and RNA). In some embodiments, a method of treatment comprises administering to a subject an effective amount of an antibody specifically recognizing an immunogenic neoantigen peptide. In some embodiments, a method of treatment comprises administering to a subject an effective amount of a soluble TCR or TCR analog specifically recognizing an immunogenic neoantigen peptide.

[00617] The methods described herein are particularly useful in the personalized medicine context, where immunogenic neoantigen peptides are used to develop therapeutics (such as vaccines or therapeutic antibodies) for the same individual. Thus, a method of treating a disease in a subject can comprise identifying an immunogenic neoantigen peptide in a subject according to the methods described herein; and synthesizing the peptide (or a precursor thereof); and administering the peptide or an antibody specifically recognizing the peptide to the subject. In some embodiments, an expression pattern of an immunogenic neoantigen can serve as the essential basis for the generation of patient specific therapuetics. In some embodiments, an expression pattern of an immunogenic neoantigen can serve as the essential basis for the generation of a therapuetic for a group of patients with a particular disease. Thus, particular diseases, e.g., particular types of tumors, can be selectively treated in a patient group.

[00618] In some embodiments, the peptides described herein are structurally normal ("shared") antigens that can be recognized by autologous anti-disease T cells in a large patient group. In some embodiments, an antigen-expression pattern of a group of diseased subjects whose disease expresses structurally normal ("shared") neoantigens is determined.

[00619] There are a variety of ways in which to produce immunogenic neoantigens. Proteins or peptides may be made by any technique known to those of skill in the art, including the expression of proteins, polypeptides or peptides through standard molecular biological techniques, the isolation of proteins or peptides from natural sources, in vitro translation, or the chemical synthesis of proteins or peptides. In general, such disease specific neoantigens may be produced either in vitro or in vivo. Immunogenic neoantigens may be produced in vitro as peptides or polypeptides, which may then be formulated into a personalized therapuetic or immunogenic composition and administered to a subject. In vitro production of immunogenic neoantigens can comprise peptide synthesis or expression of a peptide/polypeptide from a DNA or RNA molecule in any of a variety of bacterial, eukaryotic, or viral recombinant expression systems, followed by purification of the expressed peptide/polypeptide. Alternatively, immunogenic neoantigens can be produced in vivo by introducing molecules (e.g., DNA, RNA, and viral expression systems) that encode an immunogenic neoantigen into a subject, whereupon the encoded immunogenic neoantigens are expressed. In some embodiments, a

polynucleotide encoding an immunogenic neoantigen peptide can be used to produce the neoantigen peptide in vitro.

[00620] In some embodiments, a polynucleotide comprises a sequence with at least 60%, 65%, 70%1, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a polynucleotide encoding an immunogenic neoantigen.

[00621] The polynucleotide may be, e.g., DNA, cDNA, PNA, CNA, RNA, single- and/or double-stranded, native or stabilized forms of polynucleotides, or combinations thereof. A nucleic acid encoding an immunogenic neoantigen peptide may or may not contain introns so long as it codes for the peptide. In some embodiments in vitro translation is used to produce the peptide.

[00622] Expression vectors comprising sequences encoding the neoantigen, as well as host cells containing the expression vectors, are also contemplated. Expression vectors suitable for use in the present invention can comprise at least one expression control element operationally linked to the nucleic acid sequence. The expression control elements are inserted in the vector to control and regulate the expression of the nucleic acid sequence. Examples of expression control elements are well known in the art and include, for example, the lac system, operator and promoter regions of phage lambda, yeast promoters and promoters derived from polyoma, adenovirus, retrovirus or SV40. Additional operational elements include, but are not limited to, leader sequences, termination codons, polyadenylation signals and any other sequences necessary or preferred for the appropriate transcription and subsequent translation of the nucleic acid sequence in the host system. It will be understood by one skilled in the art the correct combination of expression control elements will depend on the host system chosen. It will further be understood that the expression vector should contain additional elements necessary for the transfer and subsequent replication of the expression vector containing the nucleic acid sequence in the host system. Examples of such elements include, but are not limited to, origins of replication and selectable markers.

[00623] The neoantigen peptides may be provided in the form of RNA or cDNA molecules encoding the desired neoantigen peptides. One or more neoantigen peptides of the invention may be encoded by a single expression vector. Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression, if necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognized by the desired host (e.g., bacteria), although such controls are generally available in the expression vector. The vector is then introduced into the host bacteria for cloning using standard techniques. Useful expression vectors for eukaryotic hosts, especially mammals or humans include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from E. coli, including pCR 1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as M13 and filamentous single-stranded DNA phages.

[00624] In embodiments, a DNA sequence encoding a polypeptide of interest can be constructed by chemical synthesis using an oligonucleotide synthesizer. Such oligonucleotides can be designed based on the amino

acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest is produced. Standard methods can be applied to synthesize an isolated polynucleotide sequence encoding an isolated polypeptide of interest.

[00625] Suitable host cells for expression of a polypeptide include prokaryotes, yeast, insect or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example E. coli or bacilli. Higher eukaryotic cells include established cell lines of mammalian origin. Cell-free translation systems can also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are well known in the art. Various mammalian or insect cell culture systems can be employed to express recombinant protein. Exemplary mammalian host cell lines include, but are not limited to COS-7, L cells, C127, 3T3, Chinese hamster ovary (CHO), 293, HeLa and BHK cell lines. Mammalian expression vectors can comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

[00626] The proteins produced by a transformed host can be purified according to any suitable method. Such standard methods include chromatography (e.g., ion exchange, affinity and sizing column chromatography, and the like), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexahistidine, maltose binding domain, influenza coat sequence, glutathione-S-transferase, and the like can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Isolated proteins can also be physically characterized using such techniques as proteolysis, nuclear magnetic resonance and x-ray crystallography.

[00627] A therapuetic composition can comprise an entity that binds a polypeptide sequence described herein. The entity can be an antibody. Antibody-based therapuetic can be formulated using any of the well-known techniques, carriers, and excipients as suitable and as understood in the art. In some embodiments, the peptides described herein can be used for making neoantigen specific therapeutics such as antibody therapeutics. For example, neoantigens can be used to raise and/or identify antibodies specifically recognizing the neoantigens. These antibodies can be used as therapeutics. The antibody can be a natural antibody, a chimeric antibody, a humanized antibody, or can be an antibody fragment. The antibody may recognize one or more of the polypeptides described herein. The antibody can recognize a polypeptide that has a sequence that is at most 40%, 50%, 60%, 70%, 80%, 90%, 95%, or less in sequence homology to a polypeptide described herein. The antibody can recognize a polypeptide that has a sequence that is at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more in sequence homology to a polypeptide described herein. The antibody can also recognize a polypeptide with sequence length is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more in sequence length compared to a polypeptide described herein. The antibody can recognize a polypeptide with a sequence length at most 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or less in sequence length compared to a polypeptide described herein.

[00628] The present disclosure also contemplates the use of nucleic acid molecules as vehicles for delivering neoantigen peptides/polypeptides to the subject in need thereof, in vivo, in the form of, e.g., DNA/RNA therapuetics.

[00629] In some embodiments, the therapuetic is a nucleic acid therapuetic. In some embodiments, neoantigens can be administered to a subject by use of a plasmid. Plasmids may be introduced into animal tissues by a number of different methods, e.g., injection or aerosol instillation of naked DNA on mucosal surfaces, such as the nasal and lung mucosa. In some embodiments, physical delivery, such as with a "gene-gun" may be used. The exact choice of expression vectors can depend upon the peptide/polypeptides to be expressed, and is well within the skill of the ordinary artisan.

[00630] In some embodiments, the nucleic acid encodes an immunogenic peptide or peptide precursor. In some embodiments, the nucleic acid therapuetic comprises sequences flanking the sequence coding the immunogenic peptide or peptide precursor. In some embodiments, the nucleic acid therapuetic comprises more than one immunogenic epitope. In some embodiments, the nucleic acid therapuetic is a DNA-based therapuetic. In some embodiments, the nucleic acid therapuetic is a RNA-based therapuetic. In some embodiments, the RNA-based therapuetic comprises mRNA. In some embodiments, the RNA-based therapuetic comprises naked mRNA. In some embodiments, the RNA-based therapuetic comprises modified mRNA (e.g., mRNA protected from degradation using protamine. mRNA containing modified 5' CAP structure or mRNA containing modified nucleotides). In some embodiments, the RNA-based therapuetic comprises single-stranded mRNA.

[00631] The polynucleotide may be substantially pure, or contained in a suitable vector or delivery system. Suitable vectors and delivery systems include viral, such as systems based on adenovirus, vaccinia virus, retroviruses, herpes virus, adeno-associated virus or hybrids containing elements of more than one virus. Non-viral delivery systems include cationic lipids and cationic polymers (e.g., cationic liposomes).

[00632] One or more neoantigen peptides can be encoded and expressed in vivo using a viral based system. Viral vectors may be used as recombinant vectors in the present invention, wherein a portion of the viral genome is deleted to introduce new genes without destroying infectivity of the virus. The viral vector of the present invention is a nonpathogenic virus. In some embodiments the viral vector has a tropism for a specific cell type in the mammal. In another embodiment, the viral vector of the present invention is able to infect professional antigen presenting cells such as dendritic cells and macrophages. In yet another embodiment of the present invention, the viral vector is able to infect any cell in the mammal. The viral vector may also infect tumor cells. Viral vectors used in the present invention include but is not limited to Poxvirus such as vaccinia virus, avipox virus, fowlpox virus and a highly attenuated vaccinia virus (Ankara or MVA), retrovirus, adenovirus, baculovirus and the like.

[00633] A therapuetic can be delivered via a variety of routes. Delivery routes can include oral (including buccal and sub-lingual), rectal, nasal, topical, transdermal patch, pulmonary, vaginal, suppository, or parenteral (including intramuscular, intraarterial, intrathecal, intradermal, intraperitoneal, subcutaneous and intravenous) administration or in a form suitable for administration by aerosolization, inhalation or

insufflation. The therapuetics described herein can be administered to muscle, or can be administered via intradermal or subcutaneous injections, or transdermally, such as by iontophoresis. Epidermal administration of the therapuetic can be employed.

[00634] In some instances, the therapuetic can also be formulated for administration via the nasal passages. Formulations suitable for nasal administration, wherein the carrier is a solid, can include a coarse powder having a particle size, for example, in the range of about 10 to about 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. The formulation can be a nasal spray, nasal drops, or by aerosol administration by nebulizer. The formulation can include aqueous or oily solutions of the therapuetic.

[00635] The therapuetic can be a liquid preparation such as a suspension, syrup or elixir. The therapuetic can also be a preparation for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration), such as a sterile suspension or emulsion.

[00636] The therapuetic can include material for a single immunization, or may include material for multiple immunizations (i.e. a 'multidose' kit). The inclusion of a preservative is preferred in multidose arrangements. As an alternative (or in addition) to including a preservative in multidose compositions, the compositions can be contained in a container having an aseptic adaptor for removal of material.

[00637] The therapuetic can be administered in a dosage volume of about 0.5 mL, although a half dose (i.e. about 0.25 mL) can be administered to children. Sometimes the therapuetic can be administered in a higher dose e.g. about 1 ml.

[00638] The therapuetic can be administered as a 1, 2, 3, 4, 5, 6, **7,** 8, 9, 10, or more dose-course regimen. Sometimes, the therapuetic is administered as a 1, 2, 3, or 4 dose-course regimen. Sometimes the therapuetic is administered as a 1 dose-course regimen. Sometimes the therapuetic is administered as a 2 dose-course regimen.

[00639] The administration of the first dose and second dose can be separated by about 0 day, 1 day, 2 days, 5 days, **7** days, 14 days, 21 days, 30 days, 2 months, 4 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 3 years, 4 years, or more.

[00640] The therapuetic described herein can be administered every 1, 2, 3, 4, 5, 6, **7,** 8, 9, 10, or more years. Sometimes, the therapuetic described herein is administered every 2, 3, 4, 5, 6, **7,** or more years. Sometimes, the therapuetic described herein is administered every 4, 5, 6, **7,** or more years. Sometimes, the therapuetic described herein is administered once.

[00641] The dosage examples are not limiting and are only used to exemplify particular dosing regiments for administering a therapuetic described herein. The effective amount for use in humans can be determined from animal models. For example, a dose for humans can be formulated to achieve circulating, liver, topical and/or gastrointestinal concentrations that have been found to be effective in animals. Based on animal data, and other types of similar data, those skilled in the art can determine the effective amounts of a therapuetic composition appropriate for humans.

[00642] The effective amount when referring to an agent or combination of agents will generally mean the dose ranges, modes of administration, formulations, etc., that have been recommended or approved by any of the various regulatory or advisory organizations in the medical or pharmaceutical arts (e.g., FDA, AMA) or by the manufacturer or supplier.

[00643] In some aspects, the therapuetic and kit described herein can be stored at between 2°C and 8°C. In some instances, the therapuetic is not stored frozen. In some instances, the therapuetic is stored in temperatures of such as at -20°C or -80°C. In some instances, the therapuetic is stored away from sunlight.

XXI. Computer-Implemented Aspects

[00644] As understood by those of ordinary skill in the art, the methods and information described herein can be implemented, in all or in part, as computer executable instructions on known computer readable media. For example, the methods described herein can be implemented in hardware. Alternatively, the methods can be implemented in software stored in, for example, one or more memories or other computer readable medium and implemented on one or more processors. As is known, the processors can be associated with one or more controllers, calculation units and/or other units of a computer system, or implanted in firmware as desired. If implemented in software, the routines can be stored in any computer readable memory such as in RAM, ROM, flash memory, a magnetic disk, a laser disk, or other storage medium, as is also known. Likewise, this software can be delivered to a computing device via any known delivery method including, for example, over a communication channel such as a telephone line, the Internet, a wireless connection, etc., or via a transportable medium, such as a computer readable disk, flash drive, etc.

[00645] More generally, and as understood by those of ordinary skill in the art, the various steps described above can be implemented as various blocks, operations, tools, modules and techniques which, in turn, can be implemented in hardware, firmware, software, or any combination of hardware, firmware, and/or software. When implemented in hardware, some or all of the blocks, operations, techniques, etc. can be implemented in, for example, a custom integrated circuit (IC), an application specific integrated circuit (ASIC), a field programmable logic array (FPGA), a programmable logic array (PLA), etc.

[00646] Results from sequencing data can be stored in a data storage unit, such as a data carrier, including computer databases, data storage disks, or by other convenient data storage means. In certain embodiments, the computer database is an object database, a relational database or a post-relational database. Data can be retrieved from the data storage unit using any convenient data query method.

[00647] When implemented in software, the software can be stored in any known computer readable medium such as on a magnetic disk, an optical disk, or other storage medium, in a RAM or ROM or flash memory of a computer, processor, hard disk drive, optical disk drive, tape drive, etc. Likewise, the software can be delivered to a user or a computing system via any known delivery method including, for example, on a computer readable disk or other transportable computer storage mechanism.

[00648] The steps of the claimed methods can be operational with numerous other general purpose or special purpose computing system environments or configurations. Examples of well-known computing systems, environments, and/or configurations that can be suitable for use with the methods or system of the claims

include, but are not limited to, personal computers, server computers, hand-held or laptop devices, multiprocessor systems, microprocessor-based systems, set top boxes, programmable consumer electronics, network PCs, minicomputers, mainframe computers, distributed computing environments that include any of the above systems or devices, and the like.

[00649] The steps of the claimed methods can be described in the general context of computer-executable instructions, such as program modules, being executed by a computer. Generally, program modules include routines, programs, objects, components, and/or data structures that perform particular tasks or implement particular abstract data types. The methods can also be practiced in distributed computing environments where tasks are performed by remote processing devices that are linked through a communications network. In both integrated and distributed computing environments, program modules can be located in both local and remote computer storage media including memory storage devices. Numerous alternative embodiments could be implemented, using either current technology or technology developed after the filing date of this application, which would still fall within the scope of the claims defining the disclosure.

[00650] While the methods, and other elements, have been described as preferably being implemented in software, they can be implemented in hardware, firmware, etc., and can be implemented by any other processor. Thus, the elements described herein can be implemented in a standard multi-purpose CPU or on specifically designed hardware or firmware such as an application-specific integrated circuit (ASIC) or other hard-wired device as desired. When implemented in software, the software routine can be stored in any computer readable memory such as on a magnetic disk, a laser disk, or other storage medium, in a RAM or ROM of a computer or processor, in any database, etc. Likewise, this software can be delivered to a user or a screening system via any known or desired delivery method including, for example, on a computer readable disk or other transportable computer storage mechanism or over a communication channel, for example, a telephone line, the internet, or wireless communication. Modifications and variations can be made in the techniques and structures described and illustrated herein without departing from the spirit and scope of the present disclosure.

EXAMPLES

Example 1: Obtaining T cells

[00651] Generally, T cells can be obtained by both positive selection and/or negative selection. In positive selection, an affinity agent (such as an antibody, an antibody fragment, and aptamer) can be used to bind a cell surface marker expressed on the population of cells, for example, CD3 for T cells. Using the affinity agent, the T cells can be labeled. The labeled T cells can then be enriched using various methods that are well-known in the art. Non-limiting examples of those methods include fluorescent-activated cell sorting (FACS) and (para)magnetic particle-based cell separation (e.g. MACS cell separation kits from Miltenyi Biotec).

[00652] In negative selection, affinity agents can be used to bind cell surface markers expressed on blood cells other than the desired population. For example, when attempting to isolate T cells, a cocktail of affinity agents can be used to label B cells, NK cells, monocytes, platelets, dendritic cells, granulocytes and erythrocytes. The

labeled cells can then be depleted, leaving the T cells enriched. The exemplary methods to deplete labeled cells include FACS and (para)magnetic particle-based cell separation.

[00653] In addition to labeling-based isolation, special growth condition may be used to promote the growth of one particular cell population. For example, the special growth condition can be obtained using special cytokines or growth factors. For another example, in culture media containing phytohemagglutinin (PHA), IL-2, and/or IL-15, T cells may preferentially proliferate.

[00654] In some situations, non-functional markers may be used to isolate T cells since binding of functional markers such as binding of CD3 by anti-CD3 antibody (alone or conjugated to magnetic particles) may trigger unwanted signaling events on T cells. Therefore, a cocktail of antibodies against CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a (glycophorin A) can be used to isolate T cells.

[00655] Detailed protocol can be found in published literatures (e.g. see Lefort et al., *J Vis Exp.* 2010; (40): 2017) which are incorporated by reference herein. T cell isolation kits can be obtained from, for example, STEMCELL Technologies, Thermofisher, and Miltenyi Biotec.

Example 2: Obtaining Immature Dendritic Cells (iDCs)

[00656] Primary human dendritic cells (DCs) can be differentiated from peripheral blood mononuclear cells (PBMC) from whole blood or leukophersis product. For example, $CD14^+$ monocytes can be enriched from blood sample using anti-CD14 magnetic microbeads and then cultured in XVIVO media containing Cellgenix IL-4 and GM-CSF clinical cytokines. For example, a protocol for preparing Immature dendritic cells (iDCs) can be found in Spadaro et al, Generation and Maturation of Human Monocyte-derived DCs, 2014, *Bio-protocol* 4(15): e1194.

[00657] Alternatively, patient-specific B cells can be immortalized and used as a source of RaFT APC. A cell line can also be used as an APC source for RaFT. For example, a human monocyte cell line, THP1, was engineered to be negative for all endogenous HLA class I and II alleles. Individual HLA alleles or combinations thereof can be then retored to create a cell line that complements a patient-specific HLA phenotype.

Example 3: Self-amplifiing mRNA Vector Construct

[00658] This example describes a self-amplifing mRNA vector construct. Self-amplifying mRNA replicons can be derived from single stranded positive sense RNA virus genomes in the Alphavirus family. A chimeric replicon (named VCR-Chim2.1) using genomic sequences from the Venezuelan Equine Encephalitis virus and Sindbis virus can be created according to published literature (Perri *et. al*, J Virol. 2003 Oct; 77(19): 10394-10403) with the purpose of using the resulting replicon as an infectious disease vaccine vector. To create a non-infectious self-amplifying replicon, the non-structural proteins (nsPs) of the parental viral genome can be retained but the viral capsid and envelope glycoprotein structural proteins can be deleted. In the absence of the structural proteins, a self-amplifying replicon can replicate itself but cannot form infectious particles or enter cells without another method of active delivery.

[00659] The VCR-Chim2.1 self-amplifying replicon backbone sequence include the following elements: a 5' T7 promoter sequence, VEE 5' UTR (Bases 1-44), VEE nsPl (Bases 45-1649), VEE nsP2 (Bases 1650-4031),

VEE nsP3 (Bases 4032-5741), VEE nsP4 (Bases 5742-7562), VEE 26S subgenomic promoter (Bases 7552-7575), gene or epitope of interest (Bases 7576-X), a 319 bp 3'UTR from Sindbis virus, a 117 bp 3'UTR from TC83 VEE virus, bacteria plasmid sequence colicin El, kanamycin plasmid resistance gene, and a VEE core packaging site from bp 5499-5630 to allow for viral replicon particle packaging. Other sequence elements that can be included to express multiple genes or epitopes of interest on the same molecule include viral 2A cleavage sites and internal ribosome entry sites (IRES). For example, Enterovirus EV71 IRES can be used.

Example 4: RaFT-specific Amplifier Vector and Target Vector

[00660] The parental VCR Chim2.1 self-amplifying mRNA vector was modified specifically for RaFT resulting in two distinct VCR Chim2.1-derived vectors **(FIG. 3, FIG. 6** and **FIG.** 7). The first vector, "RaFT amplifier," consists of VCR Chim2.1VEE 5' UTR, nsPl-4, and the Sindbis 3' UTR. More specifically, the RaFT amplifier vector components include the following elements: a 5' T7 promoter sequence, VEE 5' UTR (Bases 1-44), VEE nsPl (Bases 45-1649), VEE nsP2 (Bases 1650-4031), VEE nsP3 (Bases 4032-5741), VEE nsP4 (Bases 5742-7562), a 319 bp 3'UTR from Sindbis virus (Bases 7605-7919), bacteria plasmid sequence colicin El, and kanamycin plasmid resistance gene.

[00661] The amplifier vector contains no gene of interest. When the RNA form of the amplifier is introduced to cells, it is translated by host machinery and all four nsPs are expressed. The nsPs are then able to self-amplify the amplifier genome as well as recognize any co-localized RNA molecule(s) that contains the correct 26S subgenomic promoter and regulatory sequences.

[00662] A RaFT target vector that consists of the VCR Chim2.1 VEE 5' UTR, partial defective sequences for nsPl and nsP4, the VEE 26S subgenomic promoter, a cloning site to introduce an epitope or gene of interest, the VEE TC83 3' UTR, unique barcode sequences, and Illumina Read sequences for sequencing library preparation. When introduced to cells in RNA form, the target RNA molecule can be translated by host machinery but cannot self-replicate. The amplifier RNA molecule that contains nsPs 1-4 is active in the cell for the target RNA molecule to replicate.

[00663] The RaFT target vector components include the following elements: a 5' T7 promoter sequence, VEE 5' UTR (Bases 1-44), partial nsPl sequence (Bases 45-662), partial nsP4 sequence 1 (Bases 663-1030), optionally NanoString barcodes (Bases 1031-1130 for 100 bp NanoString barcodes), partial nsP4 sequence 2 (Bases 1135-1450), Illumina Read 1 (Bases 1214-1246), VEE 26S subgenomic promoter (Bases 1397-1409), gene or epitope of interest (Bases 1310-X), a unique barcode, Illumina Read 2, a 117 bp 3'UTR from TC83 VEE virus, a bacteria plasmid sequence colicin El, and kanamycin plasmid resistance gene.

[00664] Multiple genes and epitopes of interest have been inserted in the replicon under control of the 26S subgenomic promoter. These include: green fluorescent protein (GFP); immunogenic epitopes from viruses including influenza, cytomegalovirus, and Epstein-Barr virus; tumor neoantigen sequences from previously described immunogenic neoantigens (Fritsch), immunogenic neoantigens from tumor models (Gubin paper); neoantigen sequences found internally from profiling patient samples and from tumor sequence databases; human host proteins such as receptors, cytokines, and transcription factors, including but not limited to GM-CSF, HLA A01, IL-12, OX40, CD70 etc.

[00665] Other sequence modifications that have been or can be added to the replicon include internal ribosome entry sites (IRES); viral 2A cleavage sites; ribozymes; aptamers including streptavidin, small molecule, and fluorophore binding aptamers for RNA visualization in live cells such as the Spinach and Broccoli aptamers; antibody expression including full antibody sequences, camelid VHH, and single chain variable fragments (scFv).

[00666] RNA sequences encoding fewer than 50 amino acid residues can be targeted for nonsense mediated decay in mammalian cells. Therefore, the epitope sequences encoded in the RaFT target molecule encode an epitope sequence flanked by endogenous wild type coding sequence for a total of about 70 amino acid residues. It has been found that these longer epitope sequences can result in more stable, higher magnitude epitope expression.

[00667] The target vector can also contain a protein stabilizing domain such as the 10th human fibronectin domain III with the minimal epitope of interest sequence fused to its c terminus. The presence of a non-immunogenic stabilizing protein domain may further stabilize the expression of the minimal epitope sequence.

Example 5: Generation of Target RNA and Amplifier RNA

[00668] This example describes the RaFT target cloning process **(FIG. 8).** RaFT uses a rapid gene assembly cloning process to quickly generate epitope-specific target templates in a high throughput fashion. The time from *in silico* epitope calling to the generation of epitope-specific target RNA to be delivered to patient cells is 1.5 - 2 weeks.

[00669] The epitopes were identified by RNA and whole exome sequencing of the patient tumor material and normal blood. The sequencing data were then analyzed using an internal neoantigen epitope prediction algorithm, RECON, that considers factors including HLA allele expression, predicted affinity and stability, etc. Using these predictions, RECON generates composite scores that rank patient-specific epitopes to be considered for personalized neoantigen therapuetic inclusion. Some patient tumors harbor hundreds to thousands of tumor-specific mutations with many predicted immunogenic epitopes. All of these epitopes can be cloned into RaFT target vector for functional testing.

[00670] The epitopes identified above were then synthesized and assembled. Once a list of putative immunogenic neoantigen epitopes were generated by RECON, the native wildtype endogenous sequence context was added to the minimal epitope sequences so that the total sequence encodes for a protein product that is ~70 amino acid residues in length. Alternatively, a stabilizing protein domain sequence can be fused to the minimal neoantigen epitope sequence so that no sequence from the epitope's endogenous context needs to be added. This protein sequence was converted to an RNA sequence. Sequence optimization including codon optimization for mammalian expression and removal of any DNA sequence that encodes a BspQl site or other problematic restriction enzyme site was performed.

[00671] The RaFT amplifier and target vectors contain a T7 promoter and provide the DNA template for *in vitro* transcription of RaFT RNA. The vectors were linearized by cutting with a BspQl site that is located immediately downstream of the vector-encoded polyadenylation sequence. Linearized DNA templates were then subjected to RNA *in vitro* transcription, capping, and purification for mammalian cell delivery **(FIG. 6).**

[00672] The RaFT amplifier RNA can be synthesized using the following described procedure **(FIG.** 9). *In vitro* transcription (IVT) using T7 polymerase was performed according to a protocol that has been optimized for large RNA replicon synthesis. Due to the amplifier's large size **(8000** bases), selection of complete transcripts using polyadenylation selection was performed on the resulting IVT product. Otherwise, only **5-10%** of total IVT amplifier replicon molecules may contain the complete sequence through the polyA tail since the T7 RNA polymerase loses processivity after **5** kb of DNA template. Size selected polyadenylated transcripts were then capped using vaccinia virus capping enzyme to ensure that all capped structures are oriented appropriately for host translation. RNA integrity was tested by an optimized qPCR assay to quantify the abundance of intact amplifier RNA. Large amounts of amplifier RNA can be synthesized, aliquoted, and stored at **-80°** C to be used in all RaFT experiments, irrespective of target epitope sequence.

[00673] The RaFT target RNA can be synthesized according to the following procedure. Each test epitope was cloned into the target vector. Upon generation of the target DNA template, *in vitro* transcription (IVT) using T7 polymerase was performed according to a protocol that has been optimized for large RNA replicon synthesis. Since the target RNA is the size of a standard mRNA **(-1700** bases), the RNA was directly capped using vaccinia capping enzyme following IVT without an intermediate polyadenylation selection process. Target RNA integrity was assessed by the Agilent **2100** Bioanalyzer system.

Example 6: **Transferring Target RNA and Amplifier RNA into APCs**

[00674] This example describes the method to nucleofect target RNA and amplifier RNA into APCs. To achieve expression of a single epitope per dendritic cell (DC), the amplifier RNA can be delivered at least one copy per cell and the target RNA can be delivered at one copy per cell. In a preferred situation, a **20x** excess of amplifier RNA and **0.5x** of each target RNA was delivered in bulk to DCs. Calculations were based on the molarity of each RNA molecule.

[00675] Primary DCs can be poised to quickly respond to viral infection and thus express high levels of antiviral nucleic acid sensors including TLRs and RLRs. Therefore, it is more challenging to deliver a self-amplifying mRNA replicon to primary dendritic cells than to other human cells. Additionally, the replication intermediates of self-amplifying replicons make it more immunostimulatory than conventional mRNA. The *ex vivo* delivery conditions for self-amplifying replicons to primary human DCs were optimized so that they can express replicon-encoded sequences while maintaining a relatively inactivated state. RaFT amplifier and target RNA molecules were delivered to immature primary human dendritic cells using electroporation. Several electroporation technologies were tried and the highest efficiency of replicon activity was obtained when the Lonza Nucleofector system was used. Low salt nucleofection buffer was used to minimize RNA aggregation. The programmed Lonza Primary Human Monocyte electroporation protocol was used to achieve good replicon launch. Primary DCs were rested in culture for **24** hours following RNA delivery. Then, low molecular weight poly-I:C was added to DCs to enhance antigen processing and presentation for **24** hours.

[00676] An exemplary amplifiertarget titration experiment is shown in **FIG.** 10. Briefly, interferon-incompetent baby hamster kidney (BHK) cells were trypsinized, counted, and washed twice with RT Ca^{+}, Mg^{+} FREE PBS at **1500** rpm for **5** minutes. Cells were resuspended at a concentration of **50,000** BHK cells/**20**

μL cold Lonza cell line nucleofection reagent and incubated on ice for 5 min. Cells were then distributed to a 96 well plate, 50,000 cells per well in 20 μL of nucleofection buffer. RaFT amplifier and target RNA were distributed to cells as per the dose matrix below **(Table 1).** Cells and RNA were then transferred to 16 well Lonza nucleofection cuvettes and run on the stored manufacturer recommended BHK program. Immediately following nucleofection, cells were transferred to a prepared black, clear well 96 well plate containing 100 μL per well of warm BHK media (DMEM with 5% FBS, 1-glutamine, penicillin-streptomycin, and sodium pyruvate). After 24 hours, GFP expression was measured using EnVision and by standard fluorescent microscopy. Experiments were performed in triplicate.

Table 1 Dose matrix for amplifiertarget RNA titration

Amplifier RNA (ug)	Target RNA (ug)							
	0.25	**0.125**	**0.063**	**0.031**	**0.016**	**0.008**	**0.004**	**0**
0.25								
0.125								
0.063								
0.031								
0.016								
0.008								
0.004								
0								

Example 7: Culturing of T cells and APCs

[00677] One of the central concepts of RaFT is isolating DC:T cell conjugates to interrogate antigen-specific interactions **(FIG. 11).** Important variables for conjugate isolation include: timing of DC:T cell co-culture, method of cell fixation, markers for conjugate selection, and method of conjugate selection.

[00678] Autologous T cells were added to the RaFT RNA-engineered primary DCs 48 hours post RNA delivery and 24 hours post poly-I:C stimulation ("time zero"). At time zero, target RNA was expressed at very high levels (-70,000 copies per cell) and the DCs had upregulated their antigen processing and presentation machinery. The optimal timing for antigen-specific DC:T cell conjugate formation can be between 1-4 hrs.

Example 8: Isolation of Conjugates

[00679] DC:T cell conjugates can be lightly fixed to survive downstream selection and processing. However, over fixation can compromise the quality of RNA within cells and prevent RNA isolation for RNA display and/or sequencing library preparation. Fixation with 0.5% paraformaldehyde was found to be an effective strategy for light fixation. This light fixation can keep cells alive, allow for antibody recognition of surface proteins, and maintains cellular RNA.

[00680] The T cell component of conjugates can be used as the main selection tool. Extracellular markers of antigen-specific T cell signaling (CD69, CD 154 or CD40L, CD 137) appear on the cell surface later than the peak conjugate formation at time points under 4 hours of DC:T cell co-culture. Intracellular markers, such as TNF or IFNg production are upregulated later following DC:T cell interaction and also require killing cells which is prohibitive to the assay. Tetramers work well to capture antigen-specific T cells but require HLA haplotype pairing and largely only assess $CD8^+$T cell specificity with specific class I length peptide epitopes.

[00681] Following light PFA fixation, anti-CD3, anti-CD4, and anti-CD8 antibody-based selection can be used to capture T cells. To select only DC:T cell conjugates, an additional anti-CD 11c DC marker antibody is added.

[00682] Two ways were used to isolate conjugates. When flow cytometry was used for conjugate isolation, CD3/CD4/CD8 and CD 11c double-positive conjugates were sorted. In order to maintain intact conjugates, a 130 μm sorting nozzle was used. Otherwise, the conjugates may be sheared apart, precluding downstream single conjugate analysis.

[00683] When antibody-coated magnetic beads were used to perform isolation, sequential cell isolation steps was performed. Traditional magnetic bead systems cannot be used because one round of bead isolation may result in irreversibly magnetized cells (i.e. beads get internalized). Therefore, a dissolvable alginate system such as MagCloudz was used at least for the first selection step. To isolate conjugates, the first round of selection was performed with the desired anti-T cell marker, sequentially followed by a second selection for **CDllc.**

[00684] An exemplary flow cytometry gating strategy for conjugate isolation is shown in **FIGs. 12A-C.** Immature dendritic cells (iDCs) were harvested, collected and washed twice with cold HBSS (Ca^+, Mg^+ free) at 1100 rpm for 7 minutes. Cells were resuspended at a concentration of 1 million iDC/IOOμL of cold Lonza Primary cell nucleofection solution and incubated on ice for 2 minutes. RaFT amplifier and target RNA encoding epitope sequences were added to the cells and nucleofected using the Lonza Nucleofector system and run on the Human Monocyte program. Immediately after nucleofection, cells were put on ice for 2 minutes, then added to lmL of pre-equilibrated DC media (XVIVO with penicillin-streptomycin) with lOOOU/mL of GM-CSF and 800 U/mL of IL-4 and put in 37° C, 5% $C0_2$ incubator. 24 hours later, DC cultures were stimulated with 25μg/mL of Poly I:C LMW in 0.5 mL of T cell media (RPMI+ L-glutamine, penicillin-streptomycin, and sodium pyruvate) with 30% human serum and lOOOU/mL of GM-CSF and 800 U/mL and returned to the 37° C, 5% CO_2 incubator. 24 hours later, autologous PBMCs that were thawed and rested overnight were resuspended in 0.5 mL of T cell media (RPMI+ L-glutamine, penicillin-streptomycin, sodium pyruvate and 10% human serum) with 10 ng/mL of IL-7 and 100 ng/ml of IL-15 and added to DC cultures dropwise. After addition, co-cultures were returned to the 37° C, 5% CO_2 incubator for a culturing time between 0.5-5 hours. After co-culture time was complete, paraformaldehyde (PFA) was add to cultures for a final concentration of 0.5% and rocked gently on ice to immobilize conjugates. After 10 minutes of fixation, PFA was quenched by adding glycine at a final concentration of 125 mM and rocking gently for 5 minutes on ice. Using gentle scraping with a cell lifter, cells were harvested and stained for flow cytometry

with fluorescently conjugated antibodies against CD3 and CD11c. Cell pools were then sorted using an Aria fusion flow cytometer (BD Biosciences) through the 130 micron nozzle using the following gating scheme:

1. Total events were visualized on a dot plot graphing FSC-A vs. SSC-A.

2. Singlets and doublets were discriminated by visualizing FSC-H vs. FSC-A. Cells that fall on the diagonal line of the dot plot are single cells and were gated as such. This single cell gate was inverted, and this inverted gate was deemed the doublet gate and selected for isolation.

3. $CD11c^+$ vs. $CD3^+$ conjugates were gated from within the doublet gate double positive $CD11c^+/CD3^+$ conjugates were selected. These events were sorted and represent DC:T cell conjugates. The CD3 marker can be switched for other T cell markers, such as CD4 or CD8.

Example 9: Sequencing Polynucleotides Encoding Neoepitope Sequences from Conjugates and Analysis of Sequences

[00685] Upon conjugate isolation, either individual conjugate or pooled conjugate sequencing can be performed. Individual conjugate sequencing can provide single conjugate paired antigen and TCR information. Pooled conjugate sequencing can query only enriched antigen sequences. Both are useful analyses depending on what level of assay resolution is desired.

[00686] For single conjugate sequencing, sorted, lightly-fixed single conjugates can be partitioned into microfluidic droplets that contain a gel bead, unique sequencing library barcodes in each droplet, and RT reagents. All mRNA in a single droplet can be transcribed by reverse transcriptase, at which point droplet-specific barcodes can be added to each sequence. If TCR sequencing-specific library preparation reagents are used, then to capture exogenously introduced RaFT target mRNA sequences in the DCs, additional RaFT-specific primers can be introduced. The 10X Genomics single cell TCR sequencing system can be used for RaFT single conjugate partitioning and paired Antigen-TCR sequencing.

[00687] For pooled conjugate sequencing, at time points between 1-4 hours of DC:T cell co-culture, the majority of conjugates were found to be enriched for antigen-specific interactions. Therefore, simply isolating conjugates at these time points and evaluating their target RNA content in pooled format can provide information regarding the relative enrichment of T cell specificities across many presented antigens.

Example 10: Next -generation Sequencing Library Preparation

[00688] RNA is extracted from the conjugate cells and is converted to cDNA by reverse transcription.

[00689] Target vectors and the corresponding IVT mRNA obtained from them were designed with RT and forward priming sites that flank epitope sequences. These sites are constant in all constructs and designed to be immediately downstream of Illumina Read sequences **(FIG. 23).**

[00690] The RT site is the reverse compliment sequence required by Illumina Nextera for Read 2: 5'-CTGTCTCTTATACACATCTCCGAGCCCACGAGAC. This RT site was fused to 10 bp vector barcode to accommodate a very short and fast sequence process. The forward site is exact sequence required by Illumina Nextera for Read 1: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG.

[00691] Briefly, the library preparation includes the following key steps. The total RNA of selected conjugates was extracted by standard Trizol protocol. Then 10 - 100 ng RNA was reverse-transcribed with a RT primer 5'

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG. First strand cDNA was prepared by 18 cycles of PCR enrichment with RT and Forward primers: RT primer 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG, and FP primer 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG. Dual indexing of each library was prepared by 8 cycles of PCR with standard Illumina Index kit: Index 1 Read 5'-CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG, and Index 2 Read 5'-AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC. Libraries were pooled in equal molar ratio and subjected to sequencing.

[00692] There can be in general two sequencing options: 1) single end sequencing of the 10 bp barcode on MiSeq by 25 bp single read with Illumina Read 2, and 2) paired end sequencing of epitope and barcode sequencing using MiSeq 150x2 with Illumina Read 1 and Read 2.

Example 11: **NanoString Target Identification**

[00693] Nano String target identification can be used as an alternative to next-generation sequencing **(FIG.** 24). Specifically, RaFT can be developed for a NanoString-based target ID read-out. A unique 100 bp barcodes that sit upstream of the target subgenomic promoter between the partial nsPl and nsP4 sequences was introduced into the target mRNA construct. Within the unique barcodes, 50 bp of these sequences are specific for the NanoString capture probe and 50 bp are specific for hundreds of unique NanoString digital barcode probes (50 bp sequences are contiguous). Additionally, a custom library of NanoString probes for housekeeping genes, DC-specific genes, and T cell specific genes was generated to improve normalization of the data and for DC:T cell conjugate phenotyping.

[00694] Directly following conjugate sorting, total mRNA was isolated and half of them was added to the custom NanoString target plus endogenous gene probe library. The saved RNA can be used later for RNA Display or next-generation sequencing library preparation.

[00695] Standard NanoString analysis was performed to quantify enriched RaFT Target sequences as well as endogenous genes for normalization and phenotyping. This process can be automated, requires no PCR amplification, and takes about 18 hours. The potential advantages of a Nanostring read-out can be, 1) more quantitative due to direct mRNA hybridization without PCR bias, and 2) relatively faster due to 18 hour automated process instead of 3 day hands-on next-generation sequencing library preparation process.

RNA Display

Example 12: **RNA Display**

[00696] Following conjugate sequencing or Nanostring target identification, it might be necessary to bring RaFT through a round of RNA Display **(FIG.** 13). RaFT RNA Display can involve the following three steps: 1) the selective isolation of target mRNAs from enriched conjugates, 2) the regeneration of enough of these enriched target RNAs to deliver to new patient DCs, and 3) a repeated round of RaFT conjugate formation, isolation, and interrogation.

[00697] The workflow for Target RNA regeneration is as follows: 1) Total RNA of selected conjugates can be extracted by standard Trizol extraction protocol; 2) 10 - 100 ng RNA can be reverse-transcribed with a RT

primer: 5'-AATTTTAAGGCGGCATGCCA; 3) The first strand of cDNA can be prepared with 18 cycle PCR enrichment using a Reverse Primer designed to start from the 3' end of TC83 3'UTR: 5'-GAAATATTAAAAACAAAATCCGATTCGGAAAAGAAAAGAAAAATAAAATAAAAATTTTAAGGC GGCATGCCA, and Forward Primer that includes the T7 promoter sequence: 5'-TAATACGACTCACTATAGATGGGCGGCGCATGA; 4) The prepared cDNA can then be *in vitro* transcribed immediately followed by Vaccinia 5' capping; 5) Polyadenylation of IVT RNA can then be performed by *E. coli* Poly(A) Polymerase.

[00698] Following the regeneration of enriched target mRNA, all RNAs can be nucleofected in pooled format to a new preparation of the patient's DCs and a second round of RaFT can be performed exactly as previously described. If necessary, many rounds of RaFT can be performed.

Example 13: Enrichment of Viral Antigens from DC:T cell Conjugates by RaFT

[00699] This example depicts an experiment for viral antigens enrichment by RaFT assay. $CD8^+$ T cell responses to viral antigens (pp65, LMP2a, BMLF, FluA, FluM) were analyzed by combinatorial encoded multimer analysis (protocol described in Andersen, et al., 2012) **(FIG. 14).** Healthy donor PBMCs were thawed *("Ex vivo")* and analyzed directly (top row, FACS date), or analyzed after a 14 day T cell induction protocol ("14 day induction") after stimulation with short peptides encoding an HLA-A02 restricted viral antigen pool (Anaspec-CEF control peptide pool) loaded on immature DCs stimulated with PolyLC LMW for 24 hours. To compare RaFT epitope enrichment of *ex vivo* healthy donor material to the multimer analysis of autologous healthy donor material, DC:T cell conjugates were generated after 4 different co-culture time points (0.5 hr, 1 hr, 2 hr, and 3 hr) and isolated using FACS sorting as described in Example 8 based on expression of $CD11c^+CD3^+$. Total RNA was isolated from collected conjugates and eptiopes from RaFT RNA were sequenced as described in Example 9 and 10. An increase in barcoded reads can represent an enrichment of epitope expression in isolated conjugates.

Example 14: Enrichment of Neoantigens from DC:T cell Conjugates by RaFT

[00700] Healthy donor PBMCs were thawed ("£'x *vivo")* or naive T cells from that healthy donor were stimulated with short peptides (9-10 amino acids in length) encoding a pool of previously identified neoantigens (PINs) (MART, and mutant forms of ME-1, Prdx5, GAS7, ACTN4 and CDK4) ("14 day induction") **(FIG. 15).** $CD8^+$ T cell response to the PIN pool was analyzed by combinatorial encoded multimer analysis (protocol described in Andersen, et al, Parallel detection of antigen-specific T cell responses by combinatorial encoding of MHC multimers. *Nature Protocols,* 7(5), 891-902.). To compare RaFT epitope enrichment of *ex vivo* healthy donor material to the multimer analysis of autologous healthy donor material, DC:T cell conjugates were generated after 4 different co-culture time points (0.5 hr, 1 hr, 2 hr, and 3 hr) and isolated using FACS sorting as described in Example 8 based on their expression of $CDllc^+CD3^+$. Total RNA was isolated from collected conjugates and epitopes from RaFT RNA were sequenced as described in Example 9 and 10. An increase in barcoded reads can indicate an enrichment of epitope expression in isolated conjugates.

[00701] A RaFT assay was performed on *ex vivo* healthy donor material as described previously and assessed for increased sequencing reads of a shared neoantigen panel **(FIGs 16 A-C).** Combinatorial encoded multimer analysis reveals that there was $CD8^+$ T cell reactivity in two week naive T cell induction cultures from other healthy donor in response to KRAS G12C short peptide and GATA3 short peptide.

Example 15: Enrichment of Mutant ACTN4 Epitope from DC:T cell Conjugates by RAFT

[00702] Healthy donor T cells were induced with PIN peptide pools (24 amino acids in length; 24-mer) using a standard T cell induction protocol with no antibody treatment or with anti-PD-1 and anti-CTLA4 treatment for a period of two weeks. Cells were then put through a recall assay for 24 hours with individual mutant ACTN4 peptide or wildtype ACTN4 24-mer peptides at 0.8 μM or 3.2 μM in concentration. To assess CD4+ reactivity to peptide, CD4+ T cells were analyzed by flow cytometry for expression of IFN-γ, TNF, CD107a and 4-IBB. The percentage of CD4+ cells expressing 1, 2, 3, or 4 of these markers were enumerated in a pie chart **(FIG. 15B).**

Example 16: Enrichment of PIN Epitopes from Partitioned DC:$CD4^+$ T Cell Conjugates and DC:$CD8^+$ T Cell Conjugates

[00703] DC:T cell conjugates were derived as described in Example 8 and further partitioned into DC:$CD4^+$ T cell conjugates and DC:$CD8^+$ T cell conjugates using expression of CD4 and CD8 markers **(FIGs. 17A-B).** Conjugates were selected from cell pools after co-culture for 1 hour and 2 hours, and RNA was isolated and sequencing libraries were generated as outlined in Example 9 and 10. Sequencing reads were normalized between different sequencing libraries by first normalizing paired reads per condition, then normalizing barcode calling and epitope matching for each epitope in each condition, followed by normalizing each condition relative to lowest input cell number, and finally, normalizing DC input reads for each antigen.

Example 17: Enrichment of Shared Neoantigens from Partitioned DC:$CD4^+$ T Cell Conjugates and DC:$CD8^+$ T Cell Conjugates

[00704] $CD4^+$ and $CD8^+$ DC:T cell conjugates were derived and isolated and epitope sequencing was performed and normalized as described in **FIGs. 18A-B.** $CD8^+$ DC:T cell conjugate normalized epitope enrichment at 1 hour and 2 hour isolation timepoints was graphed in **FIG. 18A** for each epitope. $CD4^+$ DC:T cell normalized epitope enrichment at 1 hour and 2 hour isolation time points is shown in **FIG. 18B.**

Example 18: Frequency of DC:T Cell Conjugates Formation

[00705] Freqeuncy of total DC:T cell conjugate formation over time was assessed **(FIG. 19).** Frequency of DC:$CD3^+$ T cell conjugates in cell pools with or without RaFT RNA antigen was assessed over time **(FIG. 20).** DC:T cell conjugates were generated and cell pools were co-cultured for 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. DC:T cell conjugates were selected from cell pools based on their expression of CD11c and CD3 as described previously. Frequencies of DC:T cell conjugates with RaFT RNA antigen delivered (+ Ag) were compared to DC:T cell conjugates formed in cultures with no RaFT RNA antigen delivered (No Ag) within the doublet gate based on FACS analysis. Total frequencies of DC:T cell conjugates reported were normalized to calculate the total frequency within the cell pool. Total normalized conjugate frequencies (total events) for each timepoint = frequency of doublet formation * frequency of $CD3^+CD11c^+$ within the doublet gate. Total

conjugate frequencies from cell pools with no RaFT RNA antigen and total conjugate frequencies form cell pools with RaFT RNA antigen are shown.

[00706] Frequency of $CD4^+$ T cells or $CD8^+$ T cells in DC:T conjugates from cell pools with or without RaFT RNA antigen was assessed over time **(FIGs. 21A-B).** T cell phenotyping was performed by FACS analysis to determine the frequency of $CD4^+$ T cell and $CD8^+$ T cells within the DC:T cell conjugates that were derived as described previously. $CD4^+$ and $CD8^+$ markers were stained using fluorescent antibodies and their frequencies within were determined using FACS analysis. T cell phenotyping data was calculated to determine the total, normalized frequency of $CD4^+$ DC:T cell conjugates or $CD8^+$ DC:T cell conjugates. Normalized $CD4^+$ or $CD8^+$ conjugates were calculated at each timepoint by multiplying the frequency of doublet formation by the frequency of $CD3^+CDllc^+$ within the doublet gate by the frequency of $CD4^+/CD8^+$ T cells within the DC: T cell conjugate gate.

[00707] Frequency of T cell activation markers or T cell costimulatory markers on $CD8^+$ conjugates was assessed **(FIGs. 22A-E).** $CD8^+$ DC:T cell conjugates were isolated over a period of 1-5 hours as described previously. Frequency for cellular markers for T cell activation (4-**IBB,** PDl, and CD69) and T cell costimulatory markers (ICOS and CD27) were stained for using fluorescent antibodies and analyzed by FACS analysis on an Aria Fusion. Frequencies for each T cell activation marker were reported from within the $CD8^+$ DC:T cell conjugate gate. Frequencies of markers within $CD8^+$ DC:T cell conjugates formed from cell pools without RaFT RNA antigen (No Ag) and frequencies of markers from $CD8^+$ DC:T cell conjugates formed from cell pools given RaFT RNA antigen (+ Ag pool) are shown.

Example 19: Identification of mutant sequences with immunogenic potential

[00708] Applicants have discovered mutations that are recurrent in cancer patients. See, Tables 2 and 3.

[00709] Neoantigenic peptides described herein can be derived from the mutations listed in Tables 2 and 3. For example, a neoantigenic peptide can be derived from a substitution mutation, e.g., the KRAS G12V mutation. The neonatigenic peptide can be a peptide comprising 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, or 100 amino acid residues of the protein, e.g., KRAS including the substitution (G12V). The substitution may be positioned anywhere along the length of the neoantigenic peptide. For example, it can be located in the N terminal third of the neoantigenic peptide, the central third of the neoantigenic peptide or the C terminal third of the neoantigenic peptide. In another example, the substituted residue is located 2-5 residues away from the N terminal end or 2-5 residues away from the C terminal end. Neoantigenic peptides can similarly be derived from tumor specifc insertion mutations where the neonatigenic peptide comprises one or more, or all of the inserted residues.

[00710] Neoantigenic peptides can also be derived from frame shift mutation, fusion polypeptides, in-frame deletions, and splice variants. The tumor specific polypeptides resulting from these mutations can be characterized by the existence of a transition point between a polypeptide sequence encoded by the germline and the tumor specific mutant polypeptide encoded by the tumor specific mutation. The neonatigenic peptide can be a peptide of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, or 100 amino acid residues that encompasses the transition point between the germline encoded native

sequence and the tumor specific polypeptide sequence. The transition point can be positioned anywhere along the length of the neoantigenic peptide. For example, it can be located in the N terminal third of the neoantigenic peptide, the central third of the neoantigenic peptide or the C terminal third of the neoantigenic peptide. In another example, the transition point is located 2-5 residues away from the N terminal end or 2-5 residues away from the C terminal end. A neoantigenic peptide derived from frame shift mutation and splice variants can also be a peptide consisting of tumor specific mutant residues.

[00711] For each mutation event listed in Tables 2 and 3, the full-length amino acid sequence of the mutant protein was derived. Any constituent 9mer or lOmer not found in the germline protein sequence was flagged as a neo-peptide and scored for binding potential on six common HLA alleles (HLA-A0 1:0 1, HLA-A02:0 1. HLA-A03 :0 1, HLA-A1 1:0 1, HLA-A24:02, HLA-B07:02, and HLA-B08:0 1) using available algorithms. Any peptide scoring better than 1000 nM was nominated. The nominated peptides are listed in Tables 2 and 3 followed by the high scoring HLA alleles in parenthesis.

Table 2

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
TABLE 2A		**POINT MUTATION [1]**		
ABL1	E255K	VADGLITTLHYPAPKR NKPTVYGVSPNYDKW EMERTDITMKHKLGGG QYGKVYEGVWKKYSL TVAVKTLKEDTMEVEE FLKEAAVMKEIKHPNL VQLLGVC	GQYGKVYEG (A02.01) GQYGKVYEGV (A02.01) KLGGGQYGK (A03.01) KLGGGQYGKV (A02.01) KVYEGVWKK (A02.01, A03.01) KVYEGVWKKY (A03.01) QYGKVYEGV (A24.02) QYGKVYEGVW (A24.02)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ABL1	E255V	VADGLITTLHYPAPKR NKPTVYGVSPNYDKW EMERTDITMKHKLGGG QYGVVYEGVWKKYSL TVAVKTLKEDTMEVEE FLKEAAVMKEIKHPNL VQLLGVC	GQYGVVYEG (A02.01) GQYGVVYEGV (A02.01) KLGGGQYGV (A02.01) KLGGGQYGVV (A02.01) QYGVVYEGV (A24.02) QYGVVYEGVW (A24.02) VVYEGVWKK (A02.01, A03.01) VVYEGVWKKY (A03.01)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ABL1	M351T	LLGVCTREPPFYIITEFM	ATQISSATEY (A01.01)	Chronic myeloid

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		TYGNLLDYLRECNRQE VNAVVLLYMATQISSA TEYLEKKNFIHRDLAA RNCLVGENHLVKVADF GLSRLMTGDTYTAHAG AKF	ISSATEYLEK (A03.01) SSATEYLEK (A03.01) TQISSATEYL (A02.01) YMATQISSAT (A02.01)	leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ABL1	T315I	SLTVAVKTLKEDTMEV EEFLKEAAVMKEIKHP NLVQLLGVCTREPPFYI IIEFMTYGNLLDYLREC NRQEVNAVVLLYMAT QISSAMEYLEKKNFIHR DLA	FYIIIEFMTY (A24.02) IIEFMTYGNL (A02.01) IIIEFMTYG (A02.01) IIIEFMTYGN (A02.01) YIIIEFMTYG (A02.01)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ABL1	Y253H	STVADGLITTLHYPAPK RNKPTVYGVSPNYDK WEMERTDITMKHKLG GGQHGEVYEGVWKKY SLTVAVKTLKEDTMEV EEFLKEAAVMKEIKHP NLVQLLG	GQHGEVYEGV (A02.01) KLGGGQHGEV (A02.01)	Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Gastrointestinal stromal tumors (GIST)
ALK	G1269A	SSLAMLDLLHVARDIA CGCQYLEENHFIHRDIA ARNCLLTCPGPGRVAK IADFGMARDIYRASYY RKGGCAMLPVKWMPP EAFMEGIFTSKTDTWSF GVLL	KIADFGMAR (A03.01) RVAKIADFGM (A02.01, B07.02)	NSCLC
ALK	L1196M	QVAVKTLPEVCSEQDE LDFLMEALIISKFNHQN IVRCIGVSLQSLPRFILM ELMAGGDLKSFLRETR PRPSQPSSLAMLDLLHV ARDIACGCQYLEENHFI	FILMELMAGG (A02.01) ILMELMAGG (A02.01) ILMELMAGGD (A02.01) LMELMAGGDL (A02.01) LPRFILMEL (B07.02, B08.01) LPRFILMELM (B07.02)	NSCLC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			LQSLPRFILM (A02.01, B08.01) SLPRFILMEL (A02.01, A24.02, B07.02, B08.01)	
BRAF	V600E	MIKLIDIARQTAQGMD YLHAKSIIHRDLKSNNI FLHEDLTVKIGDFGLAT EKSRWSGSHQFEQLSG SILWMAPEVIRMQDKN PYSFQSDVYAFGIVLYE LM	LATEKSRWS (A02.01, B08.01) LATEKSRWSG (A02.01, B08.01)	CRC, GBM, KIRP, LUAD, SKCM, THCA
BTK	C481S	MIKEGSMSEDEFIEEAK VMMNLSHEKLVQLYG VCTKQRPIFIITEYMAN GSLLNYLREMRHRFQT QQLLEMCKDVCEAME YLESKQFLHRDLAARN CLVND	EYMANGSLL (A24.02) MANGSLLNY (A01.01, A03.01, A11.01) MANGSLLNYL (A02.01, B07.02, B08.01) SLLNYLREM (A02.01, B07.02, B08.01) YMANGSLLN (A02.01) YMANGSLLNY (A01.01, A03.01, A11.01)	CLL
EEF1B2	S43G	MGFGDLKSPAGLQVLN DYLADKSYIEGYVPSQ ADVAVFEAVSGPPPAD LCHALRWYNHIKSYEK EKASLPGVKKALGKYG PADVEDTTGSGAT	GPPPADLCHAL (B07.02)	BLCA, KIRP, PRAD, SKCM
EGFR	S492R	SLNITSLGLRSLKEISDG DVIISGNKNLCYANTIN WKKLFGTSGQKTKIIR NRGENSCKATGQVCH ALCSPEGCWGPEPRDC VSCRNVSRGRECVDKC NLL	IIRNRGENSCK (A03.01)	CRC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
EGFR	T790M	IPVAIKELREATSPKAN KEILDEAYVMASVDNP HVCRLLGICLTSTVQLI MQLMPFGCLLDYVRE HKDNIGSQYLLNWCVQ IAKGMNYLEDRRLVHR DLAA	CLTSTVQLIM (A01.01, A02.01) IMQLMPFGC (A02.01) IMQLMPFGCL (A02.01, A24.02, B08.01) LIMQLMPFG (A02.01) LIMQLMPFGC (A02.01) LTSTVQLIM (A01.01) MQLMPFGCL (A02.01, B07.02, B08.01) MQLMPFGCLL (A02.01, A24.02, B08.01) QLIMQLMPF (A02.01, A24.02, B08.01) QLIMQLMPFG (A02.01) STVQLIMQL (A02.01) VQLIMQLMPF (A02.01, A24.02, B08.01)	NSCLC, PRAD
ERBB3	V104M	ERCEVVMGNLEIVLTG HNADLSFLQWIREVTG YVLVAMNEFSTLPLPN LRMVRGTQVYDGKFAI FVMLNYNTNSSHALRQ LRLTQLTEILSGGVYIE KNDK		CRC, Stomach Cancer
ESR1	D538G	HLMAKAGLTLQQQHQ RLAQLLLILSHIRHMSN KGMEHLYSMKCKNVV PLYGLLLEMLDAHRLH APTSRGGASVEETDQS HLATAGSTSSHSLQKY YITGEA	GLLLEMLDA (A02.01) LYGLLLEML (A24.02) NVVPLYGLL (A02.01) PLYGLLLEM (A02.01) PLYGLLLEML (A02.01, A24.02) VPLYGLLLEM (B07.02) VVPLYGLLL (A02.01, A24.02)	Breast Cancer

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
ESR1	S463P	NQGKCVEGMVEIFDML LATSSRFRMMNLQGEE FVCLKSIILLNSGVYTFL PSTLKSLEEKDHIHRVL DKITDTLIHLMAKAGL TLQQQHQRLAQLLLILS H	FLPSTLKSL (A02.01, A24.02, B08.01) GVYTFLPST (A02.01) GVYTFLPSTL (A02.01, A24.02) TFLPSTLKSL (A24.02) VYTFLPSTL (A24.02) YTFLPSTLK (A03.01)	Breast Cancer
ESR1	Y537C	IHLMAKAGLTLQQQHQ RLAQLLLILSHIRHMSN KGMEHLYSMKCKNVV PLCDLLLEMLDAHRLH APTSRGGASVEETDQS HLATAGSTSSHSLQKY YITGE	NVVPLCDLL (A02.01) NVVPLCDLLL (A02.01) PLCDLLLEM (A02.01) PLCDLLLEML (A02.01) VPLCDLLLEM (B07.02) VVPLCDLLL (A02.01, A24.02)	Breast Cancer
ESR1	Y537N	IHLMAKAGLTLQQQHQ RLAQLLLILSHIRHMSN KGMEHLYSMKCKNVV PLNDLLLEMLDAHRLH APTSRGGASVEETDQS HLATAGSTSSHSLQKY YITGE	NVVPLNDLL (A02.01) NVVPLNDLLL (A02.01) PLNDLLLEM (A02.01) PLNDLLLEML (A02.01) VPLNDLLLEM (B07.02)	Breast Cancer
ESR1	Y537S	IHLMAKAGLTLQQQHQ RLAQLLLILSHIRHMSN KGMEHLYSMKCKNVV PLSDLLLEMLDAHRLH APTSRGGASVEETDQS HLATAGSTSSHSLQKY YITGE	NVVPLSDLL (A02.01) NVVPLSDLLL (A02.01) PLSDLLLEM (A02.01) PLSDLLLEML (A02.01) VPLSDLLLEM (B07.02) VVPLSDLLL (A02.01, A24.02)	Breast Cancer
FGFR3	S249C	HRIGGIKLRHQQWSLV MESVVPSDRGNYTCVV ENKFGSIRQTYTLDVLE RCPHRPILQAGLPANQT	VLERCPHRPI (A02.01, B08.01) YTLDVLERC (A02.01)	BLCA, HNSC, KIRP, LUSC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		AVLGSDVEFHCKVYSD AQPHIQWLKHVEVNGS KVG		
FRG1B	L52S	AVKLSDSRIALKSGYG KYLGINSDELVGHSDAI GPREQWEPVFQNGKM ALSASNSCFIRCNEAGD IEAKSKTAGEEEMIKIR SCAEKETKKKDDIPEED KG	FQNGKMALS (A02.01)	GBM, KIRP, PRAD, SKCM
HER2	V777L (Resistance)	GSGAFGTVYKGIWIPD GENVKIPVAIKVLRENT SPKANKEILDEAYVMA GLGSPYVSRLLGICLTS TVQLVTQLMPYGCLLD HVRENRGRLGSQDLLN WCM	VMAGLGSPYV (A02.01, A03.01)	BRCA
IDH1	R132H	RVEEFKLKQMWKSPN GTIRNILGGTVFREAIIC KNIPRLVSGWVKPIIIG HHAYGDQYRATDFVV PGPGKVEITYTPSDGTQ KVTYLVHNFEEGGGVA MGM	KPIIIGHHA (B07.02)	BLCA, GBM, PRAD
IDH1	R132C	RVEEFKLKQMWKSPN GTIRNILGGTVFREAIIC KNIPRLVSGWVKPIIIGC HAYGDQYRATDFVVP GPGKVEITYTPSDGTQK VTYLVHNFEEGGGVA MGM	KPIIIGCHA (B07.02)	BLCA, GBM, PRAD
IDH1	R132G	RVEEFKLKQMWKSPN GTIRNILGGTVFREAIIC	KPIIIGGHA (B07.02)	BLCA, BRCA, CRC, GBM, HNSC, LUAD,

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		KNIPRLVSGWVKPIIIG GHAYGDQYRATDFVV PGPGKVEITYTPSDGTQ KVTYLVHNFEEGGGVA MGM		PAAD, PRAD, UCEC
IDH1	R132S	RVEEFKLKQMWKSPN GTIRNILGGTVFREAIIC KNIPRLVSGWVKPIIIGS HAYGDQYRATDFVVP GPGKVEITYTPSDGTQK VTYLVHNFEEGGGVA MGM	KPIIIGSHA (B07.02)	BLCA, BRCA, GBM, HNSC, LIHC, LUAD, LUSC, PAAD, SKCM, UCEC
KIT	T670I	VAVKMLKPSAHLTERE ALMSELKVLSYLGNH MNIVNLLGACTIGGPTL VIIEYCCYGDLLNFLRR KRDSFICSKQEDHAEA ALYKNLLHSKESSCSDS TNE	IIEYCCYGDL (A02.01) TIGGPTLVII (A02.01) VIIEYCCYG (A02.01)	Gastrointestinal stromal tumors (GIST)
KIT	V654A	VEATAYGLIKSDAAMT VAVKMLKPSAHLTERE ALMSELKVLSYLGNH MNIANLLGACTIGGPTL VITEYCCYGDLLNFLRR KRDSFICSKQEDHAEA ALYK	HMNIANLLGA (A02.01) IANLLGACTI (A02.01) MNIANLLGA (A02.01) YLGNHMNIA (A02.01, B08.01) YLGNHMNIAN (A02.01)	Gastrointestinal stromal tumors (GIST)
MEK	C121S	ISELGAGNGGVVFKVS HKPSGLVMARKLIHLEI KPAIRNQIIRELQVLHES NSPYIVGFYGAFYSDGE ISICMEHMDGGSLDQV LKKAGRIPEQILGKVSI	VLHESNSPY (A03.01) VLHESNSPYI (A02.01)	Melanoma
MEK	P124L	LGAGNGGVVFKVSHKP	LQVLHECNSL (A02.01,	Melanoma

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		SGLVMARKLIHLEIKPA IRNQIIRELQVLHECNSL YIVGFYGAFYSDGEISI CMEHMDGGSLDQVLK KAGRIPEQILGKVSIAVI	B08.01) LYIVGFYGAF (A24.02) NSLYIVGFY (A01.01) QVLHECNSL (A02.01, B08.01) SLYIVGFYG (A02.01) SLYIVGFYGA (A02.01) VLHECNSLY (A03.01) VLHECNSLYI (A02.01, A03.01)	
MYC	E39D	MPLNVSFTNRNYDLDY DSVQPYFYCDEEENFY QQQQQSDLQPPAPSEDI WKKFELLPTPPLSPSRR SGLCSPSYVAVTPFSLR GDNDGG	FYQQQQQSDL (A24.02) QQQSDLQPPA (A02.01) QQSDLQPPA (A02.01) YQQQQQSDL (A02.01, B08.01)	Lymphoid Cancer; Burkitt Lymphoma
MYC	P57S	FTNRNYDLDYDSVQPY FYCDEEENFYQQQQQS ELQPPAPSEDIWKKFEL LSTPPLSPSRRSGLCSPS YVAVTPFSLRGDNDGG GGSFSTADQLEMVTEL LG	FELLSTPPL (A02.01, B08.01) LLSTPPLSPS (A02.01)	Lymphoid Cancer
MYC	T58I	TNRNYDLDYDSVQPYF YCDEEENFYQQQQQSE LQPPAPSEDIWKKFELL PIPPLSPSRRSGLCSPSY VAVTPFSLRGDNDGGG GSFSTADQLEMVTELL GG	FELLPIPPL (A02.01) IWKKFELLPI (A24.02) LLPIPPLSPS (A02.01, B07.02) LPIPPLSPS (B07.02)	Neuroblastoma
PDGFRa	T674I	VAVKMLKPTARSSEKQ ALMSELKIMTHLGPHL NIVNLLGACTKSGPIYII IEYCFYGDLVNYLHKN	IIEYCFYGDL (A02.01) IIIEYCFYG (A02.01) IYIIIEYCF (A24.02) IYIIIEYCFY (A24.02)	Chronic Eosinophilic Leukemia

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		RDSFLSHHPEKPKKELD IFGLNPADESTRSYVILS	YIIIEYCFYG (A02.01)	
PIK3CA	E542K	IEEHANWSVSREAGFS YSHAGLSNRLARDNEL RENDKEQLKAISTRDPL SKITEQEKDFLWSHRH YCVTIPEILPKLLLSVK WNSRDEVAQMYCLVK DWPP	KITEQEKDFL (A02.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, UCEC
PIK3CA	E545K	HANWSVSREAGFSYSH AGLSNRLARDNELREN DKEQLKAISTRDPLSEI TKQEKDFLWSHRHYC VTIPEILPKLLLSVKWN SRDEVAQMYCLVKDW PPIKP	STRDPLSEITK (A03.01) DPLSEITK (A03.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, SKCM, UCEC
PIK3CA	H1047R	LFINLFSMMLGSGMPE LQSFDDIAYIRKTLALD KTEQEALEYFMKQMN DARHGGWTTKMDWIF HTIKQHALN		BRCA, CESC, CRC, GBM, HNSC, LIHC, LUAD, LUSC, PRAD, UCEC
POLE	P286R	QRGGVITDEEETSKKIA DQLDNIVDMREYDVPY HIRLSIDIETTKLPLKFR DAETDQIMMISYMIDG QGYLITNREIVSEDIEDF EFTPKPEYEGPFCVFN	LPLKFRDAET (B07.02)	Colorectal adenocarcinoma; Uterine/Endometrium Adenocarcinoma; Colorectal adenocarcinoma, MSI+; Uterine/Endometrium Adenocarcinoma, MSI+; Endometrioid carcinoma; Endometrium Serous carcinoma; Endometrium Carcinosarcoma-

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
				malignant mesodermal mixed tumour; Glioma; Astrocytoma; GBM
PTEN	R130Q	KFNCRVAQYPFEDHNPPQLELIKPFCEDLDQWLSEDDNHVAAIHCKAGKGQTGVMICAYLLHRGKFLKAQEALDFYGEVRTRDKKGVTIPSQRRYVYYYSY	QTGVMICAYL (A02.01)	BRCA, CESC, CRC, GBM, KIRC, LUSC, UCEC
RAC1	P29S	MQAIKCVVVGDGAVGKTCLLISYTTNAFSGEYIPTVFDNYSANVMVDGKPVNLGLWDTAGQEDYDRLRPLSYPQTVGET	AFSGEYIPTV (A02.01, A24.02)	Melanoma
TP53	G245S	IRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGSMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEP	SMNRRPILT (A02.01, B08.01) YMCNSSCMGS (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, LUSC, PAAD, PRAD
TP53	R175H	TYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRHCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEV		BLCA, BRCA, CRC, GBM, HNSC, LUAD, PAAD, PRAD, UCEC
TP53	R248Q	EGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNQRPILTIITLEDSSGN	GMNQRPILT (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, KIRC, LIHC, LUSC, PAAD, PRAD, UCEC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		LLGRNSFEVRVCACPG RDRRTEEENLRKKGEP HHE		
TP53	R248W	EGNLRVEYLDDRNTFR HSVVVPYEPPEVGSDC TTIHYNYMCNSSCMGG MNWRPILTIITLEDSSG NLLGRNSFEVRVCACP GRDRRTEEENLRKKGE PHHE	GMNWRPILT (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, LIHC, LUSC, PAAD, SKCM, UCEC
TP53	R273C	PEVGSDCTTIHYNYMC NSSCMGGMNRRPILTII TLEDSSGNLLGRNSFEV CVCACPGRDRRTEEEN LRKKGEPHHELPPGST KRALPNNTSSSPQPKK KPL	LLGRNSFEVC (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, LUSC, PAAD, UCEC
TABLE 2B		**MSI-ASSOCIATED FRAMESHIFTS [1]**		
ACVR2A	D96fs; +1	GVEPCYGDKDKRRHCF ATWKNISGSIEIVKQGC WLDDINCYDRTDCVEK KRQP*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
ACVR2A	D96fs; -1	GVEPCYGDKDKRRHCF ATWKNISGSIEIVKQGC WLDDINCYDRTDCVEK KTALKYIFVAVRAICV MKSFLIFRRWKSHSPLQ IQLHLSHPITTSCSIPWC HLC*	ALKYIFVAV (A02.01, B08.01) ALKYIFVAVR (A03.01) AVRAICVMK (A03.01) AVRAICVMKS (A03.01) CVEKKTALK (A03.01) CVEKKTALKY (A01.01) CVMKSFLIF (A24.02, B08.01) CVMKSFLIFR (A03.01) FLIFRRWKS (A02.01, B08.01) FRRWKSHSPL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			FVAVRAICV (A02.01, B08.01) FVAVRAICVM (B08.01) IQLHLSHPI (A02.01) KSFLIFRRWK (A03.01) KTALKYIFV (A02.01) KYIFVAVRAI (A24.02) RWKSHSPLQI (A24.02) TALKYIFVAV (A02.01, B08.01) VAVRAICVMK (A03.01) VMKSFLIFR (A03.01) VMKSFLIFRR (A03.01) YIFVAVRAI (A02.01)	
C15ORF40	L132fs; +1	TAEAVNVAIAAPPSEGEANAELCRYLSKVLELRKSDVVLDKVGLALFFFFFETKSCSVAQAGVQWRSLGSLQPPPPGFKLFSCLSFLSSWDYRRMPPCLANFCIFNRDGVSPCWSGWS*	ALFFFFFET (A02.01) ALFFFFFETK (A03.01) AQAGVQWRSL (A02.01) CLANFCIFNR (A03.01) CLSFLSSWDY (A01.01, A03.01) FFETKSCSV (B08.01) FFFETKSCSV (A02.01) FKLFSCLSFL (A02.01) FLSSWDYRRM (A02.01) GFKLFSCLSF (A24.02) KLFSCLSFL (A02.01, A03.01) KLFSCLSFLS (A02.01, A03.01) LALFFFFFET (A02.01) LFFFFFETK (A03.01) LSFLSSWDY (A01.01) LSFLSSWDYR (A03.01) RMPPCLANF (A24.02) RRMPPCLANF (A24.02) SLQPPPPGFK (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			VQWRSLGSL (A02.01)	
CNOT1	L1544fs; +1	LSVIIFFFVYIWHWALP LILNNHHICLMSSIILDC NSVRQSIMSVCFFFFSVI FSTRCLTDSRYPNICWF K*	FFFSVIFST (A02.01) MSVCFFFFSV (A02.01) SVCFFFFSV (A02.01, B08.01) SVCFFFFSVI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
CNOT1	L1544fs; -1	LSVIIFFFVYIWHWALP LILNNHHICLMSSIILDC NSVRQSIMSVCFFFFCY ILNTMFDR*	FFCYILNTMF (A24.02) MSVCFFFFCY (A01.01) SVCFFFFCYI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
EIF2B3	A151fs; -1	VLVLSCDLITDVALHE VVDLFRAYDASLAML MRKGQDSIEPVPGQKG KKKQWSSVTSLEWTA QERGCSSWLMKQTWM KSWSLRDPSYRSILEYV STRVLWMPTSTV*	KQWSSVTSL (A02.01) VLWMPTSTV (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
EPHB2	K1020fs; -1	SIQVMRAQMNQIQSVE GQPLARRPRATGRTKR CQPRDVTKKTCNSNDG KKREWEKRKQILGGGG KYKEYFLKRILIRKAMT VLAGDKKGLGRFMRC VQSETKAVSLQLPLGR*	ILIRKAMTV (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
ESRP1	N512fs; +1	LDFLGEFATDIRTHGVH MVLNHQGRPSGDAFIQ MKSADRAFMAAQKCH KKKHEGQIC*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
ESRP1	N512fs; -1	LDFLGEFATDIRTHGVH MVLNHQGRPSGDAFIQ MKSADRAFMAAQKCH KKT*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
FAM111B	A273fs; -1	GALCKDGRFRSDIGEFE	RMKVPLMK (A03.01)	MSI+ CRC, MSI+

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		WKLKEGHKKIYGKQS MVDEVSGKVLEMDISK KKHYNRKISIKKLNRM KVPLMKLITRV*		Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
GBP3	T585fs; -1	RERAQLLEEQEKTLTS KLQEQARVLKERCQGE STQLQNEIQKLQKTLK KKPRDICRIS*	TLKKKPRDI (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
JAK1	P861fs; +1	VNTLKEGKRLPCPPNC PDEVYQLMRKCWEFQ PSNRTSFQNLIEGFEAL LKTSN*	LIEGFEALLK (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
JAK1	K860fs; -1	CRPVTPSCKELADLMT RCMNYDPNQRPFFRAI MRDINKLEEQNPDIVSE KNQQLKWTPHILKSAS *	QQLKWTPHI (A02.01) QLKWTPHILK (A03.01) IVSEKNQQLK (A03.01) QLKWTPHILK (A03.01) QQLKWTPHI (A24.02) NQQLKWTPHIL (B08.01) NQQLKWTPHI (B08.01) QLKWTPHIL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
LMAN1	E305fs; +1	DDHDVLSFLTFQLTEPG KEPPTPDKEISEKEKEK YQEEFEHFQQELDKKK RGIPEGPPRPPRAACGG NI*	GPPRPPRAAC (B07.02) PPRPPRAAC (B07.02)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
LMAN1	E305fs; -1	DDHDVLSFLTFQLTEPG KEPPTPDKEISEKEKEK YQEEFEHFQQELDKKK RNSRRATPTSKGSLRR KYLRV*	SLRRKYLRV (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
MSH3	N385fs; +1	TKSTLIGEDVNPLIKLD DAVNVDEIMTDTSTSY	SAACHRRGCV (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		LLCISENKENVRDKKK GQHFYWHCGSAACHR RGCV*		Cancer, MSI+ Stomach Cancer, Lynch syndrome
MSH3	K383fs; -1	LYTKSTLIGEDVNPLIK LDDAVNVDEIMTDTST SYLLCISENKENVRDK KRATFLLALWECSLPQ ARLCLIVSRTLLLVQS*	ALWECSLPQA (A02.01) CLIVSRTLL (B08.01) CLIVSRTLLL (A02.01, B08.01) FLLALWECS (A02.01) FLLALWECSL (A02.01, B08.01) IVSRTLLLV (A02.01) LIVSRTLLL (A02.01, B08.01) LIVSRTLLLV (A02.01) LLALWECSL (A02.01, B08.01) LPQARLCLI (B08.01, B07.02) LPQARLCLIV (B08.01) NVRDKKRATF (B08.01) SLPQARLCLI (A02.01, B08.01)	MSI+CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
NDUFC2	A70fs; +1	LPPPKLTDPRLLYIGFL GYCSGLIDNLIRRRPIAT AGLHRQLLYITAFFFC WILSCKT*	FFCWILSCK (A03.01) FFFCWILSCK (A03.01) ITAFFFCWI (A02.01) LYITAFFFCW (A24.02) YITAFFFCWI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
NDUFC2	F69fs; -1	SLPPPKLTDPRLLYIGFL GYCSGLIDNLIRRRPIAT AGLHRQLLYITAFFLLD IIL*	ITAFFLLDI (A02.01) LLYITAFFL (A02.01, B08.01) LLYITAFFLL (A02.01, A24.02) LYITAFFLL (A24.02) LYITAFFLLD (A24.02) YITAFFLLDI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
RBM27	Q817; +1	NQSGGAGEDCQIFSTPG HPKMIYSSSNLKTPSKL CSGSKSHDVQEVLKKK TGSNEVTTRYEEKKTG	GSNEVTTRY (A01.01) MPKDVNIQV (B07.02) TGSNEVTTRY (A01.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		SVRKANRMPKDVNIQV RKKQKHETRRKSKYNE DFERAWREDLTIKR*		
RPL22	K16fs; +1	MAPVKKLVVKGGKKK EASSEVHS*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
RPL22	K15fs; -1	MAPVKKLVVKGGKKR SKF*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SEC31A	I462fs; +1	MPSHQGAEQQQQQHH VFISQVVTEKEFLSRSD QLQQAVQSQGFINYCQ KKN*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SEC31A	I462fs; -1	MPSHQGAEQQQQQHH VFISQVVTEKEFLSRSD QLQQAVQSQGFINYCQ KKLMLLRLNLRKMCG PF*	KKLMLLRLNL (A02.01) KLMLLRLNL (A02.01, A03.01, B07.02, B08.01) KLMLLRLNLR (A03.01) LLRLNLRKM (B08.01) LMLLRLNL (B08.01) LMLLRLNLRK (A03.01) LNLRKMCGPF (B08.01) MLLRLNLRK (A03.01) MLLRLNLRKM (A02.01, A03.01, B08.01) NLRKMCGPF (B08.01) NYCQKKLMLL (A24.02) YCQKKLMLL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SEC63	K530fs; +1	AEVFEKEQSICAAEEQP AEDGQGETNKNRTKG GWQQKSKGPKKTAKS KKKETFKKKTYTCAIT	FKKKTYTCAI (B08.01) ITTVKATETK (A03.01) KSKKKETFK (A03.01) KSKKKETFKK (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		TVKATETKAGKWSRWE*	KTYTCAITTV (A02.01, A24.02) TFKKKTYTC (B08.01) TYTCAITTV (A24.02) TYTCAITTVK (A03.01) YTCAITTVK (A03.01)	
SEC63	K529fs; -1	MAEVFEKEQSICAAEEQPAEDGQGETNKNRTKGGWQQKSKGPKKTAKSKKRNL*	TAKSKKRNL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SLC35F5	C248fs; -1	NIMEIRQLPSSHALEAKLSRMSYPVKEQESILKTVGKLTATQVAKISFFFALCGFWQICHIKKHFQTHKLL*	FALCGFWQI (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SMAP1	K172fs; +1	YEKKKYYDKNAIAITNISSSDAPLQPLVSSPSLQAAVDKNKLEKEKEKKKGREKERKGARKAGKTTYS*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
SMAP1	K171fs; -1	KYEKKKYYDKNAIAITNISSSDAPLQPLVSSPSLQAAVDKNKLEKEKEKKRKRKREKRSQKSRQNHLQLKSCRRKISNWSLKKVPALKKLRSPLWIF*	LKKLRSPL (B08.01) SLKKVPAL (B08.01) RKISNWSLKK (A03.01) VPALKKLRSPL (B07.02)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TFAM	E148fs; +1	IYQDAYRAEWQVYKEEISRFKEQLTPSQIMSLEKEIMDKHLKRKAMTKKKRVNTAWKTKKTSFSL*	KRVNTAWKTK (A03.01) MTKKKRVNTA (B08.01) RVNTAWKTK (A03.01) RVNTAWKTKK (A03.01) TKKKRVNTA (B08.01) WKTKKTSFSL (B08.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TFAM	E148fs; -1	IYQDAYRAEWQVYKE		MSI+ CRC, MSI+

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		EISRFKEQLTPSQIMSLE KEIMDKHLKRKAMTK KKS*		Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TGFBR2	P129fs; +1	KPQEVCVAVWRKNDE NITLETVCHDPKLPYHD FILEDAASPKCIMKEKK KAW*		MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TGFBR2	K128fs: -1	EKPQEVCVAVWRKND ENITLETVCHDPKLPYH DFILEDAASPKCIMKEK KSLVRLSSCVPVALMS AMTTSSSQKNITPAILT CC*	ALMSAMTTS (A02.01) AMTTSSSQK (A03.01, A11.01) AMTTSSSQKN (A03.01) CIMKEKKSL (B08.01) CIMKEKKSLV (B08.01) IMKEKKSL (B08.01) IMKEKKSLV (B08.01) KSLVRLSSCV (A02.01) LVRLSSCVPV (A02.01) RLSSCVPVA (A02.01, A03.01) RLSSCVPVAL (A02.01) SAMTTSSSQK (A03.01, A11.01) SLVRLSSCV (A02.01) VPVALMSAM (B07.02) VRLSSCVPVA (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
THAP5	K99fs; -1	VPSKYQFLCSDHFTPDS LDIRWGIRYLKQTAVP TIFSLPEDNQGKDPSKK NPRRKTWKMRKKYAQ KPSQKNHLY*	KMRKKYAQK (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TTK	R854fs; -1	GTTEEMKYVLGQLVGL NSPNSILKAAKTLYEHY SGGESHNSSSSKTFEKK GEKNDLQLFVMSDTTY KIYWTVILLNPCGNLHL	FVMSDTTYK (A03.01) FVMSDTTYKI (A02.01) KTFEKKGEK (A03.01) LFVMSDTTYK (A03.01) MSDTTYKIY (A01.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		KTTSL*	VMSDTTYKI (A02.01) VMSDTTYKIY (A01.01)	
XPOT	F126fs; -1	QQLIRETLISWLQAQM LNPQPEKTFIRNKAAQ VFALLFVTEYLTKWPK FFLTFSQ*	YLTKWPKFFL (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
TABLE 2C		**FRAMESHIFT**[11]		
APC	V1352fs F1354fs Q1378fs S1398fs	AKFQQCHSTLEPNPAD CRVLVYLQNQPGTKLL NFLQERNLPPKVVLRH PKVHLNTMFRRPHSCL ADVLLSVHLIVLRVVR LPAPFRVNHAVEW*	FLQERNLPP (A02.01) FRRPHSCLA (B08.01) LIVLRVVRL (B08.01) LLSVHLIVL (A02.01, B08.01)	CRC, LUAD, UCEC, STAD
APC	S1421fs R1435fs T1438fs P1442fs P1443fs V1452fs P1453fs K1462fs E1464fs	APVIFQIALDKPCHQAE VKHLHHLLKQLKPSEK YLKIKHLLLKRERVDLS KLQ*	EVKHLHHLL (B08.01) HLHHLLKQLK (A03.01) HLLLKRERV (B08.01) KIKHLLLKR (A03.01) KPSEKYLKI (B07.02) KYLKIKHLL (A24.02) KYLKIKHLLL (A24.02) LLKQLKPSEK (A03.01) LLKRERVDL (B08.01) LLLKRERVDL (B08.01) QLKPSEKYLK (A03.01) YLKIKHLLL (A02.01, B08.01) YLKIKHLLLK (A03.01)	CRC, LUAD, UCEC, STAD
APC	T1487fs H1490fs L1488fs	MLQFRGSRFFQMLILY YILPRKVLQMDFLVHP A*	ILPRKVLQM (B08.01) KVLQMDFLV (A02.01, A24.02) LPRKVLQMDF (B07.02, B08.01) LQMDFLVHPA (A02.01) QMDFLVHPA (A02.01)	CRC, LUAD, UCEC, STAD

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			YILPRKVLQM (A02.01, B08.01)	
ARID1A	Q1306fs S1316fs Y1324fs T1348fs G1351fs G1378fs P1467fs	ALGPHSRISCLPTQTRG CILLAATPRSSSSSSSND MIPMAISSPPKAPLLAA PSPASRLQCINSNSRITS GQWMAHMALLPSGTK GRCTACHTALGRGSLS SSSCPQPSPSLPASNKLP SLPLSKMYTTSMAMPI LPLPQLLLSADQQAAP RTNFHSSLAETVSLHPL APMPSKTCHHK*	APSPASRLQC (B07.02) HPLAPMPSKT (B07.02) ILPLPQLLL (A02.01) LLLSADQQA (A02.01) LPTQTRGCI (B07.02) LPTQTRGCIL (B07.02) RISCLPTQTR (A03.01) SLAETVSLH (A03.01) TPRSSSSSS (B07.02) TPRSSSSSSS (B07.02)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS
ARID1A	S674fs P725fs R727fs I736fs	AHOGFPAAKESRVIQLS LLSLLIPPLTCLASEALP RPLLALPPVLLSLAQDH SRLLOCQATRCHLGHP VASRTASCILP*	ALPPVLLSL (A02.01) ALPPVLLSLA (A02.01) ALPRPLLAL (A02.01) ASRTASCIL (B07.02) EALPRPLLAL (B08.01) HLGHPVASR (A03.01) HPVASRTAS (B07.02) HPVASRTASC (B07.02) IIQLSLLSLL (A02.01) IQLSLLSLL (A02.01) IQLSLLSLLI (A02.01, A24.02) LLALPPVLL (A02.01) LLIPPLTCL (A02.01) LLIPPLTCLA (A02.01) LLSLLIPPL (A02.01) LLSLLIPPLT (A02.01) LPRPLLALPP (B07.02) QLSLLSLLI (A02.01) RLLQCQATR (A03.01) RPLLALPPV (B07.02)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			RPLLALPPVL (B07.02) SLAQDHSRL (A02.01) SLAQDHSRLL (A02.01) SLLIPPLTCL (A02.01) SLLSLLIPP (A02.01) SLLSLLIPPL (A02.01, B08.01)	
ARID1A	G414fs Q473fs H477fs S499fs P504fs Q548fs P549fs	PILAATGTSVRTAART WVPRAAIRVPDPAAVP DDHAGPGAECHGRPLL YTADSSLWTTRPQRVW STGPDSILQPAKSSPSA AAATLLPATTVPDPSCP TFVSAAATVSTTTAPVL SASILPAAIPASTSAVPG SIPLPAVDDTAAPPEPA PLLTATGSVSLPAAATS AASTLDALPAGCVSSA PVSAVPANCLFPAALPS TAGAISRFIWVSGILSPL NDLQ*	AAATSAASTL (B07.02) AAIPASTSAV (B07.02) AIPASTSAV (A02.01) ALPAGCVSSA (A02.01) APLLTATGSV (B07.02) APVLSASIL (B07.02) ATLLPATTV (A02.01) ATVSTTTAPV (A02.01) AVPANCLFPA (A02.01) CLFPAALPST (A02.01) CPTFVSAAA (B07.02) FPAALPSTA (B07.02) FPAALPSTAG (B07.02) GAECHGRPL (B07.02) GAISRFIWV (A02.01) ILPAAIPAST (A02.01) IWVSGILSPL (A24.02) LLTATGSVSL (A02.01) LLYTADSSL (A02.01) LPAAATSAA (B07.02) LPAAATSAAS (B07.02) LPAAIPAST (B07.02) LPAGCVSSA (B07.02) LPAGCVSSAP (B07.02) LYTADSSLW (A24.02) QPAKSSPSA (B07.02) QPAKSSPSAA (B07.02)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example!s 1	Exemplary Diseases
			RFIWVSGIL (A24.02)	
			RPQRVWSTG (B07.02)	
			RVWSTGPDSI (A02.01)	
			SAVPGSIPL (B07.02)	
			SILPAAIPA (A02.01)	
			SLPAAATSA (A02.01)	
			SLPAAATSAA (A02.01)	
			SLWTTRPQR (A03.01)	
			SLWTTRPQRV (A02.01)	
			SPSAAAATL (B07.02)	
			SPSAAAATLL (B07.02)	
			TLDALPAGCV (A02.01)	
			TVSTTTAPV (A02.01)	
			VLSASILPA (A02.01)	
			VLSASILPAA (A02.01)	
			VPANCLFPA (B07.02)	
			VPANCLFPAA (B07.02)	
			VPDPSCPTF (B07.02)	
			VPGSIPLPA (B07.02)	
			VPGSIPLPAV (B07.02)	
			WVSGILSPL (A02.01)	
			YTADSSLWTT (A02.01)	
ARID 1A	T433fs	PCRAGRRVPWAASLIH	APAGMVNRA (B07.02)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS
	A441fs	SRFLLMDNKAPAGMV	ASLHRRSYL (B08.01)	
	Y447fs	NRARLHITTSKVLTLSS	ASLHRRSYLK (A03.01)	
	P483fs	SSHPTPSNHRPRPLMPN	FLLMDNKAPA (A02.01)	
	P484fs	LRISSSHSLNHHSSSPLS	HPRRSPSRL (B07.02, B08.01)	
	P504fs	LHTPSSHPSLHISSPRLH	HPSLHISSP (B07.02)	
	S519fs	TPPSSRRHSSTPRASPPT	HRRSYLKIHL (B08.01)	
	H544fs	HSHRLSLLTSSSNLSSQ	HSRFLLMDNK (A03.01)	
	P549fs	HPRRSPSRLRILSPSLSS	KLPIPSSASL (A02.01)	
	P554fs	PSKLPIPSSASLHRRSYL	KVLTLSSSSH (A03.01)	
	Q563fs	KIHLGLRHPQPPQ*	LIHSRFLLM (B08.01)	

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			LLMDNKAPA (A02.01) LMDNKAPAGM (A02.01) LPIPSSASL (B07.02) MPNLRISSS (B07.02, B08.01) MPNLRISSSH (B07.02) NLRISSSHSL (B07.02, B08.01) PPTHSHRLSL (B07.02) RAGRRVPWAA (B08.01) RARLHITTSK (A03.01) RISSSHSLNH (A03.01) RLHTPPSSR (A03.01) RLHTPPSSRR (A03.01) RLRILSPSL (A02.01, B07.02, B08.01) RPLMPNLRI (B07.02) RPRPLMPNL (B07.02) SASLHRRSYL (B07.02, B08.01) SLHISSPRL (A02.01) SLHRRSYLK (A03.01) SLHRRSYLKI (B08.01) SLIHSRFLL (A02.01) SLIHSRFLLM (A02.01, B08.01) SLLTSSSNL (A02.01) SLNHHSSSPL (A02.01, B07.02, B08.01) SLSSPSKLPI (A02.01) SPLSLHTPS (B07.02) SPLSLHTPSS (B07.02) SPPTHSHRL (B07.02) SPRLHTPPS (B07.02) SPRLHTPPSS (B07.02) SPSLSSPSKL (B07.02)	

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			SYLKIHLGL (A24.02) TPSNHRPRPL (B07.02, B08.01) TPSSHPSLHI (B07.02)	
ARID1A	A2137fs P2139fs L1970fs V1994fs	RTNPTVRMRPHCVPFWTGRILLPSAASVCPIPFEACHLCQAMTLRCPNTQGCCSSWAS*	CVPFWTGRIL (B07.02) HCVPFWTGRIL (B07.02) ILLPSAASV (A02.01) ILLPSAASVC (A02.01) LLPSAASVCPI (A02.01) LPSAASVCPI (B07.02) MRPHCVPF (B08.01) RILLPSAASV (A02.01) RMRPHCVPF (A24.02, B07.02, B08.01) RMRPHCVPFW (A24.02) RTNPTVRMR (A03.01) SVCPIPFEA (A02.01) TVRMRPHCV (B08.01) TVRMRPHCVPF (B08.01) VPFWTGRIL (B07.02) VPFWTGRILL (B07.02) VRMRPHCVPF (B08.01)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS
ARID1A	N756fs S764fs T783fs Q799fs A817fs	TNQALPKIEVICRGTPRCPSTVPPSPAQPYLRVSLPEDRYTQAWAPTSRTPWGAMVPRGVSMAHKVATPGSQTIMPCPMPTTPVQAWLEA*	AMVPRGVSM (B07.02, B08.01) AMVPRGVSMA (A02.01) AWAPTSRTPW (A24.02) CPMPTTPVQA (B07.02) CPSTVPPSPA (B07.02) GAMVPRGVSM (B07.02, B08.01) MPCPMPTTPV (B07.02) MPTTPVQAW (B07.02)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			MPTTPVQAWL (B07.02) SLPEDRYTQA (A02.01) SPAQPYLRV (B07.02) SPAQPYLRVS (B07.02) TIMPCPMPT (A02.01) TPVQAWLEA (B07.02) TSRTPWGAM (B07.02) VPPSPAQPYL (B07.02) VPRGVSMAH (B07.02)	
β2M	N62fs E67fs L74fs F82fs T91fs E94fs	RMERELKKWSIQTCLSARTGLSISCTTLNSPPLKKMSMPAV*	CLSARTGLSI (B08.01) CTTLNSPPLK (A03.01) GLSISCTTL (A02.01) SPPLKKMSM (B07.02, B08.01) TLNSPPLKK (A03.01) TTLNSPPLK (A03.01) TTLNSPPLKK (A03.01)	CRC, STAD, SKCM, HNSC
β2M	L13fs S14fs	LCSRYSLFLAWRLSSVLQRFRFTHVIQQRMESQIS*	LQRFRFTHV (B08.01) LQRFRFTHVI (B08.01) RLSSVLQRF (A24.02) RLSSVLQRFR (A03.01) VLQRFRFTHV (A02.01, B08.01)	CRC, STAD, SKCM, HNSC
CDH1	A691fs P708fs L711fs	RSACVTVKGPLASVGRHSLSKQDCKFLPFWGFLEEFLLC*	ASVGRHSLSK (A03.01) KFLPFWGFL (A24.02) LASVGRHSL (B07.02) LPFWGFLEEF (B07.02) PFWGFLEEF (A24.02) SVGRHSLSK (A03.01)	ILC LumA Breast Cancer
CDH1	H121fs P126fs H128fs N144fs V157fs	IQWGTTTAPRPIRPPFLESKQNCSHFPTPLLASEDRRETGLFLPSAAQKMKKAHFLKTWFRSNPTKTKKARFSTASLAKELTH	APRPIRPPF (B07.02) APRPIRPPFL (B07.02) AQKMKKAHFL (B08.01) FLPSAAQKM (A02.01) GLFLPSAAQK (A03.01)	ILC LumA Breast Cancer

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
	P159fs N166fs N181fs F189fs P201fs F205fs	PLLVSLLLKEKQDG*	HPLLVSLLL (B07.02) KAHFLKTWFR (A03.01) KARFSTASL (B07.02) KMKKAHFLK (A03.01) KTWFRSNPTK (A03.01) LAKELTHPL (B07.02, B08.01) LAKELTHPLL (B08.01) NPTKTKKARF (B07.02) QKMKKAHFL (B08.01) RFSTASLAK (A03.01) RPIRPPFLES (B07.02) RSNPTKTKK (A03.01) SLAKELTHPL (A02.01, B08.01) TKKARFSTA (B08.01)	
CDH1	V114fs P127fs V132fs P160fs	PTDPFLGLRLGLHLQK VFHQSHAEYSGAPPPPP APSGLRFWNPSRIAHIS QLLSWPQKTEERLGYS SHQLPRK*	GLRFWNPSR (A03.01) ISQLLSWPQK (A03.01) RIAHISQLL (A02.01) RLGYSSHQL (A02.01) SQLLSWPQK (A03.01) SRIAHISQL (B08.01) WPQKTEERL (B07.02) YSSHQLPRK (A03.01)	ILC LumA Breast Cancer
CDH1	L731fs R749fs E757fs G759fs	FCCSCCFFGGERWSKSP YCPQRMTPGTTFITMM KKEAEKRTRTLT*	CPQRMTPGTT (B07.02) EAEKRTRTL (B08.01) GTTFITMMK (A03.01) GTTFITMMKK (A03.01) ITMMKKEAEK (A03.01) RMTPGTTFI (A02.01) SPYCPQRMT (B07.02) TMMKKEAEK (A03.01) TPGTTFITM (B07.02) TPGTTFITMM (B07.02)	ILC LumA Breast Cancer

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			TTFITMMKK (A03.01)	
CDH1	S19fs E24fs S36fs	WRRNCKAPVSLRKSVQ TPARSSPARPDRTRRLP SLGVPGQPWALGAAAS RRCCCCCRSPLGSARSR SPATLALTPRATRSRCP GATWREAASWAE*	CPGATWREA (B07.02) CPGATWREAA (B07.02) RSRCPGATWR (A03.01) TPRATRSRC (B07.02)	ILC LumA Breast Cancer
GATA3	P394fs P387fs S398fs H400fs M401fs S408fs P409fs S408fs P409fs T419fs H424fs P425fs S427fs F431fs S430fs H434fs H435fs S438fs M443fs G444fs *445fs	PGRPLQTHVLPEPHLAL QPLQPHADHAHADAPA IQPVLWTTPPLQHGHR HGLEPCSMLTGPPARV PAVPFDLHFCRSSIMKP KRDGYMFLKAESKIMF ATLQRSSLWCLCSNH*	HVLPEPHLAL (B07.02) RPLQTHVLPE (B07.02) VLWTTPPLQH (A03.01)	Breast Cancer
GATA3	P426fs H434fs P433fs T441fs	PRPRRCTRHPACPLDHT TPPAWSPPWVRALLDA HRAPSESPCSPFRLAFL QEQYHEA*	APSESPCSPF (B07.02) CPLDHTTPPA (B07.02) FLQEQYHEA (A02.01, B08.01) RLAFLQEQYH (A03.01) SPCSPFRLAF (B07.02)	Breast Cancer

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			SPPWVRALL (B07.02)	
			YPACPLDHTT (B07.02)	
MLL2	P519fs E524fs P647fs S654fs L656fs R755fs L761fs Q773fs	TRRCHCCPHLRSHPCP	ALHLRSCPC (B08.01)	STAD, BLCA, CRC, HNSC, BRCA
		HHLRNHPRPHHLRHHA	CLHHRRHLV (B08.01)	
		CHHHLRNCPHPHFLRH	CLHHRRHLVC (B08.01)	
		CTCPGRWRNRPSLRRL	CLHRKSHPHL (B08.01)	
		RSLLCLPHLNHHLFLH	CLRSHACPP (B08.01)	
		WRSRPCLHRKSHPHLL	CLRSHTCPP (B08.01)	
		HLRRLYPHHLKHRPCP	CLWCHACLH (A03.01)	
		HHLKNLLCPRHLRNCP	CPHHLKNHL (B07.02)	
		LPRHLKHLACLHHLRS	CPHHLKNLL (B07.02)	
		HPCPLHLKSHPCLHHR	CPHHLRTRL (B07.02, B08.01)	
		RHLVCSHHLKSLLCPL	CPLHLRSLPF (B07.02, B08.01)	
		HLRSLPFPHHLRHHACP	CPLPRHLKHL (B07.02,	
		HHLRTRLCPHHLKNHL	B08.01)	
		CPPHLRYRAYPPCLWC	CPLSLRSHPC (B07.02)	
		HACLHRLRNLPCPHRL	CPRHLRNCPL (B07.02,	
		RSLPRPLHLRLHASPHH	B08.01)	
		LRTPPHPHHLRTHLLPH	FPHHLRHHA (B07.02, B08.01)	
		HRRTRSCPCRWRSHPC	FPHHLRHHAC (B07.02,	
		CHYLRSRNSAPGPRGR	B08.01)	
		TCHPGLRSRTCPPGLRS	GLRSRTCPP (B08.01)	
		HTYLRRLRSHTCPPSLR	HACLHRLRNL (B08.01)	
		SHAYALCLRSHTCPPRL	HLACLHHLR (A03.01)	
		RDHICPLSLRNCTCPPR	HLCPPHLRY (A03.01)	
		LRSRTCLLCLRSHACPP	HLCPPHLRYR (A03.01)	
		NLRNHTCPPSLRSHACP	HLKHLACLH (A03.01)	
		PGLRNRICPLSLRSHPC	HLKHRPCPH (B08.01)	
		PLGLKSPLRSQANALH	HLKNHLCPP (B08.01)	
		LRSCPCSLPLGNHPYLP	HLKSHPCLH (A03.01)	
		CLESQPCLSLGNHLCPL	HLKSLLCPL (A02.01, B08.01)	
		CPRSCRCPHLGSHPCRL	HLLHLRRLY (A03.01)	
		S*	HLRNCPLPR (A03.01)	

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			HLRNCPLPRH (A03.01) HLRRLYPHHL (B08.01) HLRSHPCPL (B07.02, B08.01) HLRSHPCPLH (A03.01) HLRSLPFPH (A03.01) HLRTRLCPH (A03.01, B08.01) HLVCSHHLK (A03.01) HPCLHHRRHL (B07.02, B08.01) HPGLRSRTC (B07.02) HPHLLHLRRL (B07.02, B08.01) HRKSHPHLL (B08.01) HRRTRSCPC (B08.01) KSHPHLLHLR (A03.01) KSLLCPLHLR (A03.01) LLCPLHLRSL (A02.01, B08.01) LLHLRRLYPH (B08.01) LPRHLKHLA (B07.02) LPRHLKHLAC (B07.02, B08.01) LRRLRSHTC (B08.01) LRRLYPHHL (B08.01) LVCSHHLKSL (B08.01) NLRNHTCPPS (B08.01) PLHLRSLPF (B08.01) RLCPHHLKNH (A03.01) RLYPHHLKH (A03.01) RLYPHHLKHR (A03.01) RPCPHHLKNL (B07.02) RSHPCPLHLK (A03.01) RSLPFPHHLR (A03.01)	

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			RTRLCPHHL (B07.02) RTRLCPHHLK (A03.01) SLLCPLHLR (A03.01) SLRSHACPP (B08.01) SPLRSQANA (B07.02) YLRRLRSHT (B08.01) YPHHLKHRPC (B07.02, B08.01)	
PTEN	I122fs I135fs A148fs L152fs D162fs I168fs	SWKGTNWCNDMCIFITSGQIFKGTRGPRFLWGSKDQRQKGSNYSQSEALCVLL*	FITSGQIFK (A03.01) IFITSGQIF (A24.02) SQSEALCVL (A02.01) SQSEALCVLL (A02.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	L265fs K266fs	KRTKCFTFG*		UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	A39fs E40fs V45fs R47fs N48fs	PIFIQTLLLWDFLQKDLKAYTGTILMM*	AYTGTILMM (A24.02) DLKAYTGTIL (B08.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	T319fs T321fs K327fs A328fs A333fs	QKMILTKQIKTKPTDTFLQILR*	ILTKQIKTK (A03.01) KMILTKQIK (A03.01) KPTDTFLQI (B07.02) KPTDTFLQIL (B07.02) MILTKQIKTK (A03.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	N63fs E73fs A86fs N94fs	GFWIQSIKTITRYTIFVLKDIMTPPNLIAELHNILLKTITHHS*	ITRYTIFVLK (A03.01) LIAELHNIL (A02.01) LIAELHNILL (A02.01) MTPPNLIAEL (A02.01) NLIAELHNI (A02.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			NLIAELHNIL (A02.01) RYTIFVLKDI (A24.02) TITRYTIFVL (A02.01) TPPNLIAEL (B07.02)	
PTEN	T202fs G209fs C211fs I224fs G230fs P231fs R233fs D236fs	NYSNVQWRNLQSSVC GLPAKGEDIFLQFRTHT TGRQVHVL*	FLQFRTHTT (A02.01, B08.01) LPAKGEDIFL (B07.02) LQFRTHTTGR (A03.01) NLQSSVCGL (A02.01) SSVCGLPAK (A03.01) VQWRNLQSSV (A02.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
PTEN	G251fs E256fs K260fs Q261fs L265fs M270fs H272fs T286fs E288fs	YQSRVLPQTEQDAKKG QNVSLLGKYILHTRTR GNLRKSRKWKSM*	GQNVSLLGK (A03.01) HTRTRGNLRK (A03.01) ILHTRTRGNL (B08.01) KGQNVSLLGK (A03.01) LLGKYILHT (A02.01) LRKSRKWKSM (B08.01) SLLGKYILH (A03.01) SLLGKYILHT (A02.01)	UCEC, PRAD, SKCM, STAD, BRCA, LUSC, KIRC, LIHC, KIRP, GBM
TP53	A70fs P72fs A76fs A79fs P89fs W91fs S96fs V97fs V97fs G108fs G117fs S121fs	SSQNARGCSPRGPCTSS SYTGGPCTSPLLAPVIF CPFPENLPGQLRFPSGL LAFWDSQVCDLHVLPC PQQDVLPTGQDLPCAA VG*	CTSPLLAPV (A02.01) FPENLPGQL (B07.02) GLLAFWDSQV (A02.01) IFCPFPENL (A24.02) LLAFWDSQV (A02.01) LLAPVIFCP (A02.01) LLAPVIFCPF (A02.01, A24.02) LPCPQQDVL (B07.02) RFPSGLLAF (A24.02) RFPSGLLAFW (A24.02) SPLLAPVIF (B07.02) SPRGPCTSS (B07.02)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
	V122fs C124fs K139fs V143fs		SPRGPCTSSS (B07.02) SQVCDLHVL (A02.01) VIFCPFPENL (A02.01)	
TP53	V173fs H178fs D186fs H193fs L194fs E198fs V203fs E204fs L206fs D207fs N210fs T211fs F212fs V225fs S241fs	GAAPTMSAAQIAMVW PLLSILSEWKEICVWSI WMTETLFDIVWWCPM SRLRLALTVPPSTTTTC VTVPAWAA*	AMVWPLLSI (A02.01) AMVWPLLSIL (A02.01) AQIAMVWPL (A02.01, A24.02) AQIAMVWPLL (A02.01) CPMSRLRLA (B07.02, B08.01) CPMSRLRLAL (B07.02, B08.01) IAMVWPLLSI (A02.01, A24.02, B08.01) ILSEWKEICV (A02.01) IVWWCPMSR (A03.01) IVWWCPMSRL (A02.01) IWMTETLFDI (A24.02) LLSILSEWK (A03.01) MSAAQIAMV (A02.01) MSRLRLALT (B08.01) MSRLRLALTV (B08.01) MVWPLLSIL (A02.01) RLALTVPPST (A02.01) TLFDIVWWC (A02.01) TLFDIVWWCP (A02.01) TMSAAQIAMV (A02.01) VWSIWMTETL (A24.02) WMTETLFDI (A02.01, A24.02) WMTETLFDIV (A01.01, A02.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
TP53	R248fs P250fs	TGGPSSPSSHWKTPVVI YWDGTALRCVFVPVL	ALRCVFVPV (A02.01, B08.01) ALRCVFVPVL (A02.01,	BRCA, CRC, LUAD, PRAD, HNSC, LUSC,

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
	S260fs N263fs G266fs N268fs V272fs V274fs P278fs D281fs R282fs T284fs E285fs L289fs K292fs P301fs S303fs T312fs S314fs K319fs K320fs P322fs Y327fs F328fs L330fs R333fs R335fs R337fs E339fs	GETGAQRKRISARKGS LTTSCPQGALSEHCPTT PAPLPSQRRNHWMENI SPFRSVGVSASRCSES*	B08.01) ALSEHCPTT (A02.01) AQRKRISARK (A03.01) GAQRKRISA (B08.01) HWMENISPF (A24.02) LPSQRRNHW (B07.02) LPSQRRNHWM (B07.02, B08.01) NISPFRSVGV (A02.01) RISARKGSL (B07.02, B08.01) SPFRSVGVSA (B07.02) SPSSHWKTPV (B07.02, B08.01) TALRCVFVPV (A02.01) VIYWDGTAL (A02.01) VIYWDGTALR (A03.01) VLGETGAQRK (A03.01)	PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
TP53	S149fs P151fs P152fs V157fs Q165fs S166fs	FHTPARHPRPRHGHLQ AVTAHDGGCEALPPP*	HPRPRHGHL (B07.02, B08.01) HPRPRHGHLQ (B07.02) RPRHGHLQA (B07.02) RPRHGHLQAV (B07.02, B08.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
	H168fs V173fs			
TP53	P47fs D48fs D49fs Q52fs F54fs E56fs P58fs P60fs E62fs M66fs P72fs V73fs P75fs A78fs P82fs P85fs S96fs P98fs T102fs Y103fs G108fs F109fs R110fs G117fs	CCPRTILNNGSLKTQVQ MKLPECQRLLPPWPLH QQLLHRRPLHQPPPGPC HLLSLPRKPTRAATVSV WASCILGQPSL*	GSLKTQVQMK (A03.01) PPGPCHLLSL (B07.02) RTILNNGSLK (A03.01) SLKTQVQMK (A03.01) SLKTQVQMKL (B08.01) TILNNGSLK (A03.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
TP53	L26fs P27fs P34fs P36fs A39fs Q38fs	VRKHFQTYGNYFLKTT FCPPCRPKQWMI*	CPPCRPKQWM (B07.02) TTFCPPCRPK (A03.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
TP53	C124fs	LARTPLPSTRCFANWP	CFANWPRPAL (A24.02)	BRCA, CRC, LUAD,

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
	L130fs N131fs C135fs K139fs A138fs T140fs V143fs Q144fs V147fs T150fs P151fs P152fs G154fs R156fs R158fs A161fs	RPALCSCGLIPHPRPAP ASAPWPSTSSHST*	FANWPRPAL (B07.02, B08.01) GLIPHPRPA (A02.01) HPRPAPASA (B07.02, B08.01) HPRPAPASAP (B07.02) IPHPRPAPA (B07.02, B08.01) IPHPRPAPAS (B07.02) RPALCSCGL (B07.02) RPALCSCGLI (B07.02) TPLPSTRCF (B07.02) WPRPALCSC (B07.02) WPRPALCSCG (B07.02)	PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
VHL	L178fs D179fs L184fs T202fs R205fs D213fs G212fs	ELQETGHRQVALRRSG RPPKCAERPGAADTGA HCTSTDGRLKISVETYT VSSQLLMVLMSLDLDT GLVPSLVSKCLILRVK*	ALRRSGRPPK (A03.01) GLVPSLVSK (A03.01) KISVETYTV (A02.01) LLMVLMSLDL (A02.01, B08.01) LMSLDLDTGL (A02.01) LMVLMSLDL (A02.01) LVSKCLILRV (A02.01) QLLMVLMSL (A02.01, B08.01) RPGAADTGA (B07.02) RPGAADTGAH (B07.02) SLDLDTGLV (A02.01) SLVSKCLIL (A02.01, B08.01) SQLLMVLMSL (A02.01) TVSSQLLMV (A02.01) TYTVSSQLL (A24.02)	KIRC, KIRP

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			TYTVSSQLLM (A24.02) VLMSLDLDT (A02.01) VPSLVSKCL (B07.02) VSKCLILRVK (A03.01) YTVSSQLLM (A01.01) YTVSSQLLMV (A02.01)	
VHL	L158fs K159fs R161fs Q164fs	KSDASRLSGA*		KIRC, KIRP
VHL	P146fs I147fs F148fs L158fs	RTAYFCQYHTASVYSE RAMPPGCPEPSQA*	FCQYHTASV (B08.01)	KIRC, KIRP
VHL	S68fs S72fs I75fs S80fs P86fs P97fs I109fs H115fs L116fs G123fs T124fs N131fs L135fs V137fs G144fs D143fs I147fs	TRASPPRSSSAIAVRAS CCPYGSTSTASRSPTQR CRLARAAASTATEVTF GSSEMQGHTMGFWLT KLNYLCHLSMLTDSLF LPISHCQCIL*	CPYGSTSTA (B07.02) CPYGSTSTAS (B07.02) LARAAASTAT (B07.02) MLTDSLFLP (A02.01) PPRSSSAIAV (B07.02) RAAASTATEV (B07.02) SPPRSSSAI (B07.02) SPPRSSSAIA (B07.02) SPTQRCRLA (B07.02) TQRCRLARA (B08.01) TQRCRLARAA (B08.01)	KIRC, KIRP
VHL	K171fs P172fs	SSLRITGDWTSSGRSTK IWKTTQMCRKTWSG*	KIWKTTQMCR (A03.01) WTSSGRSTK (A03.01)	KIRC, KIRP

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
	N174fs L178fs D179fs L188fs			
VHL	V62fs V66fs Q73fs V84fs F91fs T100fs P103fs S111fs L116fs H115fs D126fs	RRRRGGVGRRGVRPGR VRPGGTGRRGGDGGR AAAARAALGELARALP GHLLQSQSARRAARM AQLRRRAAALPNAAA WHGPPHPQLPRSPLAL QRCRDTRWASG*	ALGELARAL (A02.01) AQLRRRAAA (B08.01) AQLRRRAAAL (B08.01) ARRAARMAQL (B08.01) HPQLPRSPL (B07.02, B08.01) HPQLPRSPLA (B07.02) LARALPGHL (B07.02) LARALPGHLL (B07.02) MAQLRRRAA (B07.02, B08.01) MAQLRRRAAA (B07.02, B08.01) QLRRRAAAL (B07.02, B08.01) RAAALPNAAA (B07.02) RMAQLRRRAA (B07.02, B08.01) SQSARRAARM (B08.01)	KIRC, KIRP
TABLE 2D		**CRYPTIC EXON [1]**		
AR-v7	cryptic final exon	SCKVFFKRAAEGKQKY LCASRNDCTIDKFRRK NCPSCRLRKCYEAGMT LGEKFRVGNCKHLKM TRP*	GMTLGEKFRV (A02:01) RVGNCKHLK (A03.01)	Prostate Cancer, Castration-resistant Prostate Cancer
TABLE 2E		**OUT OF FRAME FUSIONS [1,3]**		
AC011997.1: LRRC69	AC011997.1: LRRC69 *out-of-frame*	MAGAPPPASLPPCSLIS DCCASNQRDSVGVGPS EP:G:NNIKICNESASRK *	GPSEPGNNI (B07.02) KICNESASRK (A03.01)	LUSC, Breast Cancer, Head and Neck Cancer, LUAD

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
EEF1DP3	EEF1DP3:FRY *out-of-frame	HGWRPFLPVRARSRWNRRLDVTVANGR:S:**WKYGWSLLRVPQVNGIQVLNVSLKSSSNVISYE***	GIQVLNVSLK (A03.01) IQVLNVSLK (A03.01) KSSSNVISY (A01.01, A03.01) KYGWSLLRV (A24.02) RSWKYGWSL (A02.01) SLKSSSNVI (B08.01) SWKYGWSLL (A24.02) TVANGRSWK (A03.01) VPQVNGIQV (B07.02) VPQVNGIQVL (B07.02) VTVANGRSWK (A03.01) WSLLRVPQV (B08.01)	Breast Cancer
MAD1L1:MAFK	MAD1L1:MAFK	RLKEVFQTKIQEFRKACYTLTGYQIDITTENQYRLTSLYAEHPGDCLIFK::**LRVPGSSVLVTVPGL***	HPGDCLIFKL (B07.02) KLRVPGSSV (B07.02) KLRVPGSSVL (B07.02) RVPGSSVLV (A02.01) SVLVTVPGL (A02.01) VPGSSVLVTV (B07.02)	CLL
PPP1R1B:STARD3	PPP1R1B:STARD3	AEVLKVIRQSAGQKTTCGQGLEGPWERPPPLDESERDGGSEDQVEDPALS:A:**LLLRPRPPRPEVGAHQDEQAAQGADPRLGAQPACRGLPGLLTVPQPEPLLAPPSAA***	ALLLRPRPPR (A03.01) ALSALLLRPR (A03.01)	Breast Cancer
Table 2F		**IN FRAME DELETIONS and FUSIONS [1,2]**		
BCR:ABL	BCR:ABL	ERAEWRENIREQQKKCFRSFSLTSVELQMLTNSCVKLQTVHSIPLTINKE::**EALQRPVASDFEPQGLSEAARWNSKENLLA**	LTINKEEAL (A02.01, B08.01)	CML, AML

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		GPSENDPNLFVALYDFVASG		
BCR:ABL	BCR:ABL	ELQMLTNSCVKLQTVHSIPLTINKEDDESPGLYGFLNVIVHSATGFKQSS:K:**ALQRPVASDFEPQGLSEAARWNSKENLLAGPSENDPNLFVALYDFVASGD**	IVHSATGFK (A03.01) ATGFKQSSK (A03.01)	CML, AML
C11orf95:RELA	C11orf95:RELA	ISNSWDAHLGLGACGEAEGLGVQGAEEEEEEEEEEEEEGAGVPACPPKGP:E:**LFPLIFPAEPAQASGPYVEIIEQPKQRGMRFRYKCEGRSAGSIPGERSTD**	ELFPLIFPA (A02.01, B08.01) KGPELFPLI (A02.01, A24.02) KGPELFPLIF (A24.02)	Supretentorial ependyomas
CBFB:MYH11	(variant "type a")	LQRLDGMGCLEFDEERAQQEDALAQQAFEEARRRTREFEDRDRSHREEME::**VHELEKSKRALETQMEEMKTQLEELEDELQATEDAKLRLEVNMQALKGQF**		AML
CD74:ROS1	(exon6:exon32)	KGSFPENLRHLKNTMETIDWKVFESWMHHWLLFEMSRHSLEQKPTDAPPK::**AGVPNKPGIPKLLEGSKNSIQWEKAEDNGCRITYYILEIRKSTSNNLQNQ**	KPTDAPPKAGV (B07.02)	NSCLC, Crizotinib resistance
EGFR	EGFRvIII (internal deletion)	MRPSGTAGAALLALLAALCPASRALEEKK:G:NYVVTDHGSCVRACGA	ALEEKKGNYV (A02.01)	GBM

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		DSYEMEEDGVRKCKK CEGPCRKVCNGIGIGEF KD		
EGFR:SEPT14	EGFR:SEPT14	LPQPPICTIDVYMIMVK CWMIDADSRPKFRELII EFSKMARDPQRYLVIQ: :**LQDKFEHLKMIQQEE IRKLEEEKKQLEGEII DFYKMKAASEALQTQ LSTD**	IQLQDKFEHL (A02.01, B08.01) QLQDKFEHL (A02.01, B08.01) QLQDKFEHLK (A03.01) YLVIQLQDKF (A02.01, A24.02)	GBM, Glioma, Head and Neck Cancer
EML4:ALK	EML4:ALK	SWENSDDSRNKLSKIPS TPKLIPKVTKTADKHK DVIINQAKMSTREKNS Q:V:**YRRKHQELQAM QMELQSPEYKLSKLR TSTIMTDYNPNYCFAG KTSSISDL**	QVYRRKHQEL (B08.01) STREKNSQV (B08.01) VYRRKHQEL (A24.02, B08.01)	NSCLC
FGFR3:TACC3	FGFR3:TACC3	EGHRMDKPANCTHDL YMIMRECWHAAPSQRP TFKQLVEDLDRVLTVT STD::**VKATQEENRELR SRCEELHGKNLELGK IMDRFEEVVYQAMEE VQKQKELS**	VLTVTSTDV (A02.01) VLTVTSTDVK (A03.01)	Bladder Cancer, LUSC
NAB:STAT6	NAB:STAT6 (“variant 1” of Chmielecki et al.)	RDNTLLLRRVELFSLSR QVARESTYLSSLKGSRL HPEELGGPPLKKLKQE:: ATSKSQI**MSLWGLVSK MPPEKVQRLYVDFPQ HLRHLLGDWLESQP WEFLVGSDAFCC**	IMSLWGLVS (A02.01) IMSLWGLVSK (A03.01) KLKQEATSK (A03.01) QIMSLWGLV (A02.01) SQIMSLWGL (A02.01, A24.02, B08.01) SQIMSLWGLV (A02.01) TSKSQIMSL (B08.01)	Solitary fibrous tumors
NDRG1:ERG	NDRG1:ERG	MSREMQDVDLAEVKP	LLQEFDVQEA (A02.01)	Prostate Cancer

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		LVEKGETITGLLQEFDVQ::**EALSVVSEDQSLFECAYGTPHLAKTEMTASSSSDYGQTSKMSPRVPQQDW**	LQEFDVQEAL (A02.01)	
PML:RARA	PML:RARA (exon3:exon3)	VLDMHGFLRQALCRLRQEEPQSLQAAVRTDGFDEFKVRLQDLSSCITQGK:A:**IETQSSSSEEIVPSPPSPPPLPRIYKPCFVCQDKSSGYHYGVSACEGCKG**		Acute promyelocytic leukemia
PML:RARA	PML:RARA (exon6:exon3)	RSSPEQPRPSTSKAVSPPHLDGPPSPRSPVIGSEVFLPNSNHVASGAGEA:A:**IETQSSSSEEIVPSPPSPPPLPRIYKPCFVCQDKSSGYHYGVSACEGCKG**		Acute promyelocytic leukemia
RUNX1	RUNX1(ex5)-RUNX1T1(ex2)	VARFNDLRFVGRSGRGKSFTLTITVFTNPPQVATYHRAIKITVDGPREPR:N:**RTEKHSTMPDSPVDVKTQSRLTPPTMPPPPTTQGAPRTSSFTPTTLTNGT**	GPREPRNRT (B07.02) RNRTEKHSTM (B08.01)	AML
TMPRSS2:ERG	TMPRSS2:ERG	MALNS::EALSVVSEDQSLFECAYGTPHLAKTEMTASSSSDYGQTSKMSPRVPQQDW	ALNSEALSV (A02.01) ALNSEALSVV (A02.01) MALNSEALSV (A02.01, B08.01)	Prostate Cancer

Underlined AAs represent non-native AAs

[2]Bolded AAs represent native AAs of the amino acid sequence encoded by the second of the two fused genes

[3]Bolded and underlined AAs represent non-native AAs of the amino acid sequence encoded by the second of the two fused genes due to a frameshift

Table 3

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
Table 3A		**POINT MUTATIONS [1]**		
AKT1	E17K	MSDVAIVKEGWLHKRGKYIKTWRPRYFLLKNDGTFIGYKERPQDVDQREAPLNNFSVAQCQLMKTER	KYIKTWRPRY (A24.02) WLHKRGKYI (A02.01, B07.02, B08.01) WLHKRGKYIK (A03.01)	BRCA, CESC, HNSC, LUSC, PRAD, SKCM, THCA
ANAPC1	T537A	TMLVLEGSGNLVLYTGVVRVGKVFIPGLPAPSLTMSNTMPRPSTPLDGVSAPKPLSKLLGSLDEVVLLSPVPELRDSSKLHDSLYNEDCTFQQLGTYIHSI	APKPLSKLL (B07.02) GVSAPKPLSK (A03.01) VSAPKPLSK (A03.01)	GBM, LUSC, PAAD, PRAD, SKCM
FGFR3	S249C	HRIGGIKLRHQQWSLVMESVVPSDRGNYTCVVENKFGSIRQTYTLDVLERCPHRPILQAGLPANQTAVLGSDVEFHCKVYSDAQPHIQWLKHVEVNGSKVG	CPHRPILQA (B07.02)	BLCA, HNSC, KIRP, LUSC
FRG1B	I10T	MREPIYMHSTMVFLPWELHTKKGPSPPEQFMAVKLSDSRTALKSGYGKYLGINSDELVGHSDAIGPREQWEPVFQNGKMALLASNSCFIR	KLSDSRTAL (A02.01, B07.02, B08.01) KLSDSRTALK (A03.01) LSDSRTALK (A01.01, A03.01) RTALKSGYGK (A03.01) TALKSGYGK (A03.01)	KIRP, PRAD, SKCM
FRG1B	L52S	AVKLSDSRIALKSGYGKYLGINSDELVGHSDAIGPREQWEPVFQNGKMALSASNSCFIRCNEA	ALSASNSCF (A02.01, A24.02, B07.02) ALSASNSCFI (A02.01) FQNGKMALSA (A02.01,	GBM, KIRP, PRAD, SKCM

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		GDIEAKSKTAGEEEMI KIRSCAEKETKKKDDI PEEDKG	B08.01)	
HER2	L755S (Resistance)	AMPNQAQMRILKETE LRKVKVLGSGAFGTV YKGIWIPDGENVKIPV AIKVSRENTSPKANKEI LDEAYVMAGVGSPYV SRLLGICLTSTVQLVTQ LMPYGC	KVSRENTSPK (A03.01)	BRCA
IDH1	R132G	RVEEFKLKQMWKSPN GTIRNILGGTVFREAIIC KNIPRLVSGWVKPIIIG GHAYGDQYRATDFVV PGPGKVEITYTPSDGT QKVTYLVHNFEEGGG VAMGM	KPIIIGGHAY (B07.02)	BLCA, BRCA, CRC, GBM, HNSC, LUAD, PAAD, PRAD, UCEC
KRAS	G12C	MTEYKLVVVGACGVG KSALTIQLIQNHFVDE YDPTIEDSYRKQVVID GETCLLDILDTAGQE	KLVVVGACGV (A02.01) LVVVGACGV (A02.01) VVGACGVGK (A03.01, A11.01) VVVGACGVGK (A03.01)	BRCA, CESC, CRC, HNSC, LUAD, PAAD, UCEC
KRAS	G12D	MTEYKLVVVGADGVG KSALTIQLIQNHFVDE YDPTIEDSYRKQVVID GETCLLDILDTAGQE	VVGADGVGK (A11.01) VVVGADGVGK (A11.01) KLVVVGADGV (A02.01) LVVVGADGV (A02.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRP, LIHC, LUAD, PAAD, SKCM, UCEC
KRAS	G12V	MTEYKLVVVGAVGVG KSALTIQLIQNHFVDE YDPTIEDSYRKQVVID GETCLLDILDTAGQE	KLVVVGAVGV (A02.01) LVVVGAVGV (A02.01) VVGAVGVGK (A03.01, A11.01) VVVGAVGVGK (A03.01, A11.01)	BRCA, CESC, CRC, LUAD, PAAD, THCA, UCEC
KRAS	Q61H	AGGVGKSALTIQLIQN HFVDEYDPTIEDSYRK	ILDTAGHEEY (A01.01)	CRC, LUSC, PAAD, SKCM, UCEC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		QVVIDGETCLLDILDT AGHEEYSAMRDQYMR TGEGFLCVFAINNTKS FEDIHHYREQIKRVKD SEDVPM		
KRAS	Q61L	AGGVGKSALTIQLIQN HFVDEYDPTIEDSYRK QVVIDGETCLLDILDT AGLEEYSAMRDQYMR TGEGFLCVFAINNTKS FEDIHHYREQIKRVKD SEDVPM	ILDTAGLEEY (A01.01) LLDILDTAGL (A02.01)	CRC, GBM, HNSC, LUAD, SKCM, UCEC
NRAS	Q61K	AGGVGKSALTIQLIQN HFVDEYDPTIEDSYRK QVVIDGETCLLDILDT AGKEEYSAMRDQYMR TGEGFLCVFAINNSKS FADINLYREQIKRVKD SDDVPM	ILDTAGKEEY (A01.01)	BLCA, CRC, LIHC, LUAD, LUSC, SKCM, THCA, UCEC
NRAS	Q61R	AGGVGKSALTIQLIQN HFVDEYDPTIEDSYRK QVVIDGETCLLDILDT AGREEYSAMRDQYMR TGEGFLCVFAINNSKS FADINLYREQIKRVKD SDDVPM	ILDTAGREEY (A01.01)	BLCA, CRC, LUSC, PAAD, PRAD, SKCM, THCA, UCEC
PIK3CA	E542K	IEEHANWSVSREAGFS YSHAGLSNRLARDNEL RENDKEQLKAISTRDP LSKITEQEKDFLWSHR HYCVTIPEILPKLLLSV KWNSRDEVAQMYCL VKDWPP	AISTRDPLSK (A03.01)	BLCA, BRCA, CESC, CRC, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, UCEC
PTEN	R130Q	KFNCRVAQYPFEDHN	QTGVMICAY (A01.01)	BRCA, CESC, CRC,

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		PPQLELIKPFCEDLDQ WLSEDDNHVAAIHCK AGKGQTGVMICAYLL HRGKFLKAQEALDFY GEVRTRDKKGVTIPSQ RRYVYYYSY		GBM, KIRC, LUSC, UCEC
RAC1	P29S	MQAIKCVVVGDGAVG KTCLLISYTTNAFSGEY IPTVFDNYSANVMVD GKPVNLGLWDTAGQE DYDRLRPLSYPQTVGE T	FSGEYIPTV (A02.01) TTNAFSGEY (A01.01) YTTNAFSGEY (A01.01)	Melanoma
SF3B1	K700E	AVCKSKKSWQARHTG IKIVQQIAILMGCAILP HLRSLVEIIEHGLVDEQ QEVRTISALAIAALAE AATPYGIESFDSVLKPL WKGIRQHRGKGLAAF LKAI	GLVDEQQEV (A02.01)	AML associated with MDS; Chronic lymphocytic leukaemia-small lymphocytic lymphoma; Myelodysplastic syndrome; AML; Luminal NS carcinoma of breast; Chronic myeloid leukaemia; Ductal carcinoma of pancreas; Chronic myelomonocytic leukaemia; Chronic lymphocytic leukaemia-small lymphocytic lymphoma; Myelofibrosis; Myelodysplastic syndrome; PRAD; Essential thrombocythaemia; Medullomyoblastoma

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
SPOP	F133L	YLSLYLLLVSCPKSEV RAKFKFSILNAKGEET KAMESQRAYRFVQGK DWGLKKFIRRDFLLDE ANGLLPDDKLTLFCEV SVVQDSVNISGQNTM NMVKVPE	FVQGKDWGL (A02.01, B08.01)	PRAD
SPOP	F133V	YLSLYLLLVSCPKSEV RAKFKFSILNAKGEET KAMESQRAYRFVQGK DWGVKKFIRRDFLLDE ANGLLPDDKLTLFCEV SVVQDSVNISGQNTM NMVKVPE	FVQGKDWGV (A02.01)	PRAD
TP53	G245S	IRVEGNLRVEYLDDRN TFRHSVVVPYEPPEVG SDCTTIHYNYMCNSSC MGSMNRRPILTIITLED SSGNLLGRNSFEVRVC ACPGRDRRTEEENLRK KGEP	CMGSMNRRPI (A02.01, B08.01) GSMNRRPIL (B08.01) MGSMNRRPI (B08.01) MGSMNRRPIL (B08.01) SMNRRPILTI (A02.01, A24.02, B08.01)	BLCA, BRCA, CRC, GBM, HNSC, LUSC, PAAD, PRAD
TP53	R248Q	EGNLRVEYLDDRNTFR HSVVVPYEPPEVGSDC TTIHYNYMCNSSCMG GMNQRPILTIITLEDSS GNLLGRNSFEVRVCAC PGRDRRTEEENLRKKG EPHHE	CMGGMNQRPI (A02.01, B08.01) GMNQRPILTI (A02.01, B08.01) NQRPILTII (A02.01, B08.01)	BLCA, BRCA, CRC, GBM, HNSC, KIRC, LIHC, LUSC, PAAD, PRAD, UCEC
TP53	R248W	EGNLRVEYLDDRNTFR HSVVVPYEPPEVGSDC TTIHYNYMCNSSCMG GMNWRPILTIITLEDSS GNLLGRNSFEVRVCAC	CMGGMNWRPI (A02.01, A24.02, B08.01) GMNWRPILTI (A02.01, B08.01) MNWRPILTI (A02.01, A24.02, B08.01)	BLCA, BRCA, CRC, GBM, HNSC, LIHC, LUSC, PAAD, SKCM, UCEC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		PGRDRRTEEENLRKKG EPHHE	MNWRPILTII (A02.01, A24.02)	
TP53	R273C	PEVGSDCTTIHYNYMC NSSCMGGMNRRPILTII TLEDSSGNLLGRNSFE VCVCACPGRDRRTEEE NLRKKGEPHHELPPGS TKRALPNNTSSPQPK KKPL	NSFEVCVCA (A02.01)	BLCA, BRCA, CRC, GBM, HNSC, LUSC, PAAD, UCEC
TP53	R273H	PEVGSDCTTIHYNYMC NSSCMGGMNRRPILTII TLEDSSGNLLGRNSFE VHVCACPGRDRRTEEE NLRKKGEPHHELPPGS TKRALPNNTSSPQPK KKPL	NSFEVHVCA (A02.01)	BRCA, CRC, GBM, HNSC, LIHC, LUSC, PAAD, UCEC
TP53	Y220C	TEVVRRCPHHERCSDS DGLAPPQHLIRVEGNL RVEYLDDRNTFRHSV VVPCEPPEVGSDCTTIH YNYMCNSSCMGGMN RRPILTIITLEDSSGNLL GRNSF	VVPCEPPEV (A02.01) VVVPCEPPEV (A02.01)	BLCA, BRCA, GBM, HNSC, LIHC, LUAD, LUSC, PAAD, SKCM, UCEC
Table 3B		**MSI-ASSOCIATED FRAMESHIFTS [1]**		
MSH6	F1088fs; +1	YNFDKNYKDWQSAVE CIAVLDVLLCLANYSR GGDGPMCRPVILLPED TPPLLRA	ILLPEDTPPL (A02.01) LLPEDTPPL (A02.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer, Lynch syndrome
Table 3C		**FRAMESHIFT [1]**		
APC	F1354fs	AKFQQCHSTLEPNPAD CRVLVYLQNQPGTKL LNFLQERNLPPKVVLR HPKVHLNTMFRRPHS	APFRVNHAV (B07.02) CLADVLLSV (A02.01) FLQERNLPPK (A03.01) HLIVLRVVRL (A02.01, B08.01)	CRC, LUAD, UCEC, STAD

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		CLADVLLSVHLIVLRV VRLPAPFRVNHAVEW *	HPKVHLNTM (B07.02, B08.01) HPKVHLNTMF (B07.02, B08.01) KVHLNTMFR (A03.01) KVHLNTMFRR (A03.01) LPAPFRVNHA (B07.02) MFRRPHSCL (B07.02, B08.01) MFRRPHSCLA (B08.01) NTMFRRPHSC (B08.01) RPHSCLADV (B07.02) RPHSCLADVL (B07.02) RVVRLPAPFR (A03.01) SVHLIVLRV (A02.01) TMFRRPHSC (B08.01) TMFRRPHSCL (A02.01, B08.01) VLLSVHLIV (A02.01) VLLSVHLIVL (A02.01) VLRVVRLPA (B08.01) VVRLPAPFR (A03.01)	
ARID1A	Y1324fs	ALGPHSRISCLPTQTRG CILLAATPRSSSSSSSN DMIPMAISSPPKAPLLA APSPASRLQCINSNSRI TSGQWMAHMALLPSG TKGRCTACHTALGRG SLSSSSCPQPSPSLPAS NKLPSLPLSKMYTTSM AMPILPLPQLLLSADQ QAAPRTNFHSSLAETV SLHPLAPMPSKTCHHK *	AMPILPLPQL (A02.01) APLLAAPSPA (B07.02) APRTNFHSS (B07.02) APRTNFHSSL (B07.02, B08.01) CPQPSPSLPA (B07.02) GQWMAHMAL (A02.01) GQWMAHMALL (A02.01) HMALLPSGTK (A03.01) HTALGRGSL (B07.02) IPMAISSPP (B07.02) IPMAISSPPK (B07.02) KLPSLPLSK (A03.01) KLPSLPLSKM (A02.01) KMYTTSMAM (A02.01,	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			A03.01) LLAAPSPASR (A03.01) LLLSADQQAA (A02.01) LLSADQQAA (A02.01) LPASNKLPS (B07.02) LPASNKLPSL (B07.02, B08.01) LPLPQLLLSA (B07.02) LPSLPLSKM (B07.02) LSKMYTTSM (B08.01) MALLPSGTK (A03.01) MPILPLPQL (B07.02) MPILPLPQLL (B07.02) MYTTSMAMPI (A24.02) PMAISSPPK (A03.01) QWMAHMALL (A24.02) SKMYTTSMAM (B07.02) SMAMPILPL (A02.01, B07.02, B08.01) SNKLPSLPL (B08.01) SPASRLQCI (B07.02, B08.01) SPPKAPLLAA (B07.02) SPSLPASNKL (B07.02) YTTSMAMPI (A02.01) YTTSMAMPIL (A02.01)	
ARID1A	G1848fs	RSYRRMIHLWWTAQIS LGVCRSLTVACCTGGL VGGTPLSISRPTSRARQ SCCLPGLTHPAHQPLG SM*	CLPGLTHPA (A02.01) GLTHPAHQPL (A02.01) HPAHQPLGSM (B07.02) LTHPAHQPL (B07.02) RPTSRARQSC (B07.02) RQSCCLPGL (A02.01) TSRARQSCCL (B08.01)	STAD, UCEC, BLCA, BRCA, LUSC, CESC, KIRC, UCS
β2M	L13fs	QHSGRDVSLRGLSCAR ATLSFWPGGYPAYSK	ELLCVWVSSI (A02.01) EWKVKFPEL (B08.01)	CRC, STAD, SKCM, HNSC

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		DSGLLTSSSREWKVKF PELLCVWVSSIRH*	KFPELLCVW (A24.02) LLCVWVSSI (A02.01) LLTSSSREWK (A03.01) LTSSSREWK (A03.01) YPAYSKDSGL (B07.02)	
GATA3	L328fs N334fs	AQAKAVCSQESRDVL CELSDHHNHTLEEECQ WGPCLQCLWALLQAS QY*	CLQCLWALL (A02.01) CQWGPCLQCL (A02.01) QWGPCLQCL (A24.02) QWGPCLQCLW (A24.02)	Breast Cancer
GATA3	H400fs S408fs S408fs S430fs H434fs H435fs	PGRPLQTHVLPEPHLA LQPLQPHADHAHADA PAIQPVLWTTPPLQHG HRHGLEPCSMLTGPPA RVPAVPFDLHFCRSSI MKPKRDGYMFLKAES KIMFATLQRSSLWCLC SNH*	AIQPVLWTT (A02.01) ALQPLQPHA (A02.01) DLHFCRSSIM (B08.01) EPHLALQPL (B07.02, B08.01) ESKIMFATL (B08.01) FATLQRSSL (B07.02, B08.01) FLKAESKIM (B08.01) FLKAESKIMF (B08.01) GPPARVPAV (B07.02) IMKPKRDGYM (B08.01) KIMFATLQR (A03.01) KPKRDGYMF (B07.02) KPKRDGYMFL (B07.02) LHFCRSSIM (B08.01) LQHGHRHGL (B08.01) MFATLQRSSL (B07.02, B08.01) MFLKAESKI (A24.02) MLTGPPARV (A02.01) QPVLWTTPPL (B07.02) SMLTGPPARV (A02.01) TLQRSSLWCL (A02.01) VLPEPHLAL (A02.01) VPAVPFDLHF (B07.02) YMFLKAESK (A03.01)	Breast Cancer

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			YMFLKAESKI (A02.01, A03.01, A24.02, B08.01)	
MLL2	P647fs L656fs	TRRCHCCPHLRSHPCP HHLRNHPRPHHLRHH ACHHHLRNCPHPHFLR HCTCPGRWRNRPSLR RLRSLLCLPHLNHHLF LHWRSRPCLHRKSHP HLLHLRRLYPHHLKHR PCPHHLKNLLCPRHLR NCPLPRHLKHLACLHH LRSHPCPLHLKSHPCL HHRRHLVCSHHLKSLL CPLHLRSLPFPHHLRH HACPHHLRTRLCPHHL KNHLCPPHLRYRAYPP CLWCHACLHRLRNLP CPHRLRSLPRPLHLRL HASPHHLRTPPHPHHL RTHLLPHHRRTRSCPC RWRSHPCCHYLRSRN SAPGPRGRTCHPGLRS RTCPPGLRSHTYLRRL RSHTCPPSLRSHAYAL CLRSHTCPPRLRDHICP LSLRNCTCPPRLRSRT CLLCLRSHACPPNLRN HTCPPSLRSHACPPGL RNRICPLSLRSHPCPLG LKSPLRSQANALHLRS CPCSLPLGNHPYLPCL ESQPCLSLGNHLCPLC PRSCRCPHLGSHPCRL S*	APGPRGRTC (B07.02) CLRSHTCPPR (A03.01) CLWCHACLHR (A03.01) CPHLGSHPC (B07.02) CPLGLKSPL (B07.02) CPRSCRCPH (B07.02) CPRSCRCPHL (B07.02, B08.01) CSLPLGNHPY (A01.01) GLRNRICPL (A02.01, B07.02, B08.01) GLRSHTYLR (A03.01) GLRSHTYLRR (A03.01) GPRGRTCHPG (B07.02) HLGSHPCRL (B08.01) HLRLHASPH (A03.01) HLRSCPCSL (B07.02, B08.01) HLRTHLLPH (A03.01) HLRTHLLPHH (A03.01) HLRYRAYPP (B08.01) HLRYRAYPPC (B08.01) HPHHLRTHL (B07.02) HPHHLRTHLL (B07.02, B08.01) HTYLRRLRSH (A03.01) LPCPHRLRSL (B07.02, B08.01) LPHHRRTRSC (B07.02, B08.01) LPLGNHPYL (B07.02) LPRPLHLRL (B07.02, B08.01) NLRNHTCPP (B08.01) PPRLRSRTCL (B07.02, B08.01) RLHASPHHL (A02.01) RLHASPHHLR (A03.01)	STAD, BLCA, CRC, HNSC, BRCA

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			RLRDHICPL (A02.01, B07.02, B08.01) RLRNLPCPH (A03.01) RLRNLPCPHR (A03.01) RLRSHTCPP (B08.01) RLRSLPRPL (B07.02, B08.01) RLRSLPRPLH (A03.01) RLRSRTCLL (B07.02, B08.01) RNRICPLSL (B07.02, B08.01) RPLHLRLHA (B07.02) RPLHLRLHAS (B07.02) RSHACPPGLR (A03.01) RSHACPPNLR (A03.01) RSHAYALCLR (A03.01) RSHPCCHYLR (A03.01) RSHPCPLGLK (A03.01) RSHTCPPSLR (A03.01) RSLPRPLHLR (A03.01) RSRTCLLCL (B07.02) RSRTCLLCLR (A03.01) RSRTCPPGL (B07.02) RSRTCPPGLR (A03.01) RTHLLPHHRR (A03.01) RTRSCPCRWR (A03.01) RYRAYPPCL (A24.02) RYRAYPPCLW (A24.02) SLGNHLCPL (A02.01, B07.02, B08.01) SLPLGNHPYL (A02.01) SLPRPLHLRL (A02.01) SLRNCTCPPR (A03.01) SLRSHAYAL (A02.01, B07.02, B08.01) SLRSHPCPL (A02.01, B07.02,	

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			B08.01) SPHHLRTPP (B07.02) SPHHLRTPPH (B07.02) SPLRSQANAL (B07.02, B08.01) YLRRLRSHTC (B08.01) YLRSRNSAP (B08.01) YLRSRNSAPG (B08.01)	
MLL2	P2354fs	GPRSHPLPRLWHLLLQ VTQTSFALAPTLTHML SPH*	ALAPTLTHM (A02.01) ALAPTLTHML (A02.01) LLQVTQTSFA (A02.01) LQVTQTSFAL (A02.01) RLWHLLLQV (A02.01) RLWHLLLQVT (A02.01)	STAD, BLCA, CRC, HNSC, BRCA
RNF43	G659fs	PLGLVPWTRWCPQGK PRFPAMSTTTATGTTT TKSGSSGMAGSLAQK PESPSPGLLFLGHSPSQ SHLLLISKSPDPTQQPL RGGSLTHSAPGPSLSQ PLAQLTPPASAPVPAV CSTCKNPASLPDTHRG KGGGVPPSPPLALGPR MQLCTQLARFFPITPP VWHILGPQRHTP*	CTQLARFFPI (A24.02) FFPITPPVW (A24.02) FPITPPVWHI (B07.02) GPRMQLCTQL (B07.02, B08.01) ITPPVWHIL (A24.02) LALGPRMQL (B07.02) MQLCTQLARF (A24.02) RFFPITPPV (A02.01, A24.02) RFFPITPPVW (A24.02) RMQLCTQLA (A02.01) RMQLCTQLAR (A03.01) SPPLALGPRM (B07.02) TQLARFFPI (A02.01, A24.02, B08.01)	STAD
SMAP1	E169fs	KYEKKKYYDKNAIAIT NISSSDAPLQPLVSSPS LQAAVDKNKLEKEKE KKRKRKREKRSQKSR QNHLQLKSCRRKISNW	KSRQNHLQL (B07.02) ALKKLRSPL (B08.01, B07.02) HLQLKSCRRK (A03.01) KISNWSLKK (A03.01, A11.01) KISNWSLKKV (A03.01)	MSI+ CRC, MSI+ Uterine/Endometrium Cancer, MSI+ Stomach Cancer

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
		SLKKVPALKKLRSPLW IF	KLRSPLWIF (A24.02) KSRQNHLQLK (A03.01) NWSLKKVPAL (B08.01) SLKKVPALK (A03.01, A11.01) SLKKVPALKK (A03.01) SQKSRQNHL (B08.01) WSLKKVPAL (B08.01) WSLKKVPALK (A03.01)	
TP53	P58fs P72fs G108fs R110fs	CCPRTILNNGSLKTQV QMKLPECQRLLPPWPL HQQLLHRRPLHQPPPG PCHLLSLPRKPTRAAT VSVWASCILGQPSL*	KLPECQRLL (A02.01) KPTRAATVSV (B07.02) LPPWPLHQQL (B07.02) LPRKPTRAA (B07.02, B08.01) LPRKPTRAAT (B07.02) QQLLHRRPL (B08.01) RLLPPWPLH (A03.01)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
TP53	P152fs	LARTPLPSTRCFANWP RPALCSCGLIPHPRPAP ASAPWPSTSSHST*	APASAPWPST (B07.02) APWPSTSSH (B07.02) RPAPASAPW (B07.02) WPSTSSHST (B07.02)	BRCA, CRC, LUAD, PRAD, HNSC, LUSC, PAAD, STAD, BLCA, OV, LIHC, SKCM, UCEC, LAML, UCS, KICH, GBM, ACC
UBR5	K2120fs	SQGLYSSSASSGKCLM EVTVDRNCLEVLPTK MSYAANLKNVMNMQ NRQKKKGKNSPCCQK KLRVQNQGHLLMILL HN*	RVQNQGHLL (B07.02)	
VHL	L116fs G123fs	TRASPPRSSSAIAVRAS CCPYGSTSTASRSPTQ RCRLARAAASTATEVT FGSSEMQGHTMGFWL TKLNYLCHLSMLTDSL FLPISHCQCIL*	FLPISHCQCI (A02.01) FWLTKLNYL (A24.02, B08.01) HLSMLTDSL (A02.01) HTMGFWLTK (A03.01) HTMGFWLTKL (A02.01) KLNYLCHLSM (A02.01) LPISHCQCI (B07.02, B08.01)	KIRC, KIRP

Gene	Exemplary Protein Change	Mutation Sequence Context	Peptides (HLA allele example(s))	Exemplary Diseases
			LPISHCQCIL (B07.02, B08.01) LTDSLFLPI (A01.01, A02.01) LTKLNYLCHL (B08.01) MLTDSLFLPI (A01.01, A02.01, B08.01) MQGHTMGFWL (A02.01) NYLCHLSML (A24.02) SMLTDSLFL (A02.01) TMGFWLTKL (A02.01) YLCHLSMLT (A02.01)	
TABLE 3D		**INSERT**[1]		
HER2	G776insYVMA	LGSGAFGTVYKGIWIP DGENVKIPVAIKVLRE NTSPKANKEILDEAYV MAYVMAGVGSPYVSR LLGICLTSTVQLVTQL MPYGCLLDHVRENRG RLGSQDLLNW	ILDEAYVMAY (A01.01) VMAYVMAGV (A02.01) YVMAYVMAG (A02.01, B07.02, B08.01) YVMAYVMAGV (A02.01, B07.02, B08.01)	Lung Cancer

^nderlinec l AAs represent non-native AAs

[2]Bolded AAs represent native AAs of the amino acid sequence encoded by the second of the two fused genes

[3]Bolded and underlined AAs represent non-native AAs of the amino acid sequence encoded by the second of the two fused genes due to a framshift.

Example 20: Examples of Neoantigens

[00712] HLA binding assays were used to evaluate either the affinity of interaction of a given epitope with a selected HLA molecule or the stability. Each of these measurements was, e.g., indicative of the ability of an epitope to elicit a $CD8^+$ T cell response.

[00713] A subset of peptides from Table 4 (n=562) were synthesized and their affinity for their given HLA class I molecule was measured as described. The values are shown in Table 3. The measurements are plotted against the predicted affinity in Figure 31. These data show a strong correlation between prediction and measurement (dotted line represents best fit, $R^2 = 0.45$), demonstrating the value of the predictions. However, the outliers demonstrate the importance of these measurements. Thick vertical and horizontal lines are shown at 500 nM for the predicted affinity and observed affinity, respectively. 500nM is commonly accepted in the field as the maximum affinity for an epitope that is a "weak binder" to HLA class I. Therefore, the points in

the lower right quadrant (prediction greater than 500 nM, measurement less than 500 nM) are epitopes that were considered very weak binders but were observed to bind within an acceptable range. Epitopes in this quadrant (n=75) represent 30.5% of epitopes not considered to be binders by prediction (combination of bottom right and top right quadrants, n=246).

Table 4 HLA Class I Binding Affinity

Mutation	Allele	Peptide	Predicted affinity (IC_{50}; (nM))	Observed Affinity (IC_{50}; (nM))	Stability ($T_{1/2}$ (h))
ABL1, M351T	A02.01	TQISSATEYL	2921.0	2644.0	0
ABL1, T315I	A02.01	YIIIEFMTYG	3502.0	186.0	0
ABL1, T315I	A02.01	IIIEFMTYG	1991.0	779.0	0
ABL1, T315I	A02.01	IIIEFMTYGN	16793.0	1551.0	0
ABL1, T315I	A02.01	IIEFMTYGNL	2134.0	9702.0	0
ABL1, Y253H	A02.01	KLGGGQHGEV	1705.0	387.0	0.4
AKT1, E17K	B08.01	WLHKRGKYI	47.0	417.0	1.3
AKT1, E17K	A02.01	WLHKRGKYI	4972.0	1250.0	1.2
AKT1, E17K	B07.02	WLHKRGKYI	7185.0	2648.0	0
ALK, G1269A	A02.01	RVAKIADFGM	5258.0	125.0	0.5
ALK, G1269A	B07.02	RVAKIADFGM	7260.0	9723.0	0.2
ALK, L1196M	A02.01	SLPRFILMEL	94.0	26.0	0.5
ALK, L1196M	A02.01	ILMELMAGG	192.0	223.0	0.5
ALK, L1196M	A02.01	LMELMAGGDL	5617.0	311.0	8.9
ALK, L1196M	A02.01	LQSLPRFILM	2519.0	413.0	0
ALK, L1196M	B07.02	SLPRFILMEL	17.0	583.0	0.4
ALK, L1196M	B08.01	LQSLPRFILM	1288.0	1547.0	0
ALK, L1196M	A02.01	FILMELMAGG	189.0	1580.0	0
ALK, L1196M	B08.01	SLPRFILMEL	686.0	1762.0	0
ALK, L1196M	A24.02	SLPRFILMEL	5143.0	2774.0	0.2
ALK, L1196M	A02.01	ILMELMAGGD	5761.0	3451.0	0
APC, AVEW	A02.01	VLLSVHLIV	36.0	72.0	11
APC, AVEW	A02.01	CLADVLLSV	5.0	219.0	24
APC, VHPA	A02.01	KVLQMDFLV	25.0	11.0	6.4
APC, VHPA	A02.01	LQMDFLVHPA	26.0	68.0	1.5
β2M, …MPAV	A03.01	TTLNSPPLKK	62.5	14.3	not measured
β2M, …MPAV	A03.01	TTLNSPPLK	165.4	9.8	not

					measured
β2M, …MPAV	A03.01	TLNSPPLKK	27.5	5.4	not measured
β2M, …MPAV	A03.01	CTTLNSPPLK	225.3	63.6	not measured
β2M, …MPAV	B08.01	CLSARTGLSI	1106.6	149.6	not measured
β2M, …MPAV	A02.01	GLSISCTTL	669.0	114.5	not measured
β2M, …SIRH	A03.01	LTSSSREWK	413.9	117.8	not measured
β2M, …SIRH	A03.01	LLTSSSREWK	206.1	1769.8	not measured
β2M, …SIRH	B07.02	YPAYSKDSGL	41.1	79.5	not measured
β2M, …SIRH	B08.01	EWKVKFPEL	488.7	538.4	not measured
β2M, …SIRH	A24.02	KFPELLCVW	83.7	13.7	not measured
β2M, …SQIS	B08.01	LQRFRFTHV	55.5	37.3	not measured
β2M, …SQIS	A24.02	RLSSVLQRF	288.9	28.2	not measured
β2M, …SQIS	A02.01	VLQRFRFTHV	163.4	106.7	not measured
β2M, …SQIS	B08.01	VLQRFRFTHV	264.1	480.1	not measured
β2M, …SQIS	A03.01	RLSSVLQRFR	168.1	12.5	not measured
BCR:ABL (e13a2, aka b2a2)	B08.01	LTINKEEAL	4972.0	895.0	0
BCR:ABL (e13a2, aka b2a2)	A02.01	LTINKEEAL	12671.0	4413.0	0
BRAF, V600E	A02.01	LATEKSRWSG	39130.0	23337.0	0
BRAF, V600E	B08.01	LATEKSRWS	24674.0	36995.0	0
BRAF, V600E	B08.01	LATEKSRWSG	13368.0	46582.0	0

BRAF, V600E	A02.0 1	LATEKSRWS	39109.0	60997.0	0
BTK, C481S	A02.0 1	SLLNYLREM	48.0	87.0	3
BTK, C481S	A02.0 1	MANGSLLNYL	2979.0	1082.0	0
BTK, C481S	B07.02	SLLNYLREM	6544.0	1110.0	0
BTK, C481S	B08.0 1	SLLNYLREM	1091.0	1230.0	0
BTK, C481S	A02.0 1	YMANGSLLN	7856.0	4444.0	0
BTK, C481S	B07.02	MANGSLLNYL	8921.0	17715.0	0
BTK, C481S	B08.0 1	MANGSLLNYL	7639.0	19853.0	0
BTK, C481S	A03.01	MANGSLLNY	1030.3	35.6	not measured
BTK, C481S	A01.01	MANGSLLNY	285.7	439.0	not measured
BTK, C481S	A24.02	EYMANGSLL	213.2	5.0	not measured
BTK, C481S	A01.01	YMANGSLLNY	95.7	13.2	not measured
BTK, C481S	A03.01	YMANGSLLNY	109.4	95.9	not measured
C1orf95:RELA	A02.01	ELFPLIFPA	13.0	13.0	5.1
C1orf95:RELA	A24.02	KGPELFPLI	909.0	14.0	1.7
C1orf95:RELA	A02.0 1	KGPELFPLI	6840.0	101.0	0.3
C1orf95:RELA	B08.0 1	ELFPLIFPA	7316.0	449.0	0
C15ORF40(+1)	A02.01	KLFSCLSFL	6.0	6.0	14.3
C15ORF40(+1)	A03.01	KLFSCLSFL	1488.0	308.0	0.8
C15ORF40(+1)	A03.01	SLQPPPPGFK	26.3	19.0	not measured
C15ORF40(+1)	A03.01	LFFFFFETK	658.4	413.3	not measured
C15ORF40(+1)	A02.0 1	ALFFFFFET	28.9	470.7	not measured
C15ORF40(+1)	A03.01	ALFFFFFETK	31.5	216.4	not measured
C15ORF40(+1)	A02.0 1	FFFETKSCSV	754.5	61.2	not measured
C15ORF40(+1)	B08.0 1	FFETKSCSV	807.6	7.6	not measured

C15ORF40(+l)	A01.01	LSFLSSWDY	211.1	52.9	not measured
C15ORF40(+l)	A02.01	FLSSWDYRRM	62.2	323.5	not measured
C15ORF40(+l)	A03.01	LSFLSSWDYR	508.7	100.9	not measured
C15ORF40(+l)	A02.01	FKLFSCLSFL	9.9	662.9	not measured
C15ORF40(+l)	A02.01	VQWRSLGSL	986.0	4733.2	not measured
C15ORF40(+l)	A02.01	KLFSCLSFLS	65.1	0.6	not measured
C15ORF40(+l)	A03.01	KLFSCLSFLS	805.1	104.0	not measured
C15ORF40(+l)	A02.01	AQAGVQWRSL	630.2	670.0	not measured
C15ORF40(+l)	A24.02	RRMPPCLANF	253.0	141.1	not measured
C15ORF40(+l)	A03.01	CLSFLSSWDY	890.5	2705.8	not measured
C15ORF40(+l)	A24.02	GFKLFSCLSF	387.4	643.0	not measured
C15ORF40(+l)	A24.02	RMPPCLANF	34.4	8.7	not measured
C15ORF40(+l)	A03.01	CLANFCIFNR	575.4	221.8	not measured
C15ORF40(+l)	A01.01	CLSFLSSWDY	538.7	987.3	not measured
CNOTl(+l)	A02.01	SVCFFFFSV	27.0	175.0	9.4
CNOTl(+l)	B08.01	SVCFFFFSV	4940.0	10599.0	0
CNOTl(+l)	A02.01	MSVCFFFFSV	131.0	1706.4	not measured
CNOTl(+l)	A02.01	FFFSVIFST	608.9	4556.0	not measured
CNOTl(-l)	A01.01	MSVCFFFFCY	310.4	4369.3	not measured

CNOTl(-l)	A02.01	SVCFFFFCYI	237.2	519.8	not measured
CNOTl(-l)	A24.02	FFCYILNTMF	583.4	73.4	not measured
EGFR, T790M	A02.01	MQLMPFGCLL	21.0	20.0	0.4
EGFR, T790M	A02.01	MQLMPFGCL	842.0	166.0	0.4
EGFR, T790M	A02.01	LIMQLMPFGC	1984.0	177.0	0.4
EGFR, T790M	A02.01	QLIMQLMPF	2511.0	227.0	0.3
EGFR, T790M	B08.01	QLIMQLMPF	891.0	388.0	0
EGFR, T790M	B08.01	IMQLMPFGCL	1302.0	548.0	0
EGFR, T790M	A02.01	CLTSTVQLIM	3465.0	716.0	0
EGFR, T790M	A02.01	IMQLMPFGCL	143.0	837.0	0.5
EGFR, T790M	A02.01	IMQLMPFGC	1123.0	1607.0	0.4
EGFR, T790M	B07.02	MQLMPFGCL	10169.0	2270.0	0
EGFR, T790M	A24.02	QLIMQLMPF	3209.0	2389.0	0.4
EGFR, T790M	A02.01	LIMQLMPFG	4961.0	3513.0	0
EGFR, T790M	A24.02	VQLIMQLMPF	1455.0	4559.0	0
EGFR, T790M	A02.01	VQLIMQLMPF	4464.0	5492.0	0
EGFR, T790M	A02.01	QLIMQLMPFG	5751.0	5926.0	0
EGFR, T790M	B08.01	MQLMPFGCL	1105.0	7045.0	0
EGFR, T790M	A02.01	STVQLIMQL	2151.0	8537.0	0
EGFR, T790M	A01.01	CLTSTVQLIM	2998.0	11036.0	0
EGFR, T790M	B08.01	MQLMPFGCLL	970.0	14056.0	0
EGFR, T790M	B08.01	VQLIMQLMPF	3370.0	17898.0	0
EGFR, T790M	A24.02	IMQLMPFGCL	4394.0	18102.0	0
EGFR, T790M	A24.02	MQLMPFGCLL	4168.0	23572.0	0
EGFR, T790M	A01.01	LTSTVQLIM	1000.7	2891.1	not measured
EGFR: SEPT 14	B08.01	QLQDKFEHL	917.0	989.0	0
EGFR: SEPT 14	A02.01	QLQDKFEHL	422.0	1155.0	0.6
EGFR: SEPT 14	A02.01	YLVIQLQDKF	9963.0	2057.0	0
EGFR: SEPT 14	A24.02	YLVIQLQDKF	9508.0	2152.0	2.6
EGFR: SEPT 14	A02.01	IQLQDKFEHL	820.0	4265.0	0.2
EGFR: SEPT 14	B08.01	IQLQDKFEHL	4278.0	10247.0	0
EGFRvIII	A02.01	ALEEKKGNYV	2445.0	141.0	0

(internal deletion)					
EIF2B3(- 1)	A02.01	KQWSSVTSL	54.4	26.5	not measured
EML4:ALK	B08.0 1	QVYRRKHQEL	194.0	160.0	0
EPHB2(- 1)	A02.0 1	ILIRKAMTV	38.2	19.5	not measured
ESR1, D538G	A24.02	PLYGLLLEML	15 19.0	444.0	6.3
ESR1, D538G	A02.0 1	GLLLEMLDA	705 .0	558.0	0.4
ESR1, D538G	A02.0 1	PLYGLLLEM	349.0	640.0	0.7
ESR1, D538G	A24.02	VVPLYGLLL	2965.0	658.0	0.8
ESR1, D538G	A02.0 1	PLYGLLLEML	542.0	797.0	0
ESR1, D538G	A02.0 1	VVPLYGLLL	4432.0	1039.0	0.6
ESR1, D538G	A02.01	NVVPLYGLL	4835 .0	1047 1.0	0
ESR1, D538G	B07.02	VPLYGLLLEM	145 .1	27.9	not measured
ESR1, D538G	A24.02	LYGLLLEML	2 18.3	0.8	not measured
ESR1, S463P	A02.01	FLPSTLKSL	7 1.0	2 1.0	2 .1
ESR1, S463P	A02.0 1	GVYTFLPST	307.0	779.0	1.3
ESR1, S463P	A24.02	FLPSTLKSL	10723 .0	995 .0	1
ESR1, S463P	A02.0 1	GVYTFLPSTL	248.0	1197.0	0.4
ESR1, S463P	B08.0 1	FLPSTLKSL	23 14.0	1968.0	0
ESR1, S463P	A24.02	GVYTFLPSTL	954.0	7696.0	0
ESR1, Y537C	A02.0 1	PLCDLLLEM	1067.0	602.0	0.8
ESR1, Y537C	A02.0 1	VVPLCDLLL	5533 .0	1200.0	0
ESR1, Y537C	A02.0 1	NVVPLCDLLL	1964.0	1373.0	0
ESR1, Y537C	A02.0 1	PLCDLLLEML	1320.0	2008.0	0.9
ESR1, Y537C	A02.0 1	NVVPLCDLL	3473 .0	3027.0	0
ESR1, Y537C	A24.02	VVPLCDLLL	7992.0	3888.0	0.4
ESR1, Y537N	A02.0 1	PLNDLLLEM	1062.0	15 1.0	4.2
ESR1, Y537N	A02.01	NVVPLNDLL	4725 .0	2900.0	0
ESR1, Y537N	A02.01	NVVPLNDLLL	2606.0	4 190.0	0
ESR1, Y537N	A02.0 1	PLNDLLLEML	174 1.0	11957.0	0
ESR1, Y537S	A02.0 1	PLSDLLLEM	7 13 .0	404.0	2.7
ESR1, Y537S	A02.0 1	NVVPLSDLLL	25 10.0	74 1.0	0

ESR1, Y537S	A02.01	NVVPLSDLL	4259.0	916.0	0
ESR1, Y537S	A02.01	VVPLSDLLL	8320.0	2551.0	0
ESR1, Y537S	A02.01	PLSDLLLEML	1138.0	6469.0	0
ESR1, Y537S	A24.02	VVPLSDLLL	8463.0	8252.0	0.5
FAMlllB(-l)	A03.01	RMKVPLMK	58.9	33.0	not measured
FGFR3, S249C	A02.01	YTLDVLERC	3309.0	1764.0	6.6
FGFR3, S249C	B08.01	VLERCPHRPI	3629.0	7223.0	0
FGFR3, S249C	A02.01	VLERCPHRPI	4505.0	15321.0	0
FGFR3:TACC3	A02.01	VLTVTSTDV	1255.0	295.0	1.1
FRG1B, HOT	B07.02	KLSDSRTAL	225.0	9.0	6.8
FRG1B, HOT	A02.01	KLSDSRTAL	275.0	111.0	2.8
FRG1B, HOT	B08.01	KLSDSRTAL	3276.0	122.0	0
FRG1B, L52S	A02.01	ALSASNSCFI	327.0	226.0	0.8
FRG1B, L52S	B08.01	FQNGKMALSA	7796.0	425.0	0
FRG1B, L52S	B07.02	ALSASNSCF	13989.0	684.0	0
FRG1B, L52S	A02.01	ALSASNSCF	7913.0	728.0	0.3
FRG1B, L52S	A02.01	FQNGKMALS	2305.0	3276.0	0
FRG1B, L52S	A02.01	FQNGKMALSA	1205.0	6158.0	0
FRG1B, L52S	A24.02	ALSASNSCF	9672.0	16338.0	0.2
GATA3 ...CSNH	B08.01	FLKAESKIM	263.4	21.9	not measured
GATA3 ...CSNH	B08.01	LQHGHRHGL	693.0	550.4	not measured
GATA3 ...CSNH	B07.02	EPHLALQPL	106.6	17.0	not measured
GATA3 ...CSNH	B07.02	RPLQTHVLPE	968.0	2534.4	not measured
GATA3 ...CSNH	B08.01	FATLQRSSL	138.0	26.6	not measured
GATA3 ...CSNH	B07.02	MFATLQRSSL	1285.0	266.9	not measured
GATA3 ...CSNH	A24.02	MFLKAESKI	1065.7	332.1	not measured
GATA3 ...CSNH	B07.02	FATLQRSSL	261.9	14.0	not measured

GATA3 ...CSNH	A02.01	MLTGPPARV	145.4	10.6	not measured
GATA3 ...CSNH	B08.01	EPHLALQPL	1128.3	12.4	not measured
GATA3 ...CSNH	B07.02	GPPARVPAV	297.6	221.2	not measured
GATA3 ...CSNH	B08.01	MFATLQRSSL	220.5	53.4	not measured
GATA3 ...CSNH	A02.01	ALQPLQPHA	644.4	603.9	not measured
GATA3 ...CSNH	A03.01	VLWTTPPLQH	962.3	16.0	not measured
GATA3 ...CSNH	A02.01	VLPEPHLAL	140.7	16.0	not measured
GATA3 ...CSNH	B07.02	HVLPEPHLAL	1057.2	1332.6	not measured
GATA3 ...CSNH	A03.01	YMFLKAESK	53.1	79.8	not measured
GATA3 ...CSNH	B07.02	VPAVPFDLHF	1996.2	2114.2	not measured
GATA3 ...CSNH	A02.01	AIQPVLWTT	229.3	8.1	not measured
GATA3 ...CSNH	A02.01	TLQRSSLWCL	319.2	117.7	not measured
GATA3 ...CSNH	A03.01	KIMFATLQR	62.5	2.5	not measured
GATA3 ...CSNH	B07.02	QPVLWTTPPL	54.4	109.3	not measured
GATA3 ...CSNH	B08.01	ESKIMFATL	253.7	17.7	not measured
GATA3 ...CSNH	B08.01	IMKPKRDGYM	342.1	33.2	not measured
GATA3 ...CSNH	B07.02	KPKRDGYMF	109.7	28.2	not measured
GATA3 ...CSNH	B08.01	FLKAESKIMF	1539.9	82.3	not measured

GATA3 …CSNH	B07.02	KPKRDGYMFL	32.5	98. 1	not measured
GATA3 …CSNH	B08.0 1	LHFCRSSIM	2 14 1.2	118.7	not measured
GATA3 CSNH	A02.0 1	SMLTGPPARV	57.0	15 .0	2 1.7
GATA3 CSNH	B08.0 1	YMFLKAESKI	606.0	32.0	0.4
GATA3 CSNH	A02.0 1	YMFLKAESKI	163 .0	166.0	0.6
GATA3 CSNH	A03 .0 1	YMFLKAESKI	1338.0	2 1111.0	0
GATA3 YHEA	A02.0 1	FLQEQYHEA	7.0	11.0	9.5
GATA3 YHEA	B08.0 1	FLQEQYHEA	1222.0	2285 .0	0
GBP3(-1)	B08.0 1	TLKKKPRDI	286.3	3.3	not measured
HER2, G776insYVMA	A02.0 1	VMAYVMAGV	6.0	2.0	16.5
HER2, G776insYVMA	A02.0 1	YVMAYVMAGV	5.0	57.0	20.9
HER2, G776insYVMA	B07.02	YVMAYVMAG	69 10.0	170.0	0
HER2, G776insYVMA	B08.01	YVMAYVMAGV	72 1.0	353 .0	0
HER2, G776insYVMA	A02.0 1	YVMAYVMAG	84 1.0	11535 .0	2.5
HER2, G776insYVMA	B08.0 1	YVMAYVMAG	836.0	194 13 .0	1.2
HER2, G776insYVMA	B07.02	YVMAYVMAGV	11445 .0	52630.0	0
HER2, L755S	A03.0 1	KVSRENTSPK	66.0	7.0	13 .5
HER2, V777L	A02.0 1	VMAGLGSPYV	20.0	102.0	2.9
HER2, V777L	A03 .0 1	VMAGLGSPYV	395 1.0	11222.0	0
JAKl(- l)	A02.0 1	SLMPAHWSI	4.0	48.0	5
JAKl(-l)	A02.0 1	LSLMPAHWSI	2 1.0	164.0	0.4
JAKl(- l)	B08.0 1	SLMPAHWSI	282.0	177.0	0
JAKl(-l)	A02.0 1	FQMQPLSLM	33 .0	553 .0	0.3
JAKl(- l)	A24.02	SLMPAHWSI	194.0	633 .0	0.4
JAKl(- l)	B08.0 1	LSLMPAHWSI	19 14.0	860.0	0
JAKl(- l)	B07.02	SLMPAHWSI	3907.0	1040.0	0

JAKl(- l)	B08.0 1	FQMQPLSLM	226 1.0	6714.0	0
JAKl(- l)	B07.02	FQMQPLSLM	3458.0	10207.0	0
JAKl(- l)	A24.02	LSLMPAHWSI	2 125.0	12398.0	0
JAKl(- l)	A24.02	FQMQPLSLM	402 1.0	146 12.0	0
KIT, T670I	A02.0 1	VIIEYCCYG	4225 .0	19 1.0	0.5
KIT, T670I	A02.0 1	IIEYCCYGDL	39 18.0	73 10.0	0
KIT, T670I	A02.0 1	TIGGPTLVII	5425 .0	10685 .0	0
KIT, V654A	A02.0 1	YLGNHMNIA	92.0	117.0	0.6
KIT, V654A	A02.0 1	MNIANLLGA	4522.0	128.0	0.3
KIT, V654A	A02.0 1	HMNIANLLGA	294.0	430.0	0
KIT, V654A	B08.0 1	YLGNHMNIA	2480.0	872.0	0
KIT, V654A	A02.0 1	YLGNHMNIAN	7 103 .0	1342.0	0
KIT, V654A	A02.0 1	IANLLGACTI	112 14.0	64 17.0	0
KRAS, G12C	A02.0 1	KLVVVGACGV	204.0	150.0	1
KRAS, G12C	A02.0 1	LVVVGACGV	658.0	12 13.0	0.6
KRAS, G12C	A03 .0 1	VVVGACGVGK	300.7	1.6	not measured
KRAS, G12C	A03 .0 1	VVGACGVGK	182.0	4 .1	not measured
KRAS, G12D	A02.0 1	KLVVVGADGV	36 1.0	184.0	0.9
KRAS, G12D	A02.0 1	LVVVGADGV	2 120.0	1192.0	0
KRAS, G12V	A02.0 1	KLVVVGAVGV	163 .0	96.0	0.9
KRAS, G12V	A02.0 1	LVVVGAVGV	453 .0	975 .0	0.6
KRAS, G12V	A03 .0 1	VVGAVGVGK	168.9	1.9	not measured
KRAS, Q6 1H	A0 1.0 1	ILDTAGHEEY	13 1.8	64.2	not measured
KRAS, Q6 1L	A0 1.0 1	ILDTAGLEEY	65 .9	8.6	not measured
KRAS, Q6 1L	A02.0 1	LLDILDTAGL	113 .4	7 15 .7	not measured
LMAN1(+1)	B07.02	GPPRPPRAAC	69.3	48.6	not measured
LMAN1(+1)	B07.02	PPRPPRAAC	263 .7	32.8	not measured
LMANl(-l)	B08.0 1	SLRRKYLRV	28.0	0.4	not

					measured
MEK, cms	A02.01	VLHESNSPYI	189.0	131.0	1.9
MEK, P124L	A02.01	VLHECNSLYI	67.0	10.0	5.1
MEK, P124L	A02.01	SLYIVGFYGA	104.0	390.0	0.4
MEK, P124L	A02.01	SLYIVGFYG	2987.0	1063.0	0
MEK, P124L	A02.01	LQVLHECNSL	5803.0	4723.0	0
MEK, P124L	A02.01	QVLHECNSL	8695.0	7774.0	0.5
MEK, P124L	A03.01	VLHECNSLYI	4733.0	10500.0	0
MEK, P124L	B08.01	QVLHECNSL	6854.0	14532.0	0
MEK, P124L	B08.01	LQVLHECNSL	2782.0	19316.0	0
MLL2, ...LSPH	A02.01	LLQVTQTSFA	1935.0	676.9	not measured
MLL2, ...LSPH	A02.01	RLWHLLLQV	8.3	1.3	not measured
MLL2, ...LSPH	A02.01	LQVTQTSFAL	1147.4	718.3	not measured
MLL2, ...LSPH	A02.01	RLWHLLLQVT	140.8	50.9	not measured
MLL2, ...LSPH	A02.01	ALAPTLTHM	98.4	59.0	not measured
MLL2, ...LSPH	A02.01	ALAPTLTHML	66.4	39.0	not measured
MLL2, CRLS	B08.01	SLGNHLCPL	136.0	6.0	0.5
MLL2, CRLS	A02.01	SLGNHLCPL	28.0	18.0	3.5
MLL2, CRLS	B07.02	SLGNHLCPL	3967.0	2590.0	0
MSH3(-1)	A02.01	LLALWECSL	46.0	15.0	4
MSH3(-1)	A02.01	FLLALWECSL	17.0	114.0	10.8
MSH3(-1)	B08.01	LLALWECSL	1454.0	154.0	0
MSH3(-1)	B08.01	FLLALWECSL	671.0	13100.0	0
MSH3(-1)	A02.01	LIVSRTLLL	755.0	173.5	not measured
MSH3(-1)	A02.01	LIVSRTLLLV	146.6	10920.6	not measured
MSH3(-1)	B08.01	LIVSRTLLL	270.7	881.7	not measured
MSH3(-1)	A02.01	IVSRTLLLV	166.2	12.7	not

					measured
MSH3(-1)	B08.01	SLPQARLCLI	632.3	4313.9	not measured
MSH3(-1)	B08.01	CLIVSRTLLL	835.7	1100.4	not measured
MSH3(-1)	B08.01	LPQARLCLI	136.5	15.0	not measured
MSH3(-1)	A02.01	SLPQARLCLI	782.4	112.9	not measured
MSH3(-1)	A02.01	CLIVSRTLLL	560.5	2005.1	not measured
MSH3(-1)	A02.01	FLLALWECS	686.6	93.2	not measured
MSH3(-1)	A02.01	FLLALWECSL	16.6	0.9	not measured
MSH3(-1)	A02.01	LLALWECSL	46.1	12.5	not measured
MSH3(-1)	B07.02	LPQARLCLI	134.6	72.6	not measured
MSH3(-1)	B08.01	CLIVSRTLL	915.0	126.7	not measured
MSH3(-1)	A02.01	ALWECSLPQA	24.6	9.0	not measured
MSH3(-1)	B08.01	LPQARLCLIV	591.4	152.1	not measured
MSH6(+1)	A02.01	LLPEDTPPL	8.9	2.8	not measured
MSH6(+1)	A02.01	ILLPEDTPPL	16.3	6.7	not measured
MYC, E39D	A02.01	QQSDLQPPA	4930.0	70.0	0
MYC, E39D	A02.01	QQQSDLQPPA	11835.0	646.0	0
MYC, E39D	A02.01	YQQQQQSDL	8842.0	799.0	0.4
MYC, E39D	B08.01	YQQQQQSDL	5259.0	18868.0	0
MYC, P57S	A02.01	FELLSTPPL	2509.0	225.0	0
MYC, P57S	A02.01	LLSTPPLSPS	5226.0	1770.0	0
MYC, P57S	B08.01	FELLSTPPL	4208.0	3179.0	0

MYC, T58I	A02.0 1	LLPIPPLSPS	207 1.0	449.0	0
MYC, T58I	A02.0 1	FELLPIPPL	2472.0	553.0	0
NAB:STAT6 ("variant 1" of Chmielecki et al.)	A02.0 1	SQIMSLWGL	14.0	62.0	1
NAB:STAT6 ("variant 1" of Chmielecki et al.)	A02.0 1	IMSLWGLVS	3630.0	732 1.0	0
NAB:STAT6 ("variant 1" of Chmielecki et al.)	A24.02	SQIMSLWGL	1604.0	85 16.0	0
NAB:STAT6 ("variant 1" of Chmielecki et al.)	B08.0 1	SQIMSLWGL	4587.0	15997.0	0
NDRG1 :ERG	A02.0 1	LLQEFDVQEA	200.0	45.0	2.5
NDRG1 :ERG	A02.0 1	LQEFDVQEAL	2229.0	50280.0	0
NDUFC2(-KCDT14)(+1)	A02.0 1	ITAFFFCWI	437.7	7490.4	not measured
NDUFC2(-KCDT14)(+1)	A24.02	LYITAFFFCW	46.6	45.3	not measured
NDUFC2(-KCDT14)(+1)	A03 .0 1	FFFCWILSCK	325 .9	597. 1	not measured
NDUFC2(-KCDT14X+1)	A03.0 1	FFCWILSCK	985 .7	184.9	not measured
NDUFC2(-KCDT14)(-1)	A02.0 1	LLYITAFFL	24.0	7 13 .0	17
NDUFC2(-KCDT14)(-1)	B08.01	LLYITAFFL	3588.0	9592.0	0
NDUFC2(-KCDT14)(-1)	A02.0 1	ITAFFLLDI	699.0	78.7	not measured
NDUFC2(-KCDT14)(-1)	A02.0 1	YITAFFLLDI	157.0	64.5	not measured
NDUFC2(-KCDT14X-1)	A24.02	LYITAFFLL	15 .6	0 .1	not measured
NDUFC2(-KCDT14)(-1)	A02.0 1	LLYITAFFLL	43.7	323 .2	not measured

NDUFC2(-KCDT14)(-1)	A24.02	LLYITAFFLL	59.7	60.1	not measured
NDUFC2(-KCDT14)(-1)	A24.02	LYITAFFLLD	414.3	0.4	not measured
NPvAS, Q61K	A01.01	ILDTAGKEEY	272.6	14.3	not measured
NPvAS, Q61R	A01.01	ILDTAGREEY	255.8	7.0	not measured
PDGFRa, T674I	A02.01	IIIEYCFYG	693.0	16.0	1.2
PDGFRa, T674I	A02.01	YIIIEYCFYG	1529.0	113.0	0
PDGFRa, T674I	A02.01	IIEYCFYGDL	3049.0	1090.0	0
PIK3CA, E542K	A02.01	KITEQEKDFL	12548.0	1397.0	0
PIK3CA, E542K	A03.01	AISTRDPLSK	41.3	57.5	not measured
PTEN, R130Q	A02.01	QTGVMICAYL	3786.0	9760.0	0
RAC1, P29S	A02.01	FSGEYIPTV	21.0	3.0	6.8
RAC1, P29S	A02.01	AFSGEYIPTV	1008.0	781.0	0
RAC1, P29S	A01.01	TTNAFSGEY	23.0	4.4	not measured
RAC1, P29S	A01.01	YTTNAFSGEY	20.0	10.5	not measured
RBM27(+1)	B07.02	MPKDVNIQV	291.6	12.5	not measured
RBM27(+1)	A01.01	TGSNEVTTRY	343.9	15545.2	not measured
RBM27(+1)	A01.01	GSNEVTTRY	151.6	605.5	not measured
RNF43, RHTP	A02.01	TQLARFFPI	17.0	19.0	0
RNF43, RHTP	A24.02	TQLARFFPI	268.0	52.0	0
RNF43, RHTP	B08.01	TQLARFFPI	41.0	9150.0	0
SEC31A(-1)	A02.01	KLMLLRLNL	58.0	17.0	16.9
SEC31A(-1)	B08.01	KLMLLRLNL	421.0	29.0	0
SEC31A(-1)	B07.02	KLMLLRLNL	2969.0	133.0	1.5
SEC31A(-1)	A03.01	KLMLLRLNL	4664.0	210.0	0
SEC31A(-1)	B08.01	LLRLNLRKM	185.1	68.2	not measured

SEC3 1A(-1)	A03.01	MLLRLNLRKM	171.4	116.9	not measured
SEC3 1A(-1)	A03.01	KLMLLRLNLR	95.4	48.4	not measured
SEC3 1A(-1)	A03.01	MLLRLNLRK	14.6	1.3	not measured
SEC3 1A(-1)	A03.01	LMLLRLNLRK	23.9	6.0	not measured
SEC3 1A(-1)	A02.01	MLLRLNLRKM	508.5	2507.4	not measured
SEC3 1A(-1)	B08.01	MLLRLNLRKM	565.9	95.3	not measured
SEC3 1A(-1)	A02.01	KLMLLRLNL	57.6	2.6	not measured
SEC3 1A(-1)	B08.01	LMLLRLNL	116.0	9.3	not measured
SEC3 1A(-1)	B08.01	KLMLLRLNL	420.6	58.4	not measured
SEC3 1A(-1)	A02.01	KKLMLLRLNL	275.4	288.9	not measured
SEC3 1A(-1)	B08.01	NLRKMCGPF	163.5	35.9	not measured
SEC3 1A(-1)	B08.01	YCQKKLMLL	203.1	222.1	not measured
SEC3 1A(-1)	B08.01	LNLRKMCGPF	782.2	438.7	not measured
SEC63(+1)	A03.01	YTCAITTVK	279.0	122.4	not measured
SEC63(+1)	A03.01	TYTCAITTVK	556.4	2362.2	not measured
SEC63(+1)	A03.01	ITTVKATETK	795.8	1245.3	not measured
SEC63(+1)	A03.01	KSKKKETFKK	744.0	39.6	not measured
SEC63(+1)	B08.01	TFKKKTYTC	648.2	77.9	not measured

SEC63(+1)	A03 .0 1	KSKKKETFK	4 11.0	74.3	not measured
SEC63(+1)	B08.0 1	FKKKTYTCAI	562.8	384.9	not measured
SEC63(- 1)	B08.01	TAKSKKRNL	2 13 .8	30.6	not measured
SF3B 1, K700E	A02.0 1	GLVDEQQEV	50.0	44.0	7.4
SLC35F5(-1)	A02.0 1	FALCGFWQI	10.5	0.4	not measured
SMAP l(-l)	A03.0 1	KSRQNHLQLK	88. 1	4.7	not measured
SMAP l(-l)	B07.02	KSRQNHLQL	329.5	78.0	not measured
SMAP l(-l)	A24.02	KLRSPLWIF	504.5	828.2	not measured
SMAP l(-l)	A03.0 1	KISNWSLKK	11.5	8.8	not measured
SMAP l(-l)	A 11.0 1	KISNWSLKK	15 .3	9.8	not measured
SMAP l(-l)	A 11.0 1	SLKKVPALK	117.6	129. 1	not measured
SMAP l(-l)	B08.0 1	SLKKVPAL	66.8	7.9	not measured
SMAP l(-l)	A03 .01	WSLKKVPALK	148.9	94.9	not measured
SMAP l(-l)	A03 .0 1	KISNWSLKKV	168.3	114.6	not measured
SMAP l(-l)	A03 .0 1	RKISNWSLKK	20.8	130.6	not measured
SMAP l(-l)	A03 .0 1	SLKKVPALK	29.6	4.4	not measured
SMAP l(-l)	B08.01	SQKSRQNHL	305 .0	44.6	not measured
SMAP l(-l)	B07.02	ALKKLRSPL	355 .5	223 .2	not measured
SMAP l(-l)	B08.01	ALKKLRSPL	58.9	0.5	not

					measured
SMAP l(-l)	B08.0 1	WSLKKVPAL	110.7	12.5	not measured
SMAP l(-l)	A03 .0 1	HLQLKSCRRK	2 16.7	96.9	not measured
SMAP l(-l)	B08.01	LKKLRSPL	139.6	0.6	not measured
SMAP l(-l)	A03 .0 1	SLKKVPALKK	43 .1	9.6	not measured
SPOP, F133L	A02.0 1	FVQGKDWGL	12 1.0	34.0	2 .1
SPOP, F133L	B08.0 1	FVQGKDWGL	140 1.0	207.0	0
TFAM(+1)	A03 .0 1	RVNTAWKTK	136.4	8.6	not measured
TFAM(+1)	A03 .0 1	RVNTAWKTKK	70.6	2.3	not measured
TFAM(+1)	B08.0 1	TKKKRVNTA	3 12.4	159.4	not measured
TFAM(+1)	A03 .0 1	KRVNTAWKTK	304. 1	33 1.6	not measured
TFAM(+1)	B08.0 1	WKTKKTSFSL	930.6	112.2	not measured
TFAM(+1)	B08.0 1	MTKKKRVNTA	534.2	186.9	not measured
TGFBPv2(-l)	A02.0 1	RLSSCVPVA	83 .0	4.0	18.7
TGFBPv2(-l)	A03.0 1	RLSSCVPVA	4264.0	439.0	0
TGFBPv2(-l)	A03 .0 1	AMTTSSSQK	48.5	8.3	not measured
TGFBPv2(-l)	A03.0 1	AMTTSSSQKN	887.2	2336.5	not measured
TGFBPv2(-l)	B08.0 1	IMKEKKSL	69.8	14. 1	not measured
TGFBPv2(-l)	A02.0 1	KSLVRLSSCV	903 .1	279.8	not measured
TGFBPv2(-l)	A02.0 1	SLVRLSSCV	177.3	29.9	not measured
TGFBPv2(-l)	A 11.0 1	SAMTTSSSQK	36.4	15 .8	not

					measured
TGFBR2(-1)	B08.01	IMKEKKSLV	80.8	16.8	not measured
TGFBR2(-1)	A11.01	AMTTSSSQK	89.9	161.6	not measured
TGFBR2(-1)	A03.01	SAMTTSSSQK	96.7	15.7	not measured
TGFBR2(-1)	A02.01	RLSSCVPVAL	84.5	54.2	not measured
TGFBR2(-1)	A02.01	VRLSSCVPVA	640.6	1206.8	not measured
TGFBR2(-1)	A02.01	RLSSCVPVA	82.7	49.5	not measured
TGFBR2(-1)	B08.01	CIMKEKKSL	218.5	7.5	not measured
TGFBR2(-1)	A02.01	ALMSAMTTS	320.4	139.1	not measured
TGFBR2(-1)	A02.01	LVRLSSCVPV	132.7	1237.6	not measured
THAP5(-1)	A03.01	KMRKKYAQK	23.7	5.7	not measured
TMPRSS2:ERG	A02.01	ALNSEALSV	66.0	14.0	9.1
TMPRSS2:ERG	A02.01	ALNSEALSVV	84.0	15.0	2.9
TMPRSS2:ERG	A02.01	MALNSEALSV	198.0	129.0	0.7
TMPRSS2:ERG	B08.01	MALNSEALSV	8512.0	13457.0	0
TP53, AAVG	A02.01	GLLAFWDSQV	57.0	10.0	14.2
TP53, AAVG	A02.01	LLAFWDSQV	13.0	68.0	12.8
TP53, AWAA	A02.01	WMTETLFDI	7.0	14.0	4
TP53, AWAA	A02.01	WMTETLFDIV	15.0	40.0	0.4
TP53, AWAA	A24.02	WMTETLFDI	4936.0	713.0	0
TP53, AWAA	A01.01	WMTETLFDIV	4046.0	14394.0	0
TP53, CSES	B07.02	LPSQRRNHWM	89.0	10.0	6.5
TP53, CSES	B08.01	LPSQRRNHWM	325.0	47.0	0.7
TP53, CSES	A02.01	ALSEHCPTT	208.0	79.0	27.3
TP53, G245S	B08.01	CMGSMNRRPI	1204.0	80.0	0
TP53, G245S	A02.01	YMCNSSCMGS	2485.0	81.0	0.8

TP53, G245S	B08.01	SMNRRPILTI	260.0	337.0	0
TP53, G245S	A02.01	SMNRRPILTI	1644.0	1198.0	0.3
TP53, G245S	B08.01	SMNRRPILT	2536.0	1282.0	0
TP53, G245S	A02.01	CMGSMNRRPI	7822.0	1989.0	0
TP53, G245S	A02.01	SMNRRPILT	7251.0	3839.0	0
TP53, G245S	A24.02	SMNRRPILTI	10308.0	16292.0	0
TP53, G245S	B08.01	GSMNRRPIL	636.7	15.5	not measured
TP53, G245S	B08.01	MGSMNRRPIL	89.1	6.3	not measured
TP53, G245S	B08.01	MGSMNRRPI	324.2	29.1	not measured
TP53, QPSL	B07.02	LPRKPTRAAT	47.0	3.0	3.7
TP53, QPSL	B07.02	LPRKPTRAA	8.0	8.0	5.5
TP53, QPSL	B07.02	KPTRAATVSV	12.0	8.0	3
TP53, QPSL	B08.01	LPRKPTRAA	873.0	1158.0	0
TP53, R248Q	B08.01	NQRPILTII	3433.0	20.0	0
TP53, R248Q	A02.01	GMNQRPILTI	1787.0	709.0	0.4
TP53, R248Q	A02.01	GMNQRPILT	8115.0	3029.0	0
TP53, R248Q	A02.01	CMGGMNQRPI	3025.0	3673.0	0
TP53, R248Q	A02.01	NQRPILTII	10855.0	9606.0	0
TP53, R248Q	B08.01	CMGGMNQRPI	6364.0	18766.0	0
TP53, R248Q	B08.01	GMNQRPILTI	3266.0	29251.0	0
TP53, R248W	B08.01	MNWRPILTI	6447.0	1.0	0
TP53, R248W	A02.01	GMNWRPILTI	189.0	282.0	0.5
TP53, R248W	A02.01	CMGGMNWRPI	354.0	346.0	0.4
TP53, R248W	A02.01	MNWRPILTI	5834.0	516.0	3.8
TP53, R248W	A02.01	MNWRPILTII	8158.0	1026.0	0.4
TP53, R248W	B08.01	GMNWRPILTI	3990.0	1045.0	0
TP53, R248W	A02.01	GMNWRPILT	3416.0	1130.0	0
TP53, R248W	B08.01	CMGGMNWRPI	3218.0	2248.0	0
TP53, R248W	A24.02	CMGGMNWRPI	9521.0	4453.0	0
TP53, R248W	A24.02	MNWRPILTI	3634.0	6977.0	0.2
TP53, R248W	A24.02	MNWRPILTII	1517.0	44901.0	0
TP53, R273C	A02.01	LLGRNSFEVC	1272.0	2081.0	0

TP53, R273C	A02.01	NSFEVCVCA	4239.0	2200.0	0
TP53, R273H	A02.01	NSFEVHVCA	6768.0	503.0	0
TP53, SHST	B07.02	HPRPAPASA	13.0	11.0	4.9
TP53, SHST	B08.01	HPRPAPASA	1718.0	25.0	0
TP53, Y220C	A02.01	VVPCEPPEV	1268.0	187.0	0.9
TTK(-l)	A02.01	VMSDTTYKI	15.8	19.6	not measured
TTK(-l)	A03.01	LFVMSDTTYK	57.9	749.4	not measured
TTK(-l)	A02.01	FVMSDTTYKI	16.0	62.4	not measured
TTK(-l)	A03.01	FVMSDTTYK	63.1	66.9	not measured
TTK(-l)	A03.01	KTFEKKGEK	81.3	32.2	not measured
TTK(-l)	A01.01	VMSDTTYKIY	245.1	375.8	not measured
TTK(-l)	A01.01	MSDTTYKIY	18.9	10.2	not measured
UBR5(-1)	B07.02	RVQNQGHLL	429.1	826.5	not measured
VHL, QCIL	A02.01	MLTDSLFLPI	8.0	16.0	1
VHL, QCIL	A02.01	SMLTDSLFL	14.0	31.0	9.8
VHL, QCIL	B08.01	MLTDSLFLPI	2581.0	110.0	0
VHL, QCIL	A01.01	MLTDSLFLPI	429.0	7673.0	0
XPOT(-l)	A02.01	YLTKWPKFFL	10.7	42.9	not measured

CLAIMS

WHAT IS CLAIMED IS:

1. A method for identifying a subject-specific and tumor-specific polypeptide sequence or polynucleotide sequence encoding the subject-specific and tumor-specific polypeptide sequence for preparing an immunogenic compositon, the method comprising:
   (a) contacting an antigen presenting cell (APC) of a plurality of APCs with a T cell of a plurality of T cells,
   (b) forming one or more APC:T cell conjugates, wherein the APC expresses a target polypeptide encoded by a target polynucleotide;
   (c) isolating an APCT cell conjugate from non-conjugated APCs, non-conjugated T cells, or both; and
   (d) sequencing the target polynucleotide that encodes the target polypeptide from the APC of the APCT cell conjugate;
   wherein the target polypeptide comprises a neoantigen sequence encoded by a nucleic acid of a tumor cell sample of a subject and that is not encoded by a nucleic acid of a non-tumor cell sample of the subject.
2. A method comprising:
   (a) contacting an antigen presenting cell (APC) of a plurality of APCs with a T cell of a plurality of T cells,
   (b) forming one or more APCT cell conjugates, wherein the APC expresses a target polypeptide encoded by a target polynucleotide; and
   (c) isolating an APCT cell conjugate from non-conjugated APCs, non-conjugated T cells, or both.
3. The method of claim 1 or 2, wherein the method further comprises sequencing a target polynucleotide from the APC of the APCT cell conjugate that encodes the target polypeptide.
4. The method of any one of claim 1 or 3, wherein the method further comprises selecting a sequenced target polynucleotide that encodes an immunogenic target polypeptide.
5. The method of any one of claims 1-4, further comprising partitioning a first APCT cell conjugate of the one or more APCT cell conjugates into a first vessel and a second APCT cell conjugate of one or more APCT cell conjugates into a second vessel.
6. The method of claim 5, wherein the APC of the first APCT cell conjugate comprises a first APC polynucleotide and the APC of the second APCT cell conjugate comprises a second APC polynucleotide; and wherein the T cell of the first APCT cell conjugate comprises at least one first T cell polynucleotide and the T cell of the second APCT cell conjugate comprises at least one second T cell polynucleotide.
7. The method of any one of claims 1-6, wherein the method further comprises preparing an immunogenic compositon or a vaccine composition comprising (i) a peptide comprising a neoantigen sequence that is encoded by a target polynucleotide from the APC of the APCT cell conjugate or (ii) a polynucleic acid encoding a neoantigen sequence that is encoded by a target polynucleotide from the APC of the APCT cell conjugate.

8. The method of any one of claims 1-6, wherein the method further comprises preparing a therapeutic composition comprising (i) T cell receptor (TCR) or cell comprising a TCR, wherein the TCR is specific for a complex of an HLA protein expressed by the subject and a peptide with a neoantigen sequence that is encoded by a target polynucleotide from the APC of the APC:T cell conjugate or (ii) one or more APCs comprising a peptide with a neoantigen sequence that is encoded by a target polynucleotide from the APC of the APC:T cell conjugate.
9. The method of any one of claims 1-8, wherein the method comprises predicting target polypeptides that bind to a protein encoded by an HLA allele of the subject and/or predicting predicting target polypeptides that are presented by a protein encoded by an HLA allele of the subject
10. The method of claim 9, wherein predicting comprises predicting binding affinities of a plurality of candidate target polpeptide sequences to a protein encoded by an HLA allele expressed by the subject.
11. The method of claim 9 or 10, wherein predicting comprises using a program implemented on computer system.
12. The method of any one of claims 1-11, wherein the target polynucleotide is DNA.
13. The method of any one of claims 1-11, wherein the target polynucleotide is RNA.
14. The method of claim 13, wherein the target polynucleotide is mRNA.
15. The method of any one of claims 1-14, wherein the target polynucleotide is linear.
16. The method of any one of claims 1-15, wherein the target polypeptide comprises from 20 to 100 amino acids.
17. The method of any one of claims 1-16, wherein the target polypeptide or a fragment thereof is presented on an APC.
18. The method of any one of claims 4-17, wherein the immunogenic target polypeptide elicits and/or increases an immune response when administered to a subject with a disease or condition.
19. The method of any one of claims 4-18, wherein the method further comprises contacting the selected polynucleotide encoding the immunogenic target polypeptide or the immunogenic target polypeptide to second plurality of APCs.
20. The method of claim 19, wherein the method further comprises administering one or more APCs of the second plurality of APCs to a subject with a disease or condition.
21. The method of claim 19, wherein the method further comprises contacting a second plurality of T cells to the second plurality of APCs.
22. The method of claim 22, wherein the method further comprises administering one or more T cells of the second plurality of T cells to a subject with a disease or condition.
23. The method of any one of claims 1-21, wherein the plurality of APCs is from a subject, a cell line or engineered cells; and/or the plurality of T cells is from a subject, a cell line or engineered cells.
24. The method of any one of claims 1-23, wherein the plurality of T cells and the plurality of APCs are from the same subject.
25. The method of any one of claims 1-24, wherein the method is performed *ex vivo*.

26. The method of any one of claims 1-25, wherein the method further comprises enriching the target polynucleotide from cellular RNA from an APC of an APC:T cell conjugate.
27. The method of any one of claims 26, wherein enriching comprises transcribing or amplifying the target polynucleotide using a primer that hybridizes to a heterologous promoter region of the target polynucleotide.
28. The method of any one of claims 1-27, wherein the plurality of APCs and/or the plurality of T cells is from a subject with a disease or condition.
29. The method of any one of claims 1-28, wherein the target polypeptide comprises a neoantigen, a neoepitope, an immunogenic peptide sequence, an immunogenic epitope, a cancer peptide, a viral peptide, an infectious disease peptide, and/or a non-cancer disease-associated peptide.
30. The method of any one of claims 1-29, wherein the target polypeptide activates a T cell or stimulates a T cell to proliferate.
31. The method of any one of claims 1-30, wherein the plurality of APCs is a CRISPR-Cas engineered APC comprising the target polynucleotide.
32. The method of any one of claims 1-31, wherein the target polynucleotide further comprises a sequence encoding a reporter tag or wherein the target polypeptide is linked to a reporter tag.
33. The method of claim 32, wherein the method comprises detecting the reporter tag.
34. The method of claim 32 or 33, wherein the reporter tag comprises a fluorescent reporter tag.
35. The method of any one of claims 32-34, wherein the method comprises selectively isolating an APC:T cell conjugate comprising the reporter tag.
36. The method of any one of claims 1-35, wherein the plurality of APCs comprises an amplifier polynucleotide comprising a sequence encoding an amplifier polypeptide that transcribes the target polynucleotide.
37. The method of claim 36, wherein the amplifier polynucleotide is linear.
38. The method of claim 36 or 37, wherein the amplifier polynucleotide is an *in vitro* transcribed RNA.
39. The method of any one of claims 1-38, wherein the target polynucleotide is an *in vitro* transcribed RNA.
40. The method of claim 4-39, wherein selecting comprises selecting a target polynucleotide based on a frequency of the target polynucleotide or a barcode of a target polynucleotide in the plurality of APC:T cell conjugates.
41. The method of any one of claims 1-40, wherein the method comprises identifying a target polypeptide that interacts with a TCR of interest expressed by an engineered T cell.
42. The method of any one of claims 1-41, wherein the method further comprises
    (i) expressing a polypeptide from a selectively transcribed target RNA in a second antigen presenting cell (APC);
    (ii) contacting the second APC to a second T cell, thereby forming a second APC:T cell conjugate;
    (iii) isolating cellular RNA from the second APC of the second APC:T cell conjugate; and
    (iv) enriching for the target RNA from the cellular RNA.

43. The method of any one of claims 1-42, wherein the method comprises identifying a target RNA enriched from cellular RNA isolated from an APC of an APC:T cell conjugate to encode for a polypeptide that elicits an immune response when administered to a subject with a disease or condition.
44. The method of any one of claims 1-43, wherein the isolated APC:T cell conjugate is isolated by flow cytometry or magnetic beads.
45. The method of any one of claims 1-44, wherein the method further comprises reverse transcribing a target polynucleotide from the APC of an APC:T cell conjugate.
46. The method of any one of claims 1-45, wherein the method comprises transcribing or amplifying a target polynucleotide using a primer that hybridizes to a heterologous promoter region of the target polynucleotide.
47. The method of any one of claims 1-46, wherein the method comprises identifying a TCRthat specifically interacts with the target polypeptide and an HLA, or an identifying an antibody that specifically interacts with the target polypeptide.
48. An isolated, purified, antibody or TCR identified by the method of claim 47.
49. A method of treating a subject in need thereof, comprising administering the TCR of claim 48, a cell comprising thee TCR of claim 48, or an antibody of claim 48, or fragment thereof, to a subject in need thereof.
50. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the TCR of claim 48, a cell comprising the TCR of claim 48, or an antibody of claim 48, or fragment thereof, to a subject in need thereof.
51. A method of treating a subject with a disease or condition comprising administering to the subject a polypeptide comprising a sequence of an immunogenic target polypeptide identified according to the method of any one of claims 1-47, or a polynucleotide comprising a sequence encoding the immunogenic target polypeptide.
52. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a polypeptide comprising a sequence of an immunogenic target polypeptide identified according to the method of any one of claims 1-47, or a polynucleotide comprising a sequence encoding the immunogenic target polypeptide.
53. An isolated immunogenic target polypeptide identified according to the method of any one of claims 1-47, or a polynucleotide comprising a sequence encoding the immunogenic target polypeptide.
54. A method of treating a subject with a disease or condition comprising administering to the subject an APC comprising an immunogenic target polypeptide identified according to the method of any one of claims 1-47, or a polynucleotide comprising a sequence encoding the immunogenic target polypeptide.
55. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an APC comprising an immunogenic target polypeptide identified according to the method of any one of claims 1-47, or a polynucleotide comprising a sequence encoding the immunogenic target polypeptide.
56. A method comprising:

(a) contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a polypeptide encoded by a target polynucleotide that is an *in vitro* transcribed target RNA;

(b) isolating the APC:T cell conjugate from non-conjugated APCs, non-conjugated T cells, or both within at most about 12 hours after contacting the APC with the T cell.

57. A method comprising:

(a) contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a target polypeptide encoded by a target polynucleotide, wherein the target polynucleotide contains a region comprising a probe binding region and a reporter barcode region;

(b) binding the target polynucleotide with a probe and a reporter probe.

58. A method comprising:

(a) forming a plurality of vessels each comprising

(i) a single APC:T cell conjugate from a sample comprising a plurality of APC:T cell conjugates, and

(ii) a vessel barcoded polynucleotide comprising a vessel barcode sequence;

(b) producing:

(i) a complementary APC polynucleotide that is complementary to an APC polynucleotide from the APC of the APC:T cell conjugate,

(ii) a first complementary T cell polynucleotide that is complementary to a first T cell polynucleotide from the T cell of the APC:T cell conjugate, and

(iii) optionally, a second complementary T cell polynucleotide that is complementary to a second T cell polynucleotide from the T cell of the APC:T cell conjugate

wherein the complementary APC polynucleotide, the first complementary T cell polynucleotide and optionally, the second complementary T cell polynucleotide comprise the vessel barcode sequence or a complement thereof.

59. A composition comprising an APC:T cell conjugate isolated from non-conjugated APCs and T cells, wherein the APC of the APC:T cell conjugate comprises an *in vitro* transcribed target RNA, and wherein the *in vitro* transcribed target RNA comprises a sequence encoding a polypeptide that interacts with the T cell of the APC:T cell conjugate.

60. A composition comprising a plurality of vessels, each comprising

(a) a single APC:T cell conjugate from a sample comprising a plurality of APC:T cell conjugates,

(b) a vessel barcoded polynucleotide comprising a vessel barcode sequence;

(c) an APC polynucleotide from the APC of the single APC:T cell conjugate,

(d) a first T cell polynucleotide from the T cell of the single APC:T cell conjugate; and

(e) optionally, a second T cell polynucleotide from the T cell of the single APC:T cell conjugate

wherein the APC polynucleotide, the first T cell polynucleotide, and optionally, the second T cell polynucleotide each comprise the vessel barcode sequence or a complement thereof.

61. An antigen presenting cell (APC) comprising:
    (a) a first *in vitro* transcribed RNA comprising a sequence encoding a first polypeptide, wherein the first polypeptide amplifies a second *in vitro* transcribed RNA;
    (b) the second *in vitro* transcribed RNA, and
    (c) a second polypeptide expressed from the second *in vitro* transcribed RNA, wherein the second polypeptide or a fragment thereof interacts with a T cell when presented by the APC.
62. An antigen presenting cell (APC) comprising
    (a) a linear amplifier polynucleotide comprising a sequence encoding a first polypeptide that amplifies a linear target polynucleotide;
    (b) the linear target polynucleotide; and
    (c) a second polypeptide expressed from the linear target polynucleotide, wherein the second polypeptide or a fragment thereof interacts with a T cell when presented by the APC.
63. An antigen presenting cell (APC) comprising:
    (a) an amplifier polynucleotide comprising a sequence encoding a first polypeptide that amplifies a target polynucleotide, and
    (b) the target polynucleotide, wherein the target polynucleotide comprises a sequence encoding a second polypeptide comprising at most about 100 amino acids; and
    (c) the second polypeptide, wherein the second polypeptide is expressed from the target polynucleotide, wherein the second polypeptide or a fragment thereof interacts with a T cell when presented by the APC.
64. A method comprising:
    (a) contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a target polypeptide encoded by a target polynucleotide;
    (b) sequencing a target polynucleotide that encodes the target polypeptide from the APC of the APC:T cell conjugate; and
    (c) selecting a sequenced target polynucleotide that encodes an immunogenic target polypeptide.
65. A method comprising:
    (a) contacting a population of antigen presenting cells (APCs) with a population of T cells, thereby forming a plurality of APC:T cell conjugates each comprising a single APC and at least one T cell, wherein a plurality of APCs of the population of APCs express a target polypeptide encoded by a target polynucleotide sequence; and
    (b) partitioning a first APC:T cell conjugate of the plurality of APC:T cell conjugates into a first vessel and a second APC:T cell conjugate of the plurality of APC:T cell conjugates into a second vessel;

    wherein the APC of the first APC:T cell conjugate comprises a first APC polynucleotide and the APC of the second APC:T cell conjugate comprises a second APC polynucleotide; and

wherein the T cell of the first APC:T cell conjugate comprises at least one first T cell polynucleotide and the T cell of the second APC:T cell conjugate comprises at least one second T cell polynucleotide.

66. A method comprising
    (a) contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a polypeptide encoded by a target polynucleotide that is an *in vitro* transcribed target RNA; and
    (b) enriching the target polynucleotide from cellular nucleic acids of the APC of the APC:T cell conjugate.
67. A method comprising:
    (a) contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a target polypeptide encoded by a target polynucleotide; and
    (b) selectively enriching for the target polynucleotide from the APC of the APC:T cell conjugate, wherein the enriching comprises transcribing or amplifying the target polynucleotide using a primer that hybridizes to a heterologous promoter region of the target polynucleotide.
68. A method comprising:
    (a) contacting an antigen presenting cell (APC) with a T cell, thereby forming an APC:T cell conjugate, wherein the APC expresses a target polypeptide comprising at most about 100 amino acids encoded by a target polynucleotide, wherein the target polynucleotide is an *in vitro* transcribed target RNA, and wherein the APC and/or the T cell is from a subject with a disease or condition; and
    (b) identifying target RNA enriched from cellular RNA isolated from the APC of the APC:T cell conjugate to encode for an immunogenic polypeptide.
69. A method comprising:
    (a) contacting an antigen presenting cell (APC) with:
        (i) an amplifier polynucleotide that is an *in vitro* transcribed RNA comprising a sequence encoding an amplifier polypeptide that transcribes a target polynucleotide, and
        (ii) the target polynucleotide, wherein the target polynucleotide is an *in vitro* transcribed RNA comprising a sequence encoding a target polypeptide; and
    (b) expressing the target polypeptide in the APC.
70. A method comprising:
    (a) contacting an antigen presenting cell (APC) with:
        (i) an amplifier polynucleotide comprising a sequence encoding an amplifier polypeptide that transcribes a target polynucleotide, and
        (ii) the target polynucleotide which comprises a sequence encoding a target polypeptide comprising at most about 100 amino acids; and
    (b) presenting the target polypeptide or a fragment thereof on the APC.
71. A method comprising:
    (a) contacting an antigen presenting cell (APC) with:

(i) a linear amplifier polynucleotide comprising a sequence encoding an amplifier polypeptide that transcribes a linear target polynucleotide; and

(ii) the linear target polynucleotide,

(b) expressing a target polypeptide encoded by the linear target polynucleotide in the APC.

72. A therapeutic, immunogenic or vaccine composition comprising

(a) a peptide comprising a neoantigen sequence that is encoded by the target polynucleotide from the APC of the APC:T cell conjugate identifed according to the method of any one of claims 1-43 and 56-71;

(b) a polynucleotide encoding the peptide comprising a neoantigen sequence that is encoded by the target polynucleotide from the APC of the APC:T cell conjugate identifed according to the method of any one of claims 1-43 and 56-71;

(c) one or more APCs comprising a peptide comprising a neoantigen sequence that is encoded by the target polynucleotide from the APC of the APC:T cell conjugate identifed according to the method of any one of claims 1-43 and 56-71; or

(d) a T cell receptor (TCR) specific for a peptide in complex with an HLA protein, wherein the peptide comprises a neoantigen sequence that is encoded by the target polynucleotide from the APC of the APC:T cell conjugate identifed according to the method of any one of claims 1-43 and 56-71.

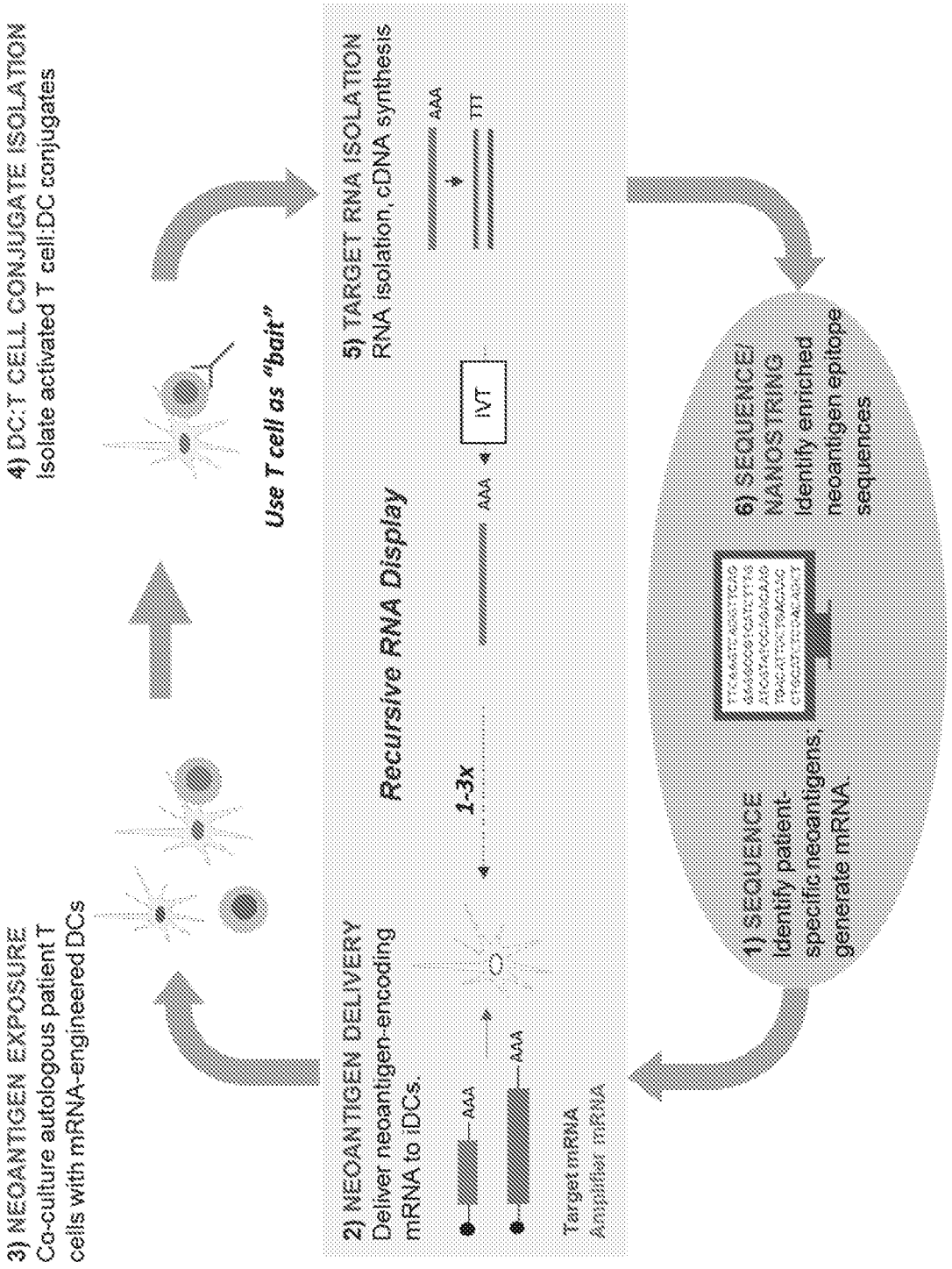
3) NEOANTIGEN EXPOSURE
Co-culture autologous patient T cells with mRNA-engineered DCs
4) DC:T CELL CONJUGATE ISOLATION
Isolate activated T cell:DC conjugates
Use T cell as "bait"
5) TARGET RNA ISOLATION
RNA isolation, cDNA synthesis
2) NEOANTIGEN DELIVERY
Deliver neoantigen-encoding mRNA to iDCs.
Recursive RNA Display
1-3x
IVT
AAA
TTT
Target mRNA
Amplified mRNA
1) SEQUENCE
Identify patient-specific neoantigens, generate mRNA
6) SEQUENCE/ NANOSTRING
Identify enriched neoantigen epitope sequences

FIG. 1

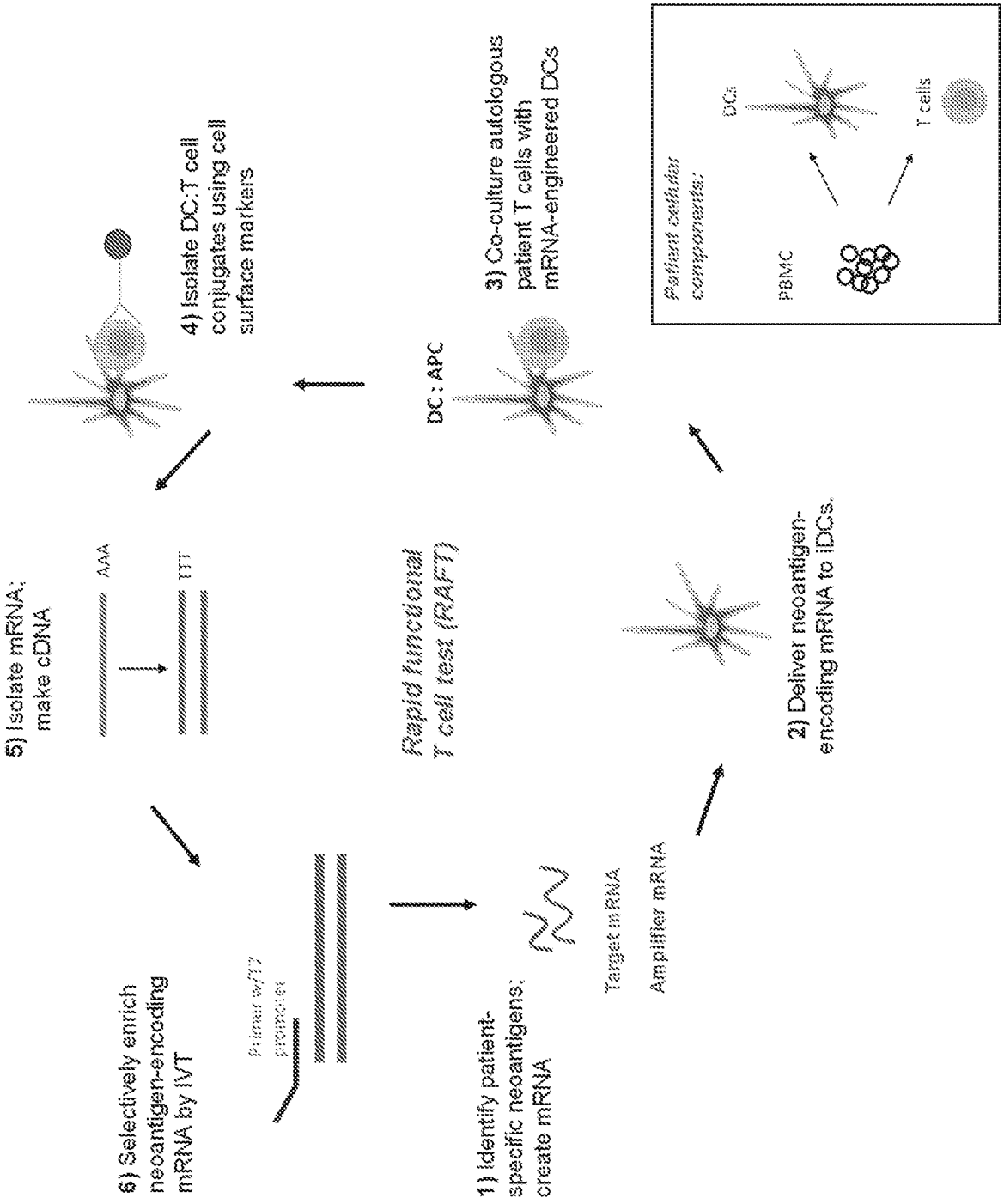
5) Isolate mRNA; make cDNA
AAA
TTT
4) Isolate DC:T cell conjugates using cell surface markers
6) Selectively enrich neoantigen-encoding mRNA by IVT
Primer w/T7 promoter
Rapid functional T cell test (RAFT)
DC : APC
3) Co-culture autologous patient T cells with mRNA-engineered DCs
Patient cellular components:
DCs
PBMC
T cells
1) Identify patient-specific neoantigens; create mRNA
Target mRNA
Amplified mRNA
2) Deliver neoantigen-encoding mRNA to iDCs.

FIG. 2

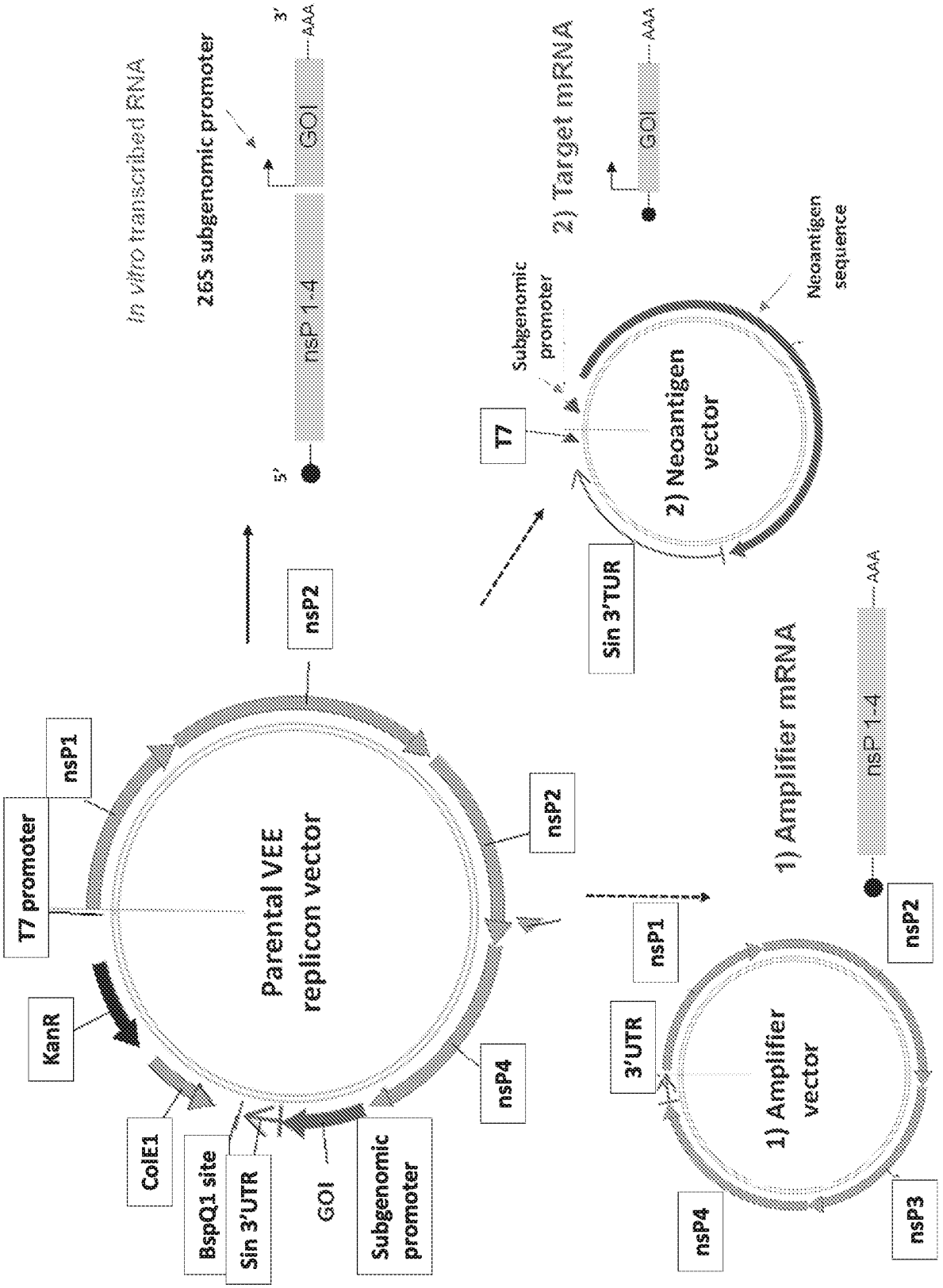
In vitro transcribed RNA
26S subgenomic promoter
5'
nsP 1-4
GOI
3'
AAA
nsP2
nsP1
T7 promoter
KanR
ColE1
BspQ1 site
Sin 3'UTR
GOI
Subgenomic promoter
Parental VEE replicon vector
nsP2
nsP4
2) Target mRNA
GOI
AAA
T7
Subgenomic promoter
2) Neoantigen vector
Neoantigen sequence
Sin 3'TUR
1) Amplifier mRNA
nsP 1-4
AAA
nsP2
nsP1
3'UTR
1) Amplifier vector
nsP4
nsP3

FIG. 3

Empty Target mRNA
5'
3'UTR
3'
AAA

FIG. 4A

Target mRNA assembly scheme:
Neoantigen
4 bp overlap
Oligo1
Oligo2
5'
3'UTR
3'
AAA
1
Pre-generated 5' sequence elements (dsDNA)
2
Synthesized neoantigen sequence with overlapping 5' and 3' vector sequences and Type IIS RE (ssDNA oligos)
3
Pre-generated 3' sequence elements (dsDNA)
Finished construct:
5'
Neoantigen
3'UTR
3'
AAA

FIG. 4B

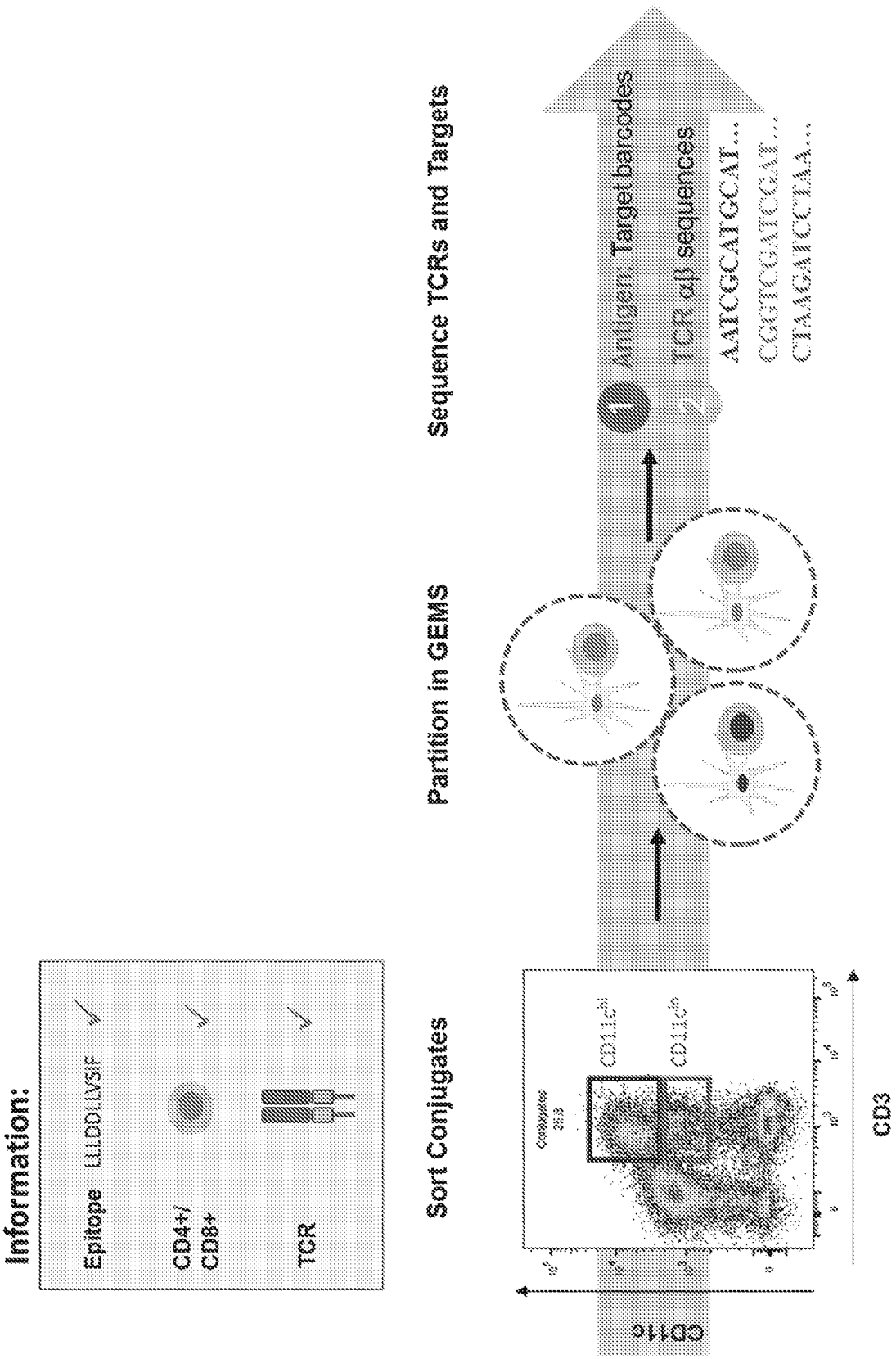
Information:
Epitope LLLDDLLVSIF
CD4+/ CD8+
TCR
Sort Conjugates
Partition in GEMS
Sequence TCRs and Targets
Antigen Target barcodes
TCR αβ sequences
AATCGCATGCAT...
CGGTCGATCGAT...
CTAAGATCCTAA...
CD11c
CD3
Conjugates 25.8
CD11c^hi
CD11c^lo

FIG. 5

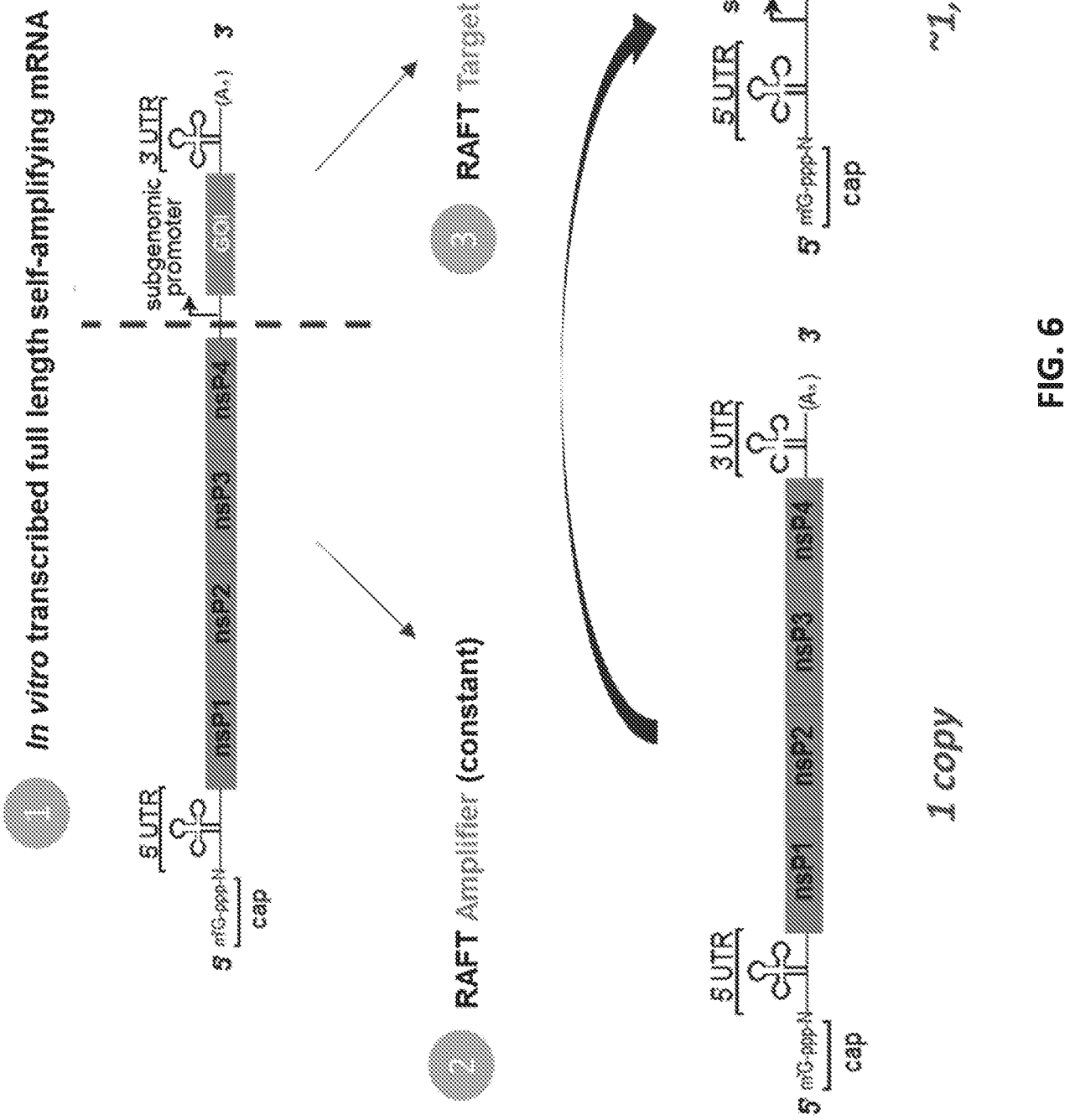
1
In vitro transcribed full length self-amplifying mRNA
5' UTR
mG-ppp-N
cap
nsP1
nsP2
nsP3
nsP4
subgenomic promoter
3' UTR
(A30)
3'
2
RAFT Amplifier (constant)
3
RAFT Target (epitope specific)
5' UTR
mG-ppp-N
cap
nsP1
nsP2
nsP3
nsP4
subgenomic promoter
3' UTR
(A30)
3'
1 copy
~1,000 copies

FIG. 6

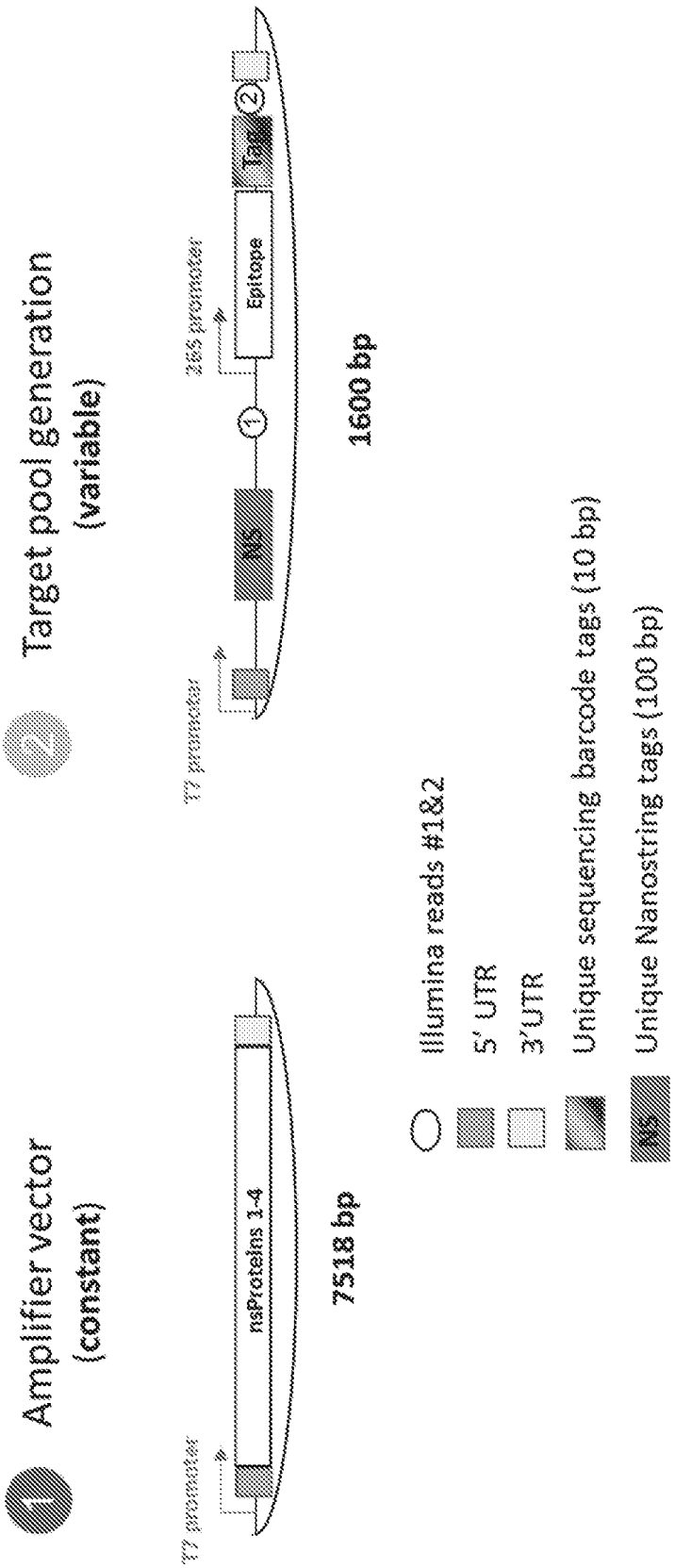
1
Amplifier vector
(constant)
T7 promoter
nsProteins 1-4
7518 bp
2
Target pool generation
(variable)
T7 promoter
NS
1
26S promoter
Epitope
Tag
2
1600 bp
Illumina reads #1&2
5' UTR
3'UTR
Unique sequencing barcode tags (10 bp)
NS
Unique Nanostring tags (100 bp)

FIG. 7

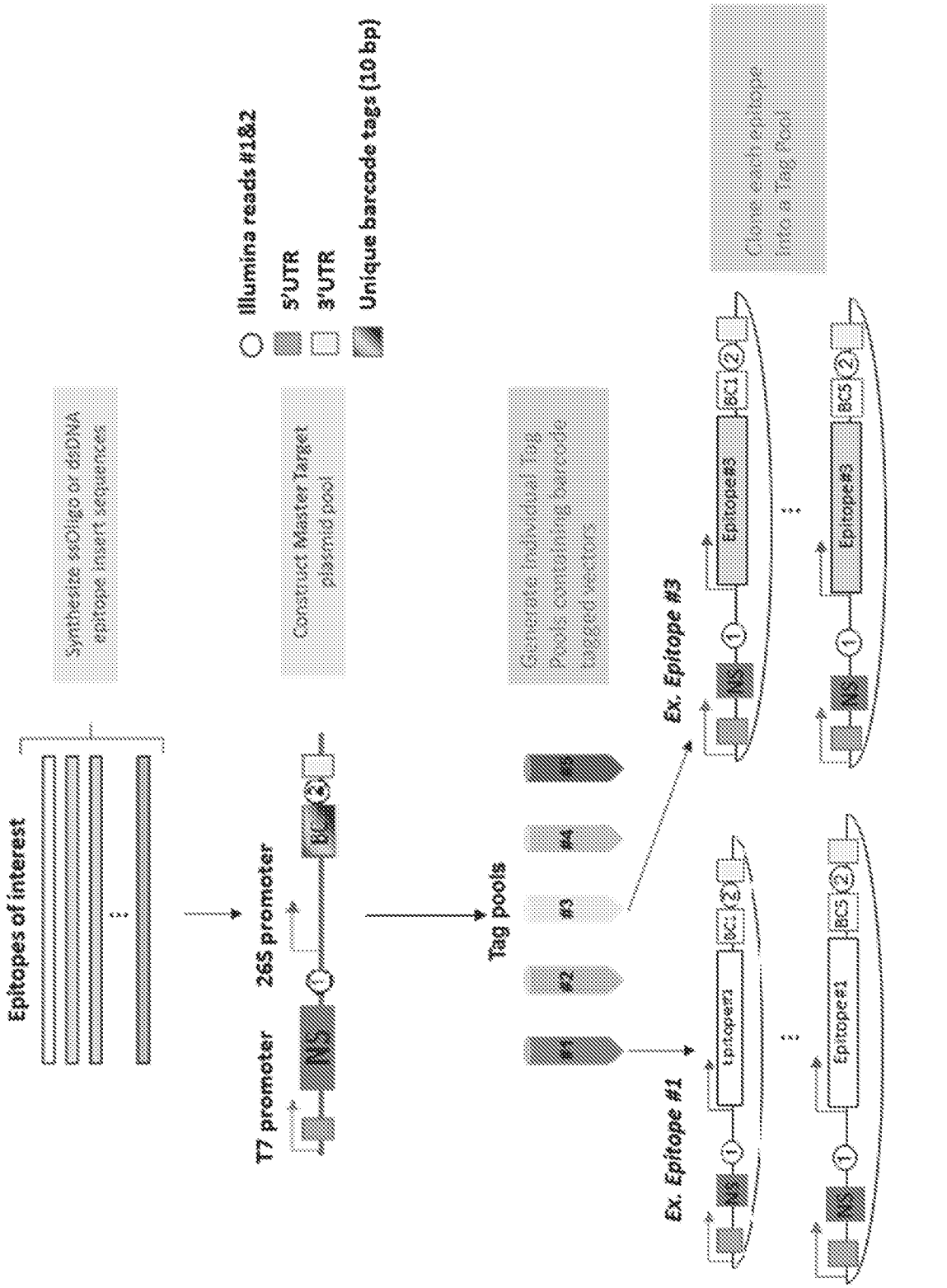
Epitopes of interest
Synthesize oligo or dsDNA epitope insert sequences
T7 promoter
26S promoter
Construct Master Target plasmid pool
Tag pools
Generate individual Tag Pools containing barcode labeled vectors
Ex. Epitope #1
Ex. Epitope #3
Epitope#1
Epitope#3
Illumina reads #1&2
5'UTR
3'UTR
Unique barcode tags (10 bp)

FIG. 8

1. Target epitope cloning (~1.5 week)
Prepared Target vector
Design epitope insert sequence with 5' and 3' end sequences homologous to vector → Assemble → transform, grow → sequence verify
2. In vitro transcription (0.5 day)
T7 promoter
In vitro transcription with T7 polymerase
3. In vitro Vaccinia capping (0.5 day)
In vitro vaccinia capping → QC

FIG. 9

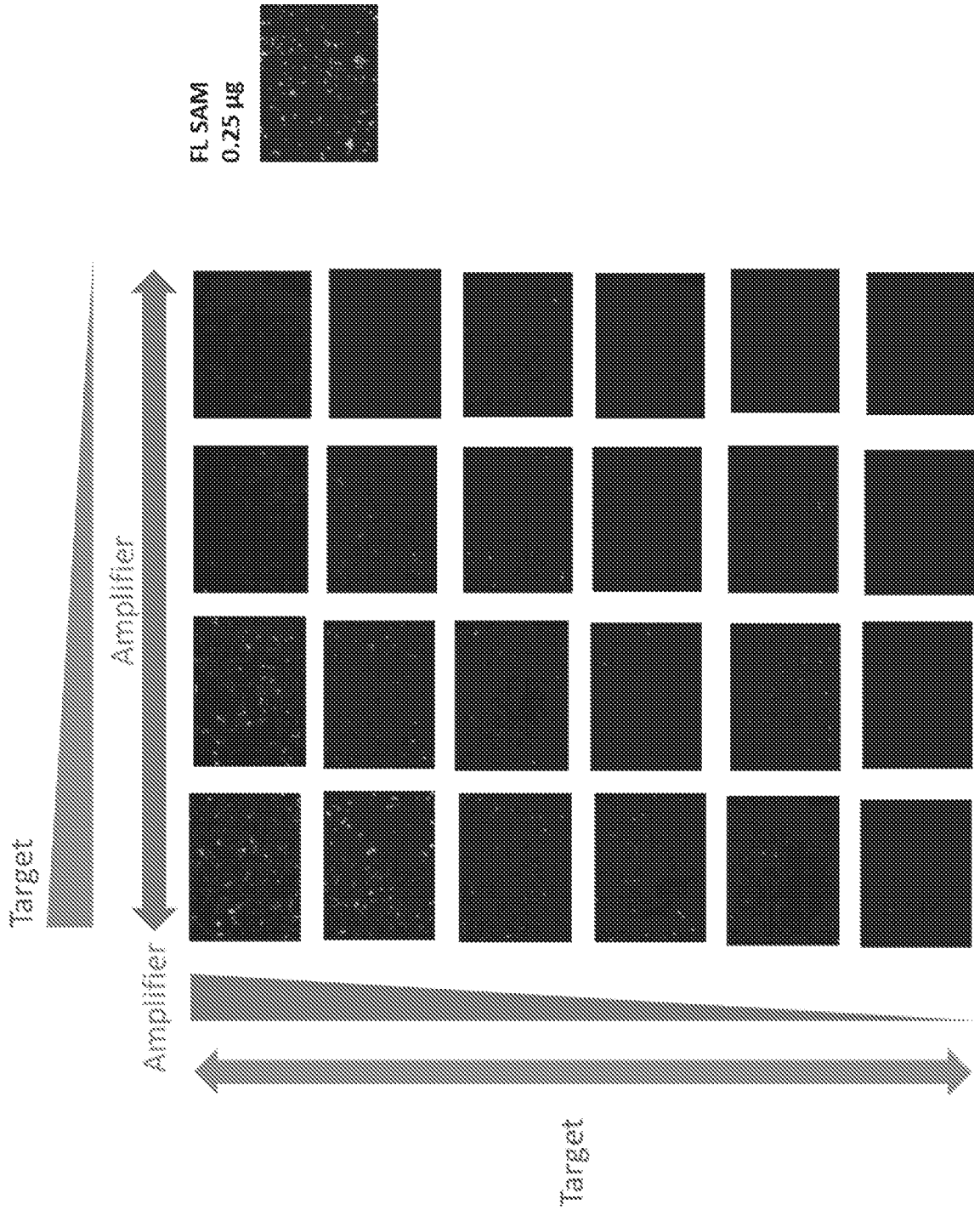

FIG. 10

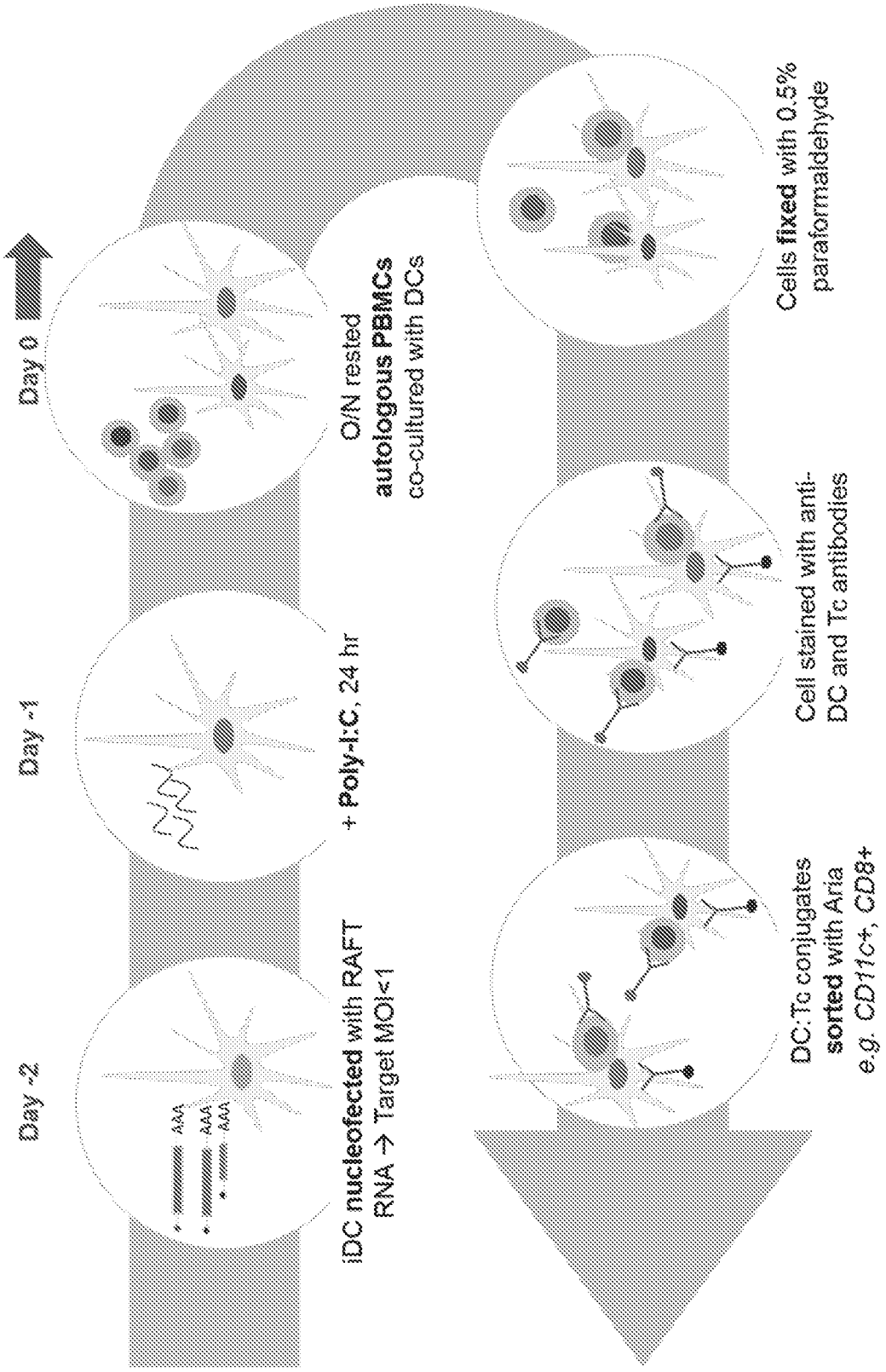
Day -2
Day -1
Day 0
AAA
AAA
AAA
iDC **nucleofected** with RAFT
RNA → Target MOI<1
+ **Poly-I:C**, 24 hr
O/N rested
autologous PBMCs
co-cultured with DCs
Cells **fixed** with 0.5%
paraformaldehyde
Cell stained with anti-
DC and Tc antibodies
DC:Tc conjugates
sorted with Aria
e.g. CD11c+, CD8+

FIG. 11

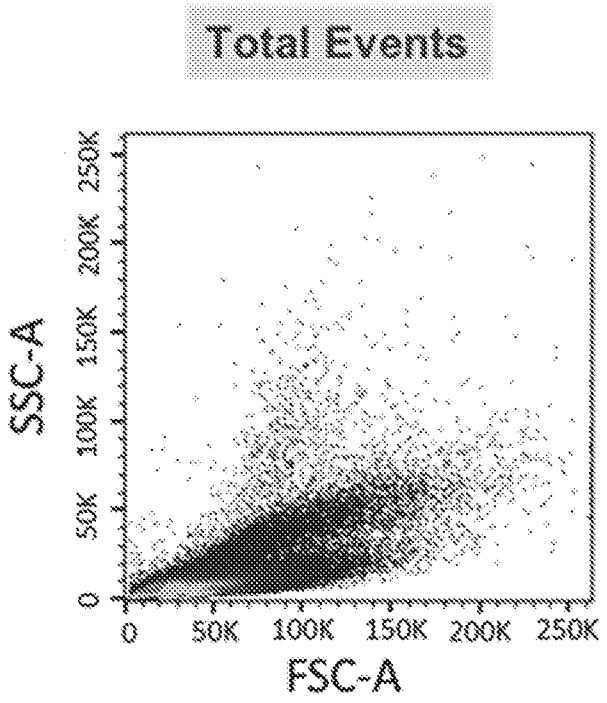

FIG. 12A

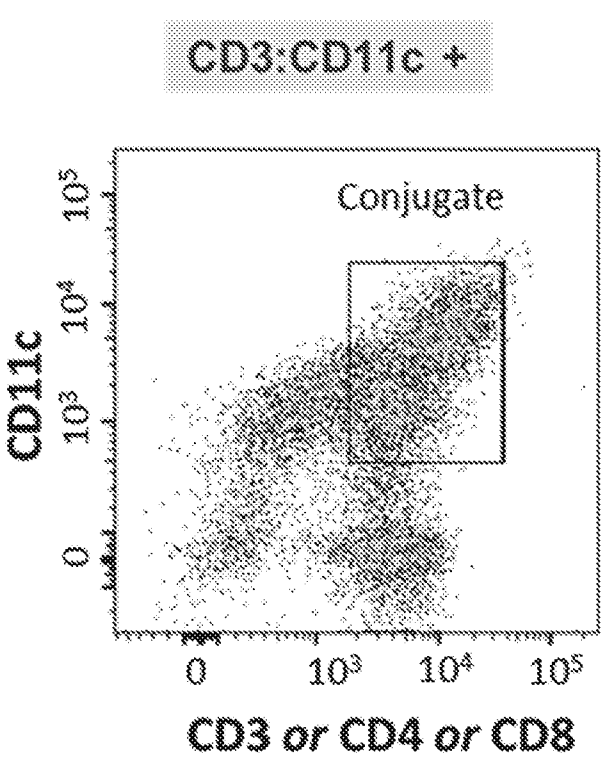

FIG. 12B

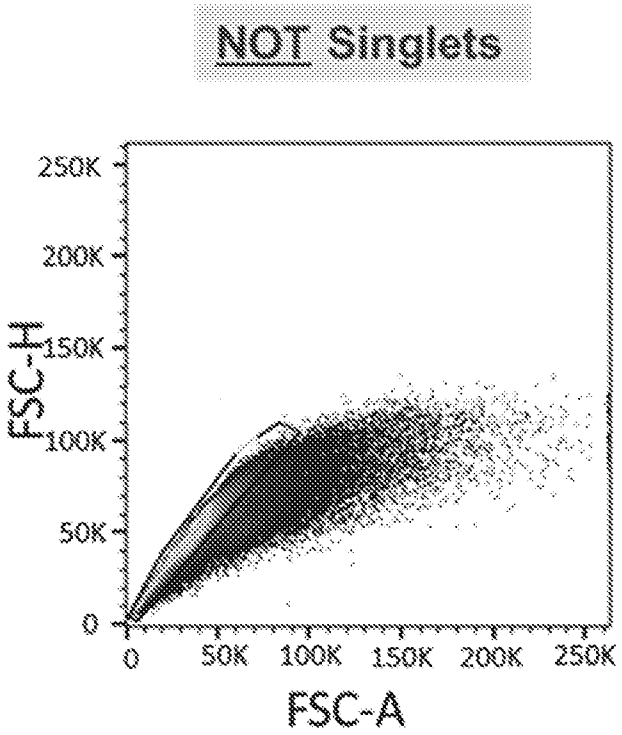

FIG. 12C

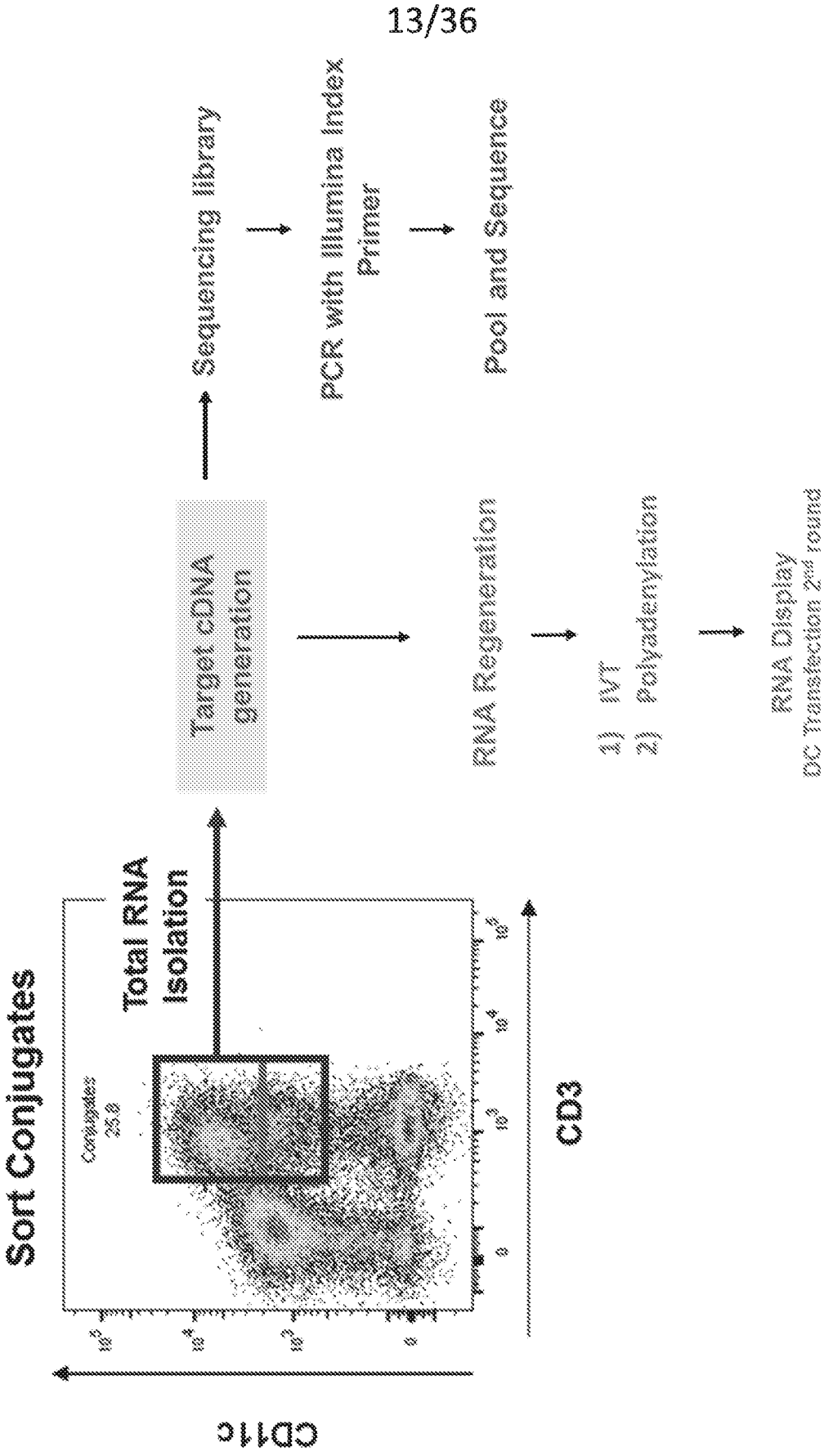
Sort Conjugates
Total RNA Isolation
Conjugates
25.8
CD11c
CD3
Target cDNA generation
RNA Regeneration
1) IVT
2) Polyadenylation
RNA Display
DC Transfection 2nd round
PCR with Illumina Index Primer
Pool and Sequence
Sequencing library

FIG. 13A

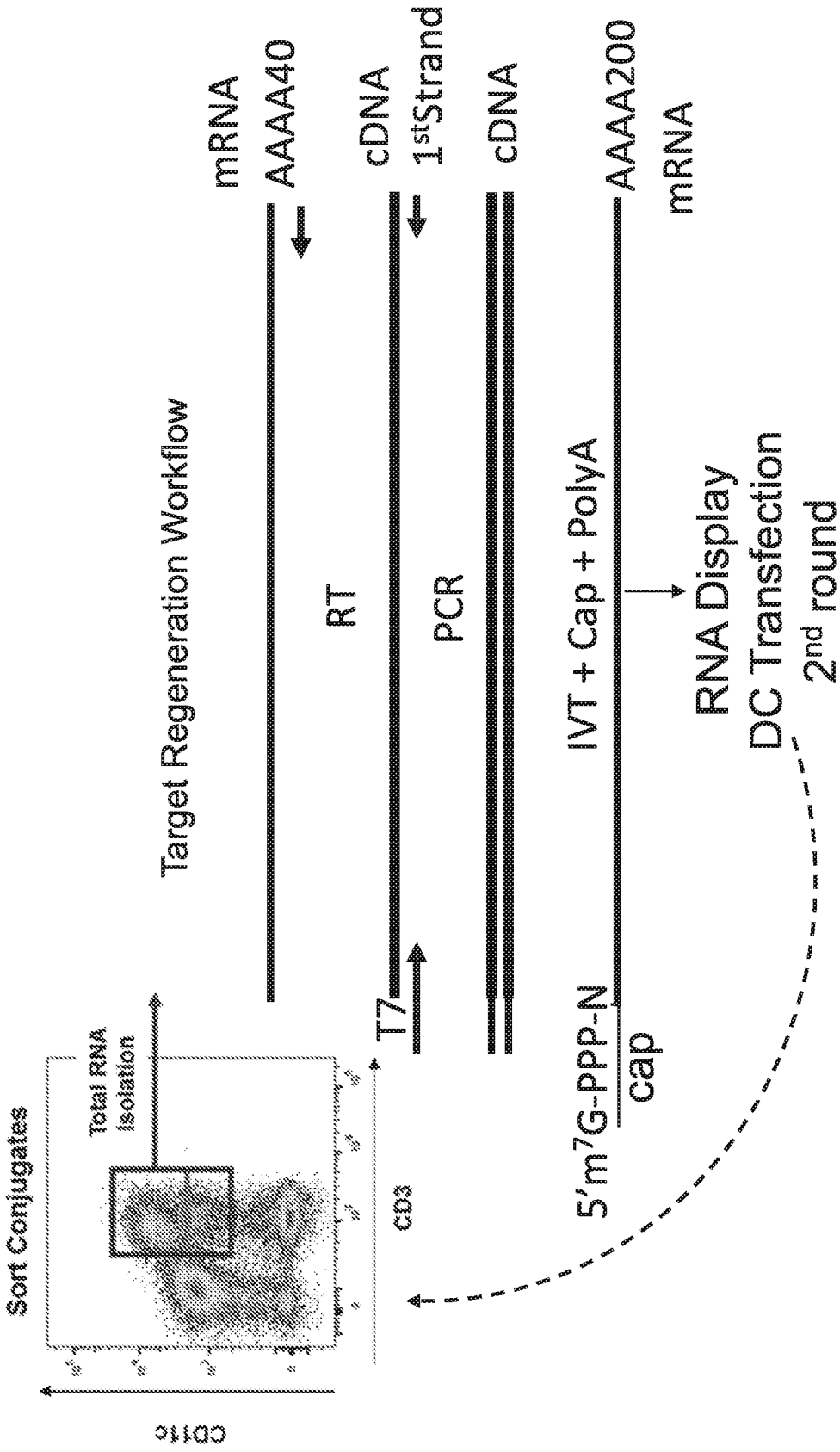
Sort Conjugates
Total RNA Isolation
cd11c
cd3
Target Regeneration Workflow
mRNA
AAAA40
RT
cDNA
1stStrand
PCR
T7
cDNA
AAAA200
mRNA
IVT + Cap + PolyA
5'm7G-PPP-N
cap
RNA Display
DC Transfection
2nd round

FIG. 13B

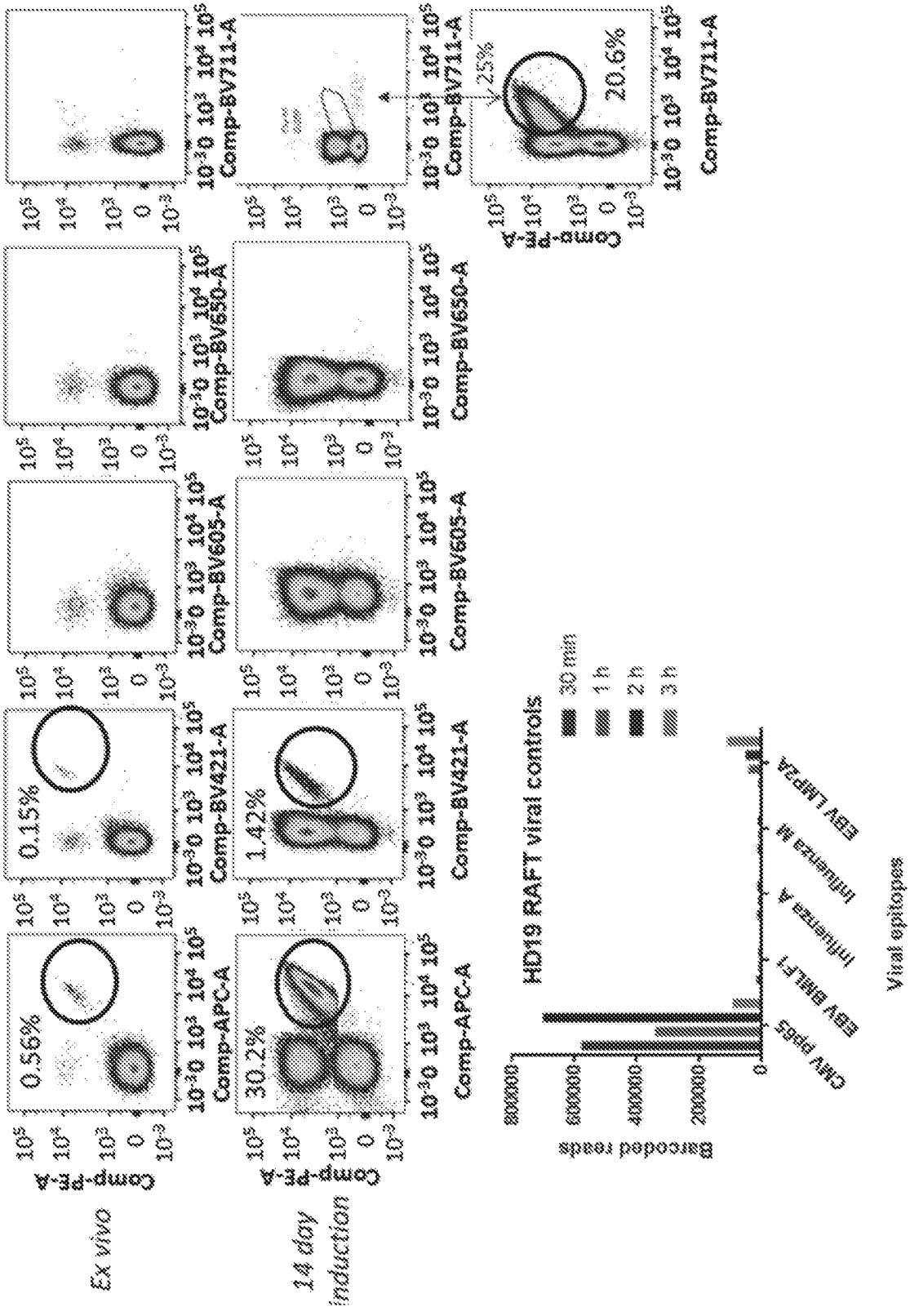
pp65
LMP2a
BMLF
FluA
FluM
Ex vivo
14 day induction
0.56%
0.15%
30.2%
1.42%
20.6%
Comp-APC-A
Comp-BV421-A
Comp-BV605-A
Comp-BV650-A
Comp-BV711-A
HD19 RAFT viral controls
30 min
1 h
2 h
3 h
Barcoded reads
CMV pp65
EBV BMLF1
Influenza A
Influenza M
EBV LMP2A
Viral epitopes

FIG. 14

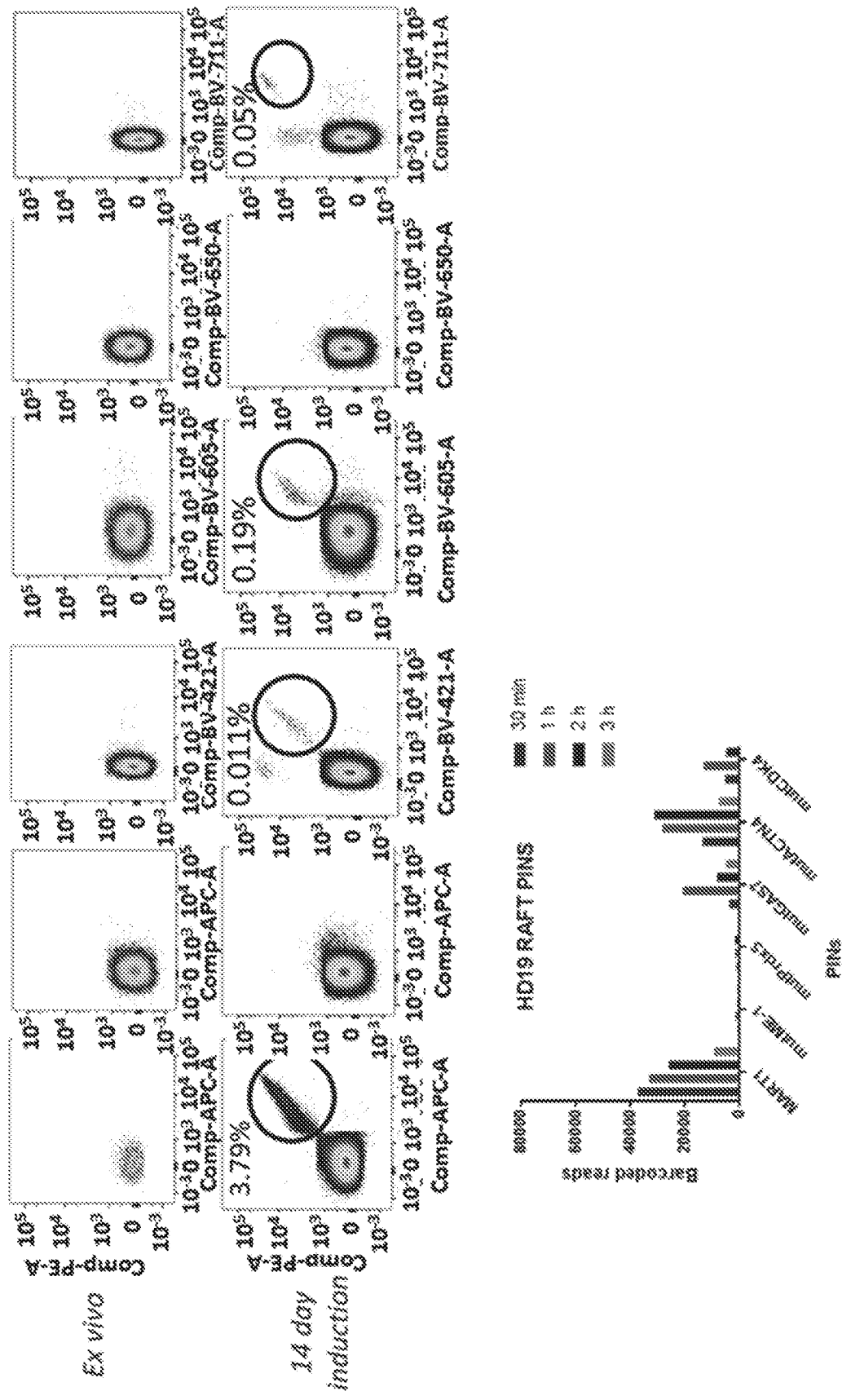
MART-1
mME-1
mPrdx5
mGAS7
mACTN4
mCDK4
Ex vivo
14 day induction
Comp-PE-A
Comp-APC-A
Comp-BV-421-A
Comp-BV-605-A
Comp-BV-650-A
Comp-BV-711-A
3.79%
0.011%
0.19%
0.05%
HD19 RAFT PINS
30 min
1 h
2 h
3 h
Barcoded reads
PINs
MART1
mME-1
mPRDX5
mGAS7
mACTN4
mCDK4

FIG. 15A

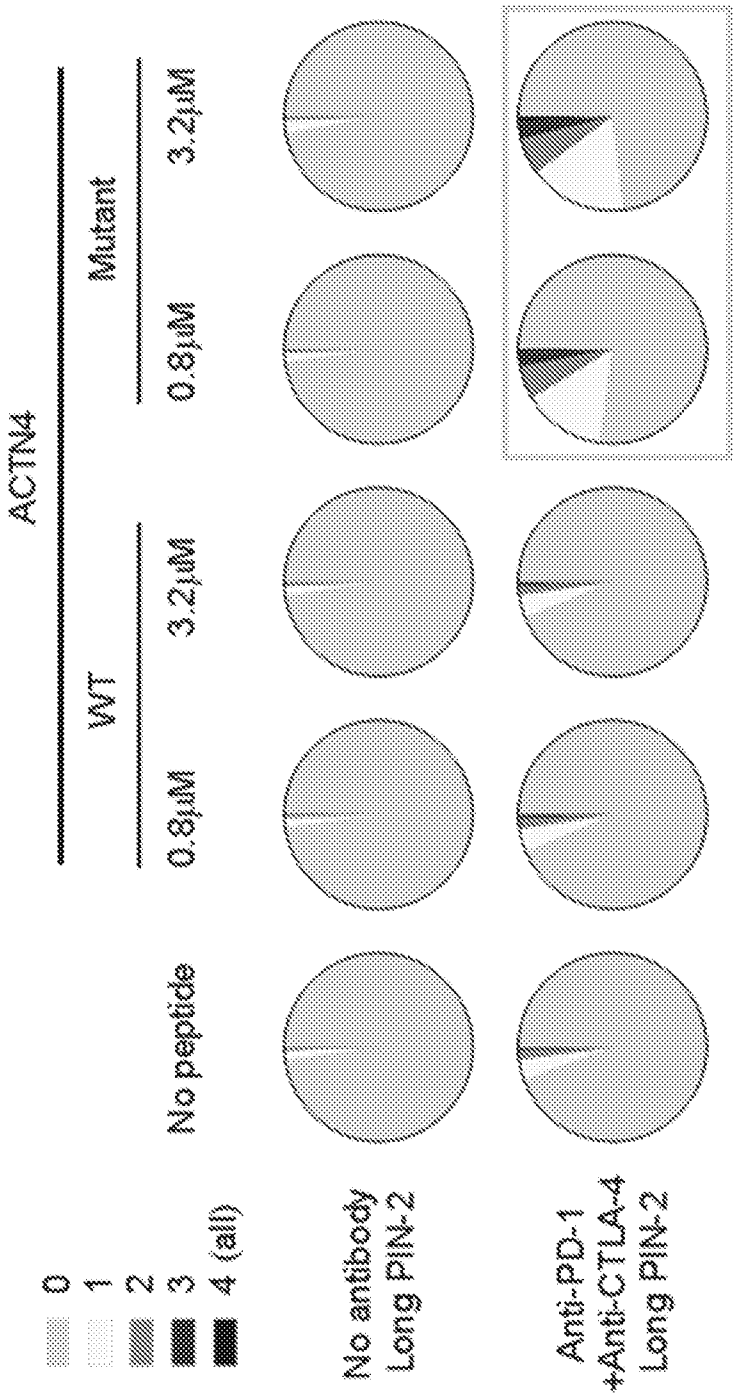
ACTN4
WT
Mutant
0.8μM
3.2μM
0.8μM
3.2μM
No peptide
No antibody
Long PIN-2
Anti-PD-1
+Anti-CTLA-4
Long PIN-2
0
1
2
3
4 (all)

FIG. 15B

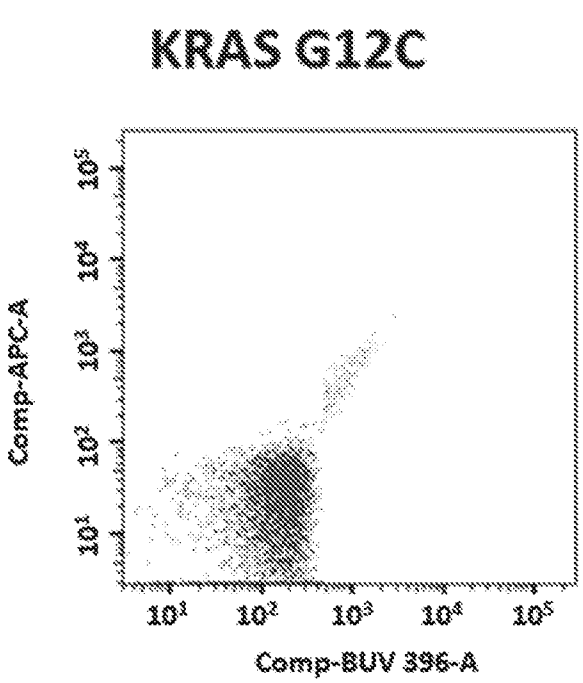

FIG. 16A

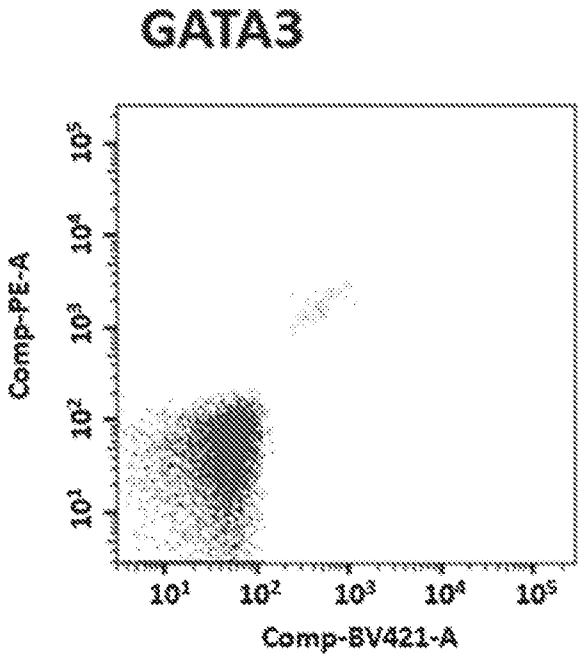

FIG. 16B

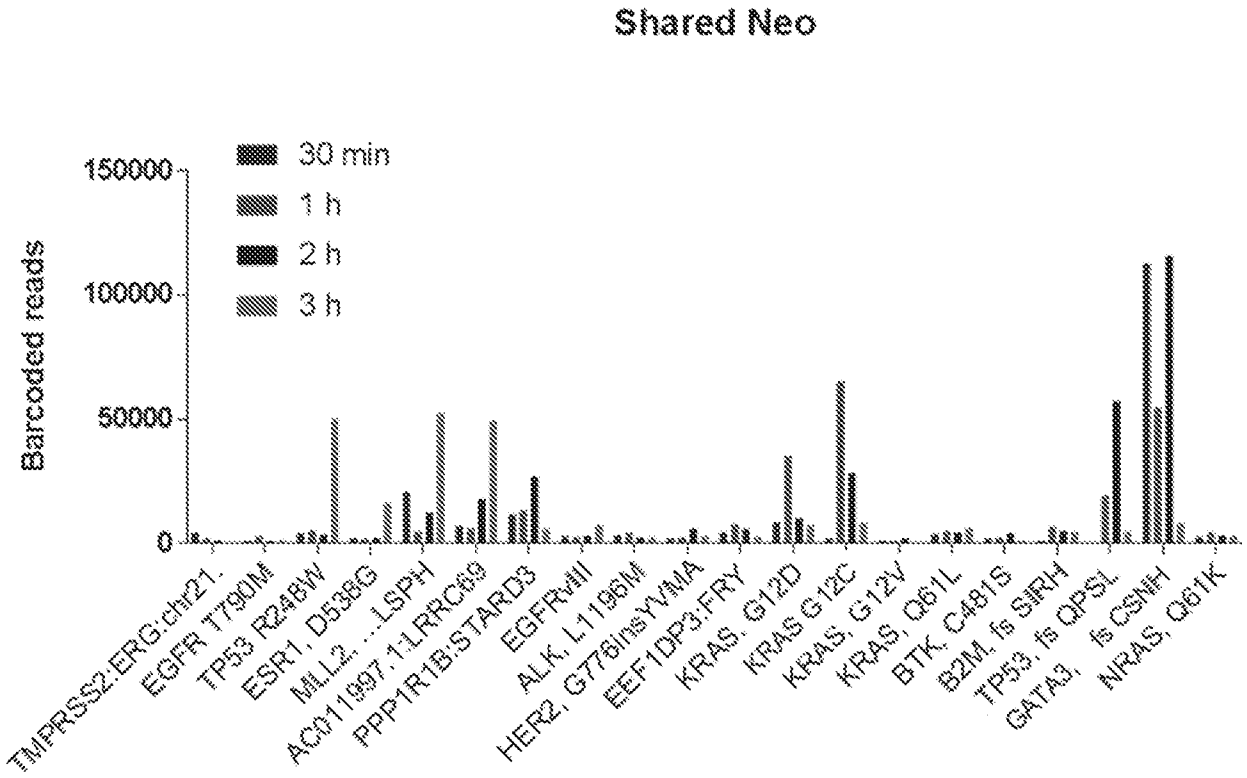

FIG. 16C

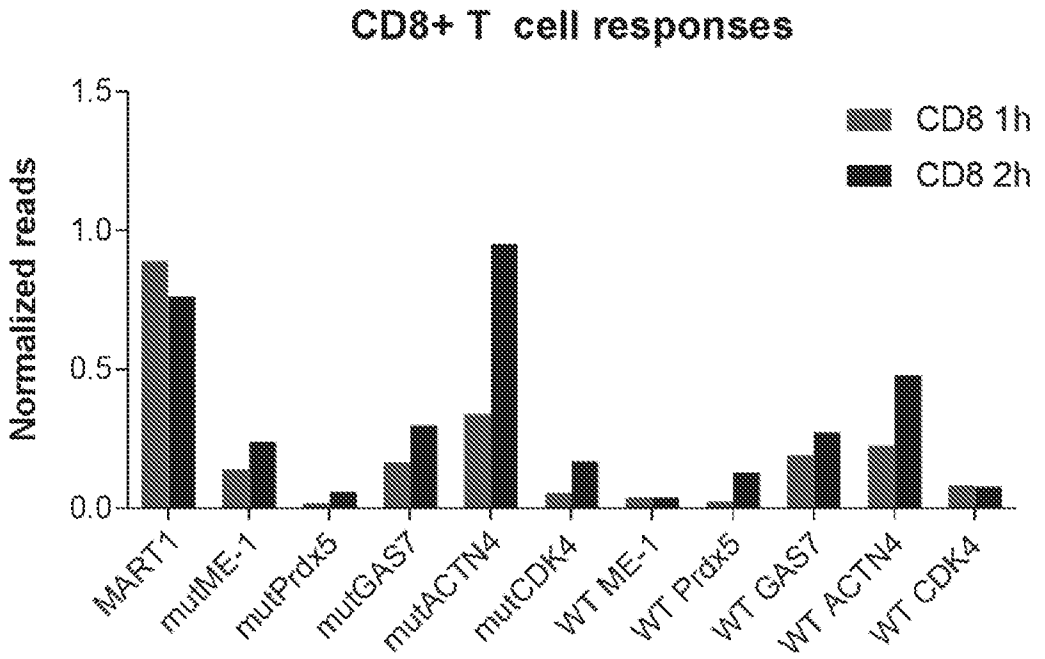

FIG. 17A

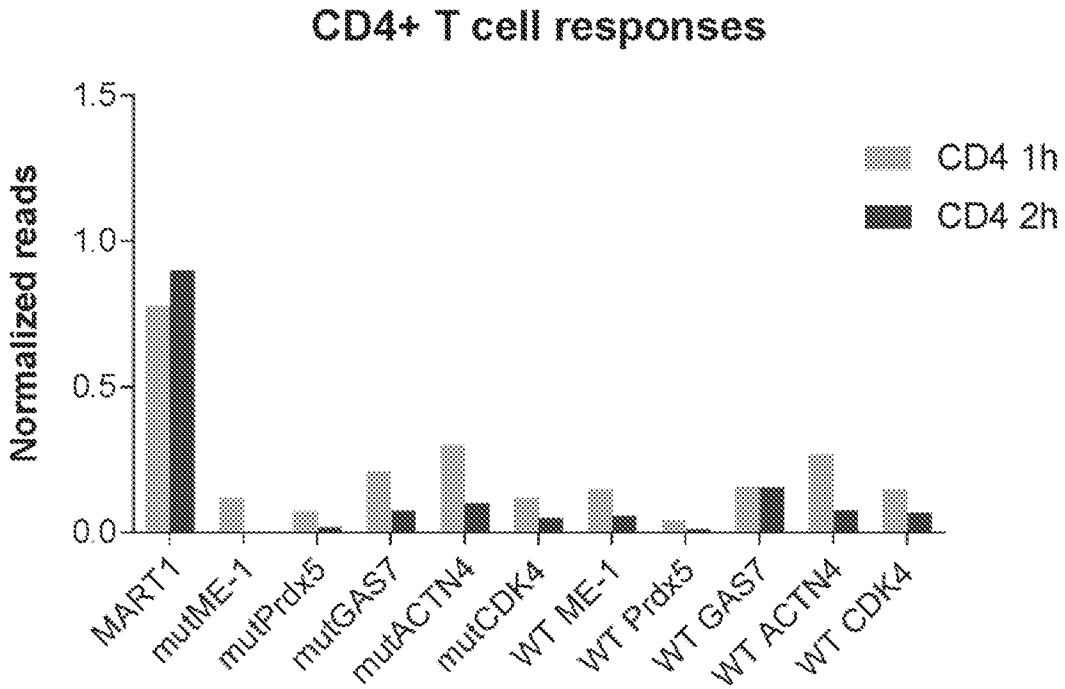

FIG. 17B

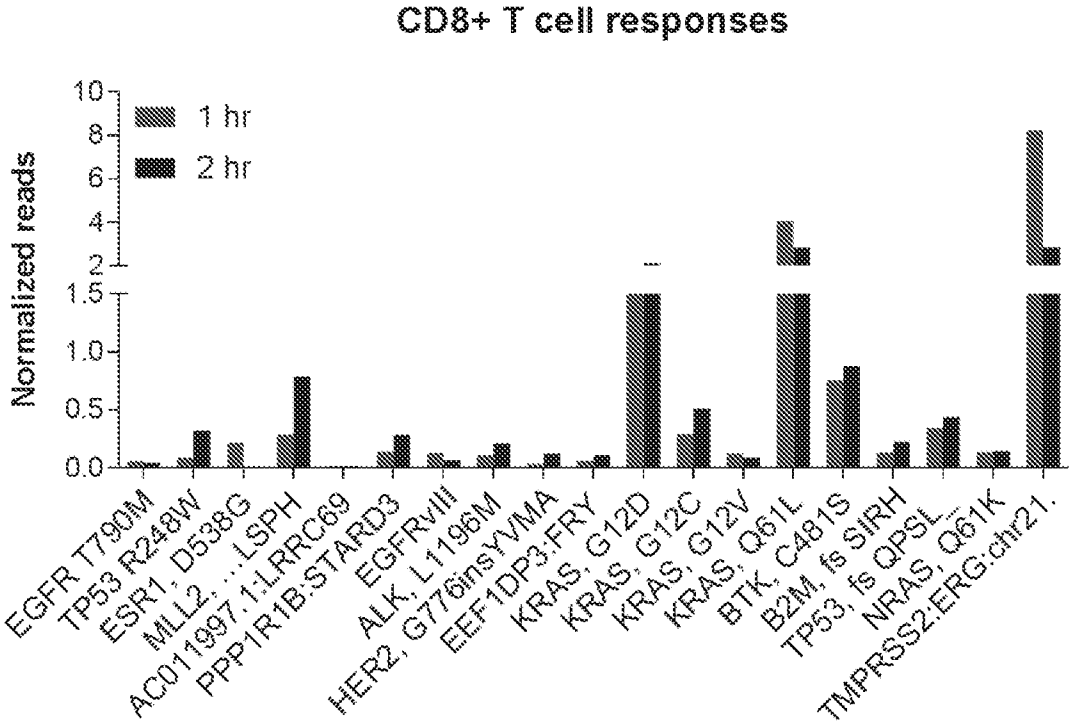

FIG. 18A

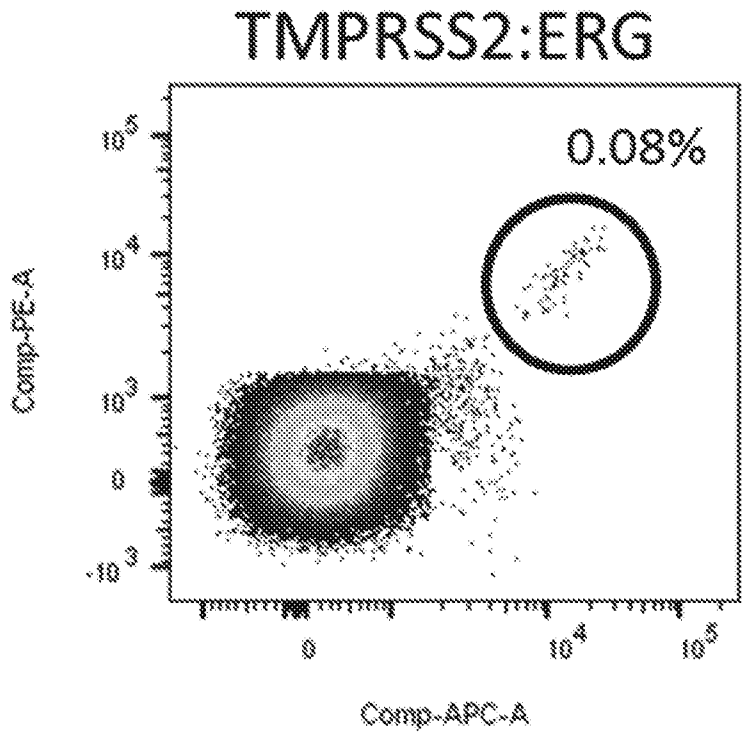

FIG. 18B

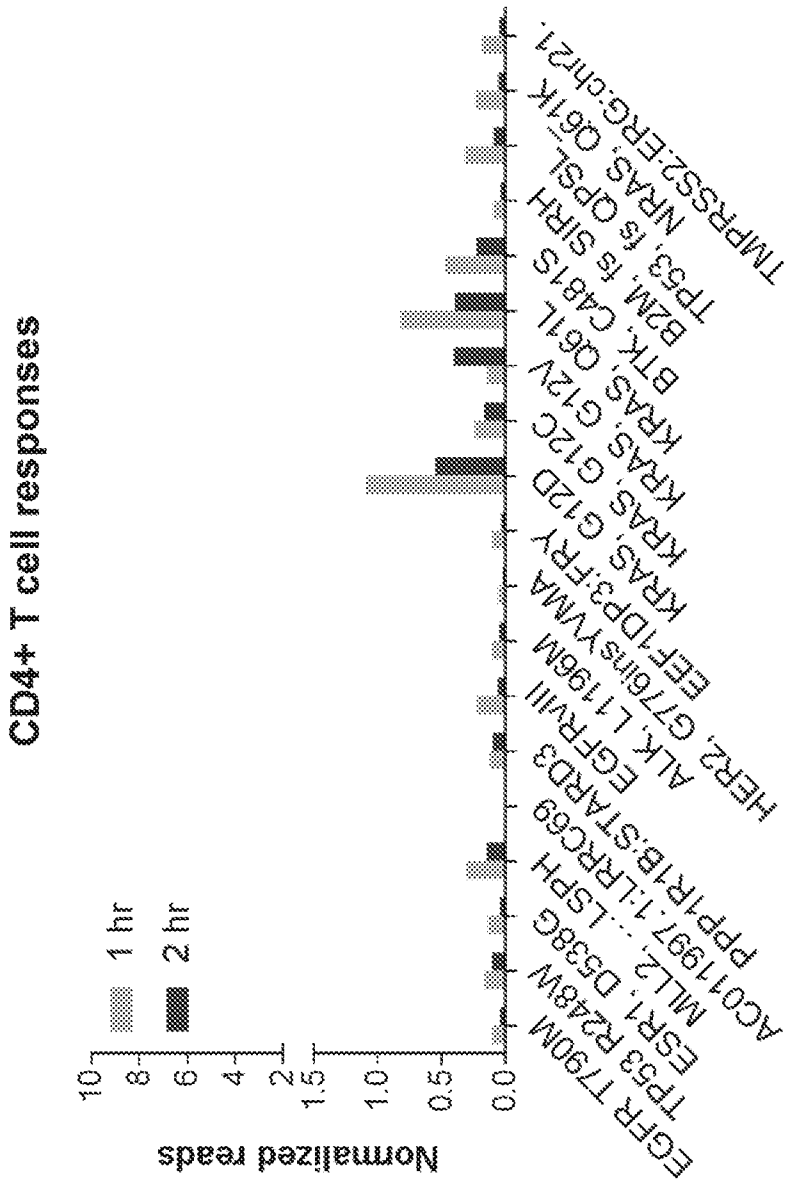
CD4+ T cell responses
Normalized reads
1 hr
2 hr
10
8
6
4
2
1.5
1.0
0.5
0.0
EGFR T790M
TP53 R248W
ESR1 D538G
MLL2_...LSPH
AC011997.1:LRRC69
PPP1R18:STARD3
EGFRvIII
ALK L1196M
HER2, G776insVVMA
EEF1DP3:FRY
KRAS G12D
KRAS G12C
KRAS G12V
BTK Q61L
B2M, fs CA81S
TP53, fs SIRH
NRAS Q61K
TMPRSS2:ERG.chr21.

FIG. 18C

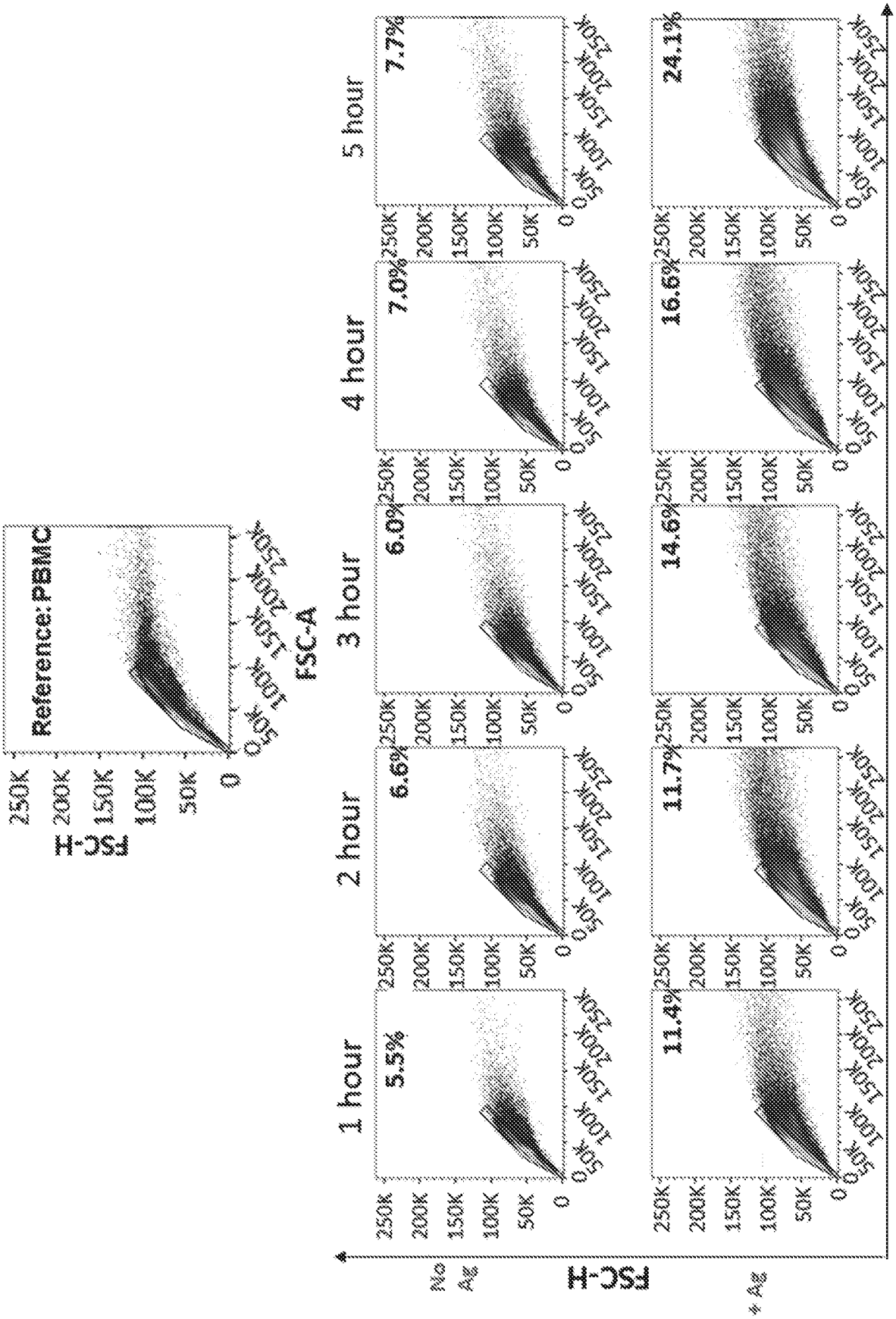
Reference: PBMC
FSC-H
FSC-A
1 hour
2 hour
3 hour
4 hour
5 hour
5.5%
6.6%
6.0%
7.0%
7.7%
11.4%
11.7%
14.6%
16.6%
24.1%
No Ag
+ Ag
250K
200K
150K
100K
50K
0

FIG. 19

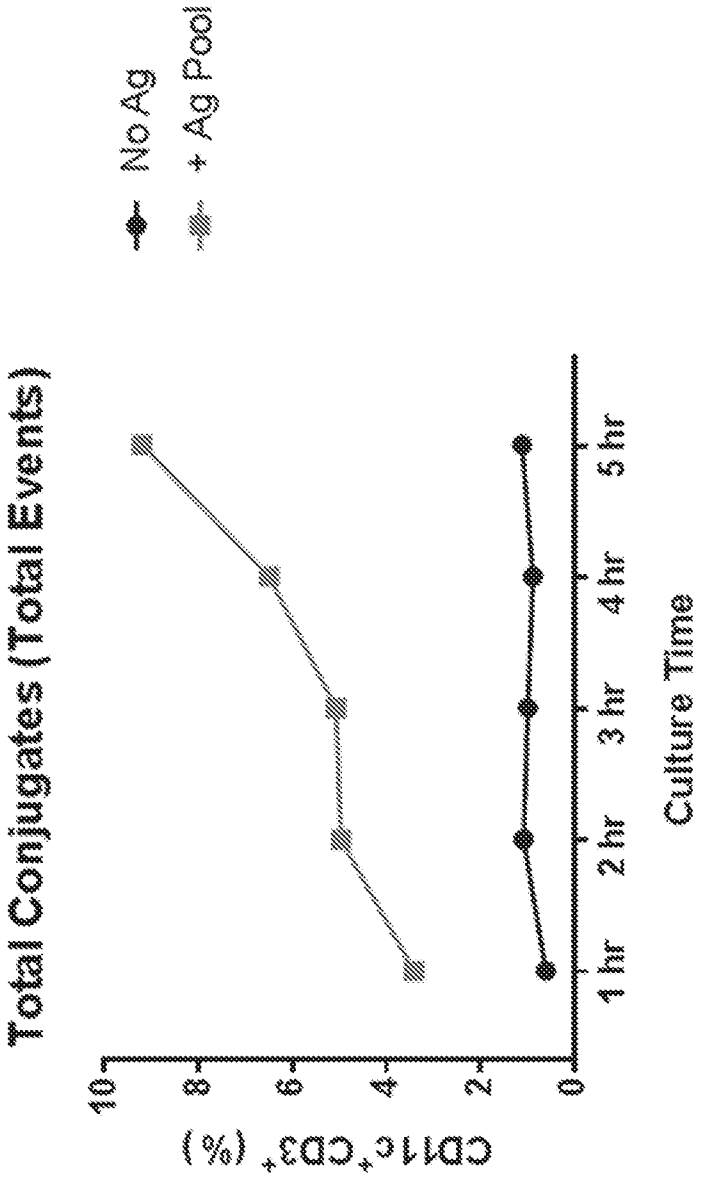
No Ag
+ Ag Pool
Total Conjugates (Total Events)
CD11c+CD3+ (%)
10
8
6
4
2
0
1 hr
2 hr
3 hr
4 hr
5 hr
Culture Time

FIG. 20

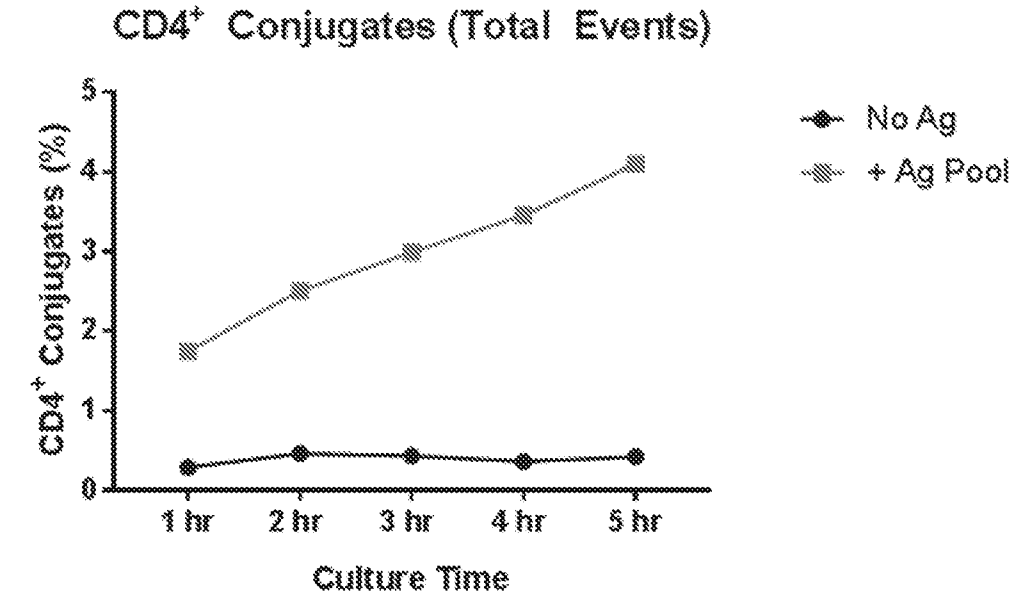

FIG. 21A

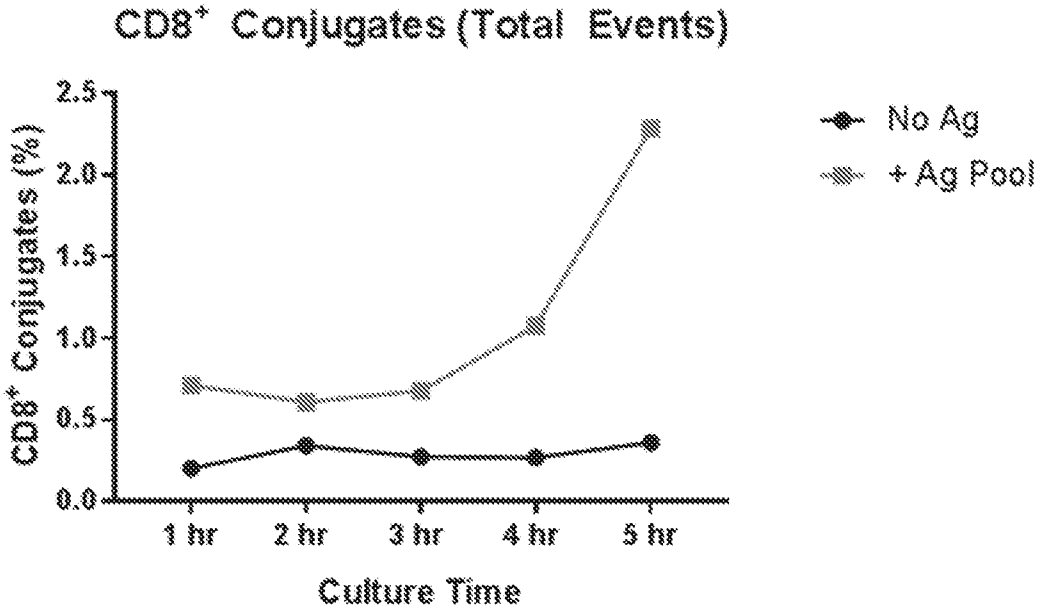

FIG. 21B

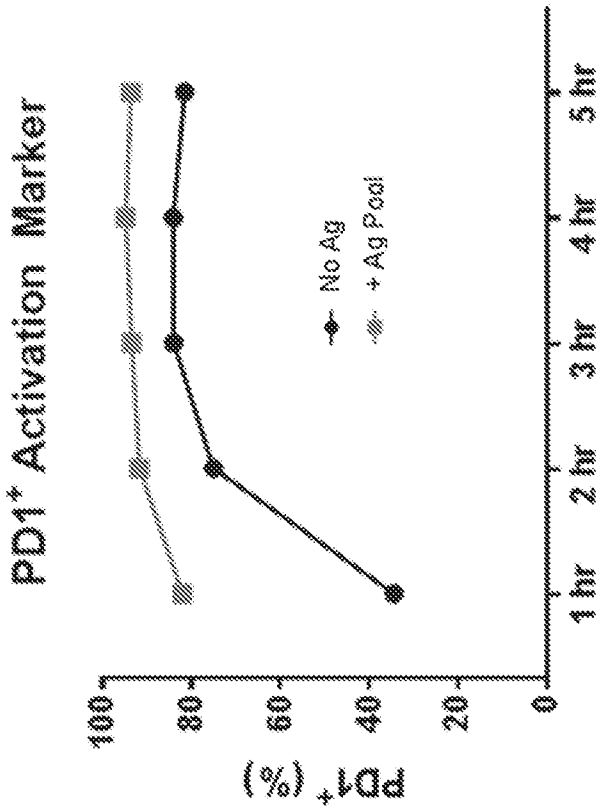

FIG. 22B

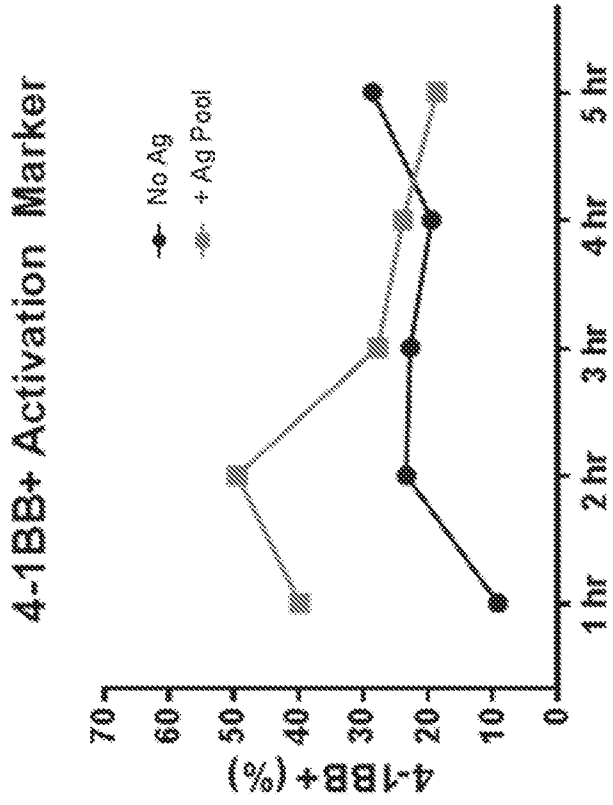

FIG. 22A

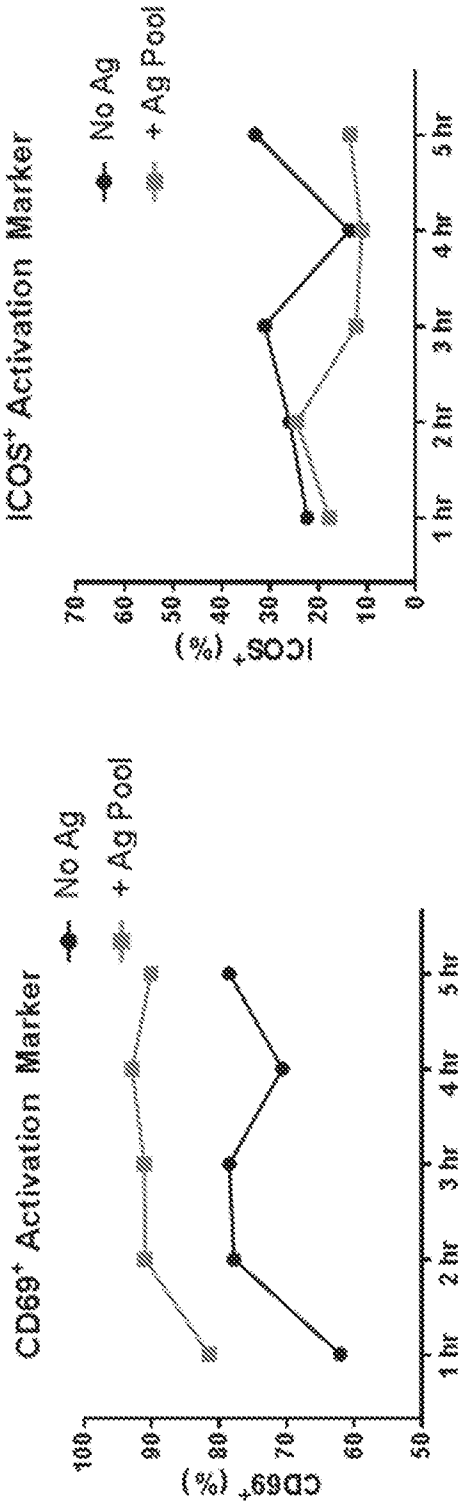

FIG. 22C

FIG. 22D

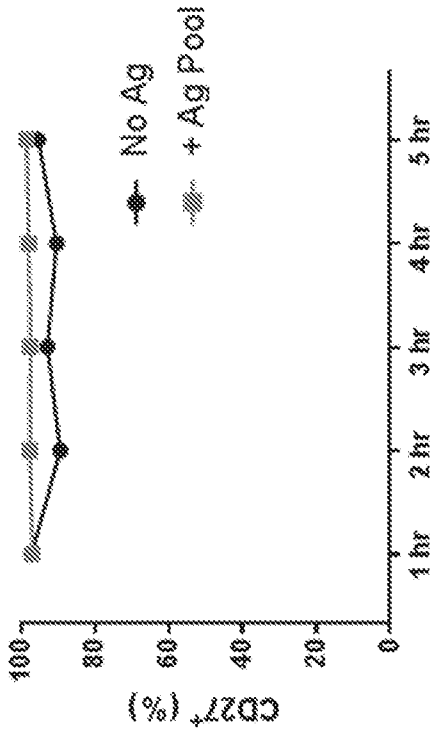

FIG. 22E

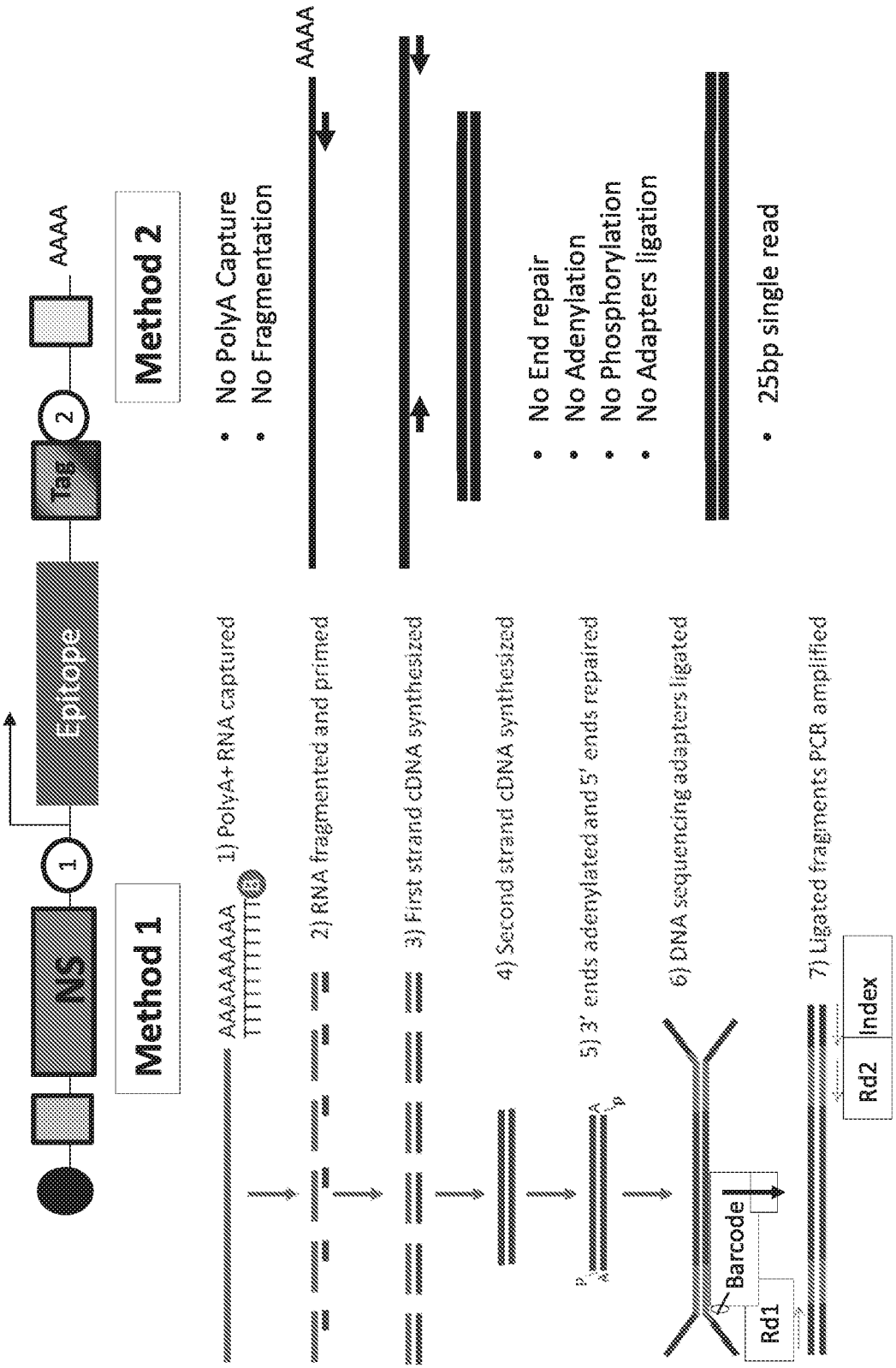
Method 1
NS
1
Epitope
Tag
2
AAAA
Method 2
• No PolyA Capture
• No Fragmentation
• No End repair
• No Adenylation
• No Phosphorylation
• No Adapters ligation
• 25bp single read
AAAAAAAAA
TTTTTTTTTT
1) PolyA+ RNA captured
2) RNA fragmented and primed
3) First strand cDNA synthesized
4) Second strand cDNA synthesized
5) 3' ends adenylated and 5' ends repaired
6) DNA sequencing adapters ligated
7) Ligated fragments PCR amplified
Barcode
Rd1
Rd2
Index

FIG. 23

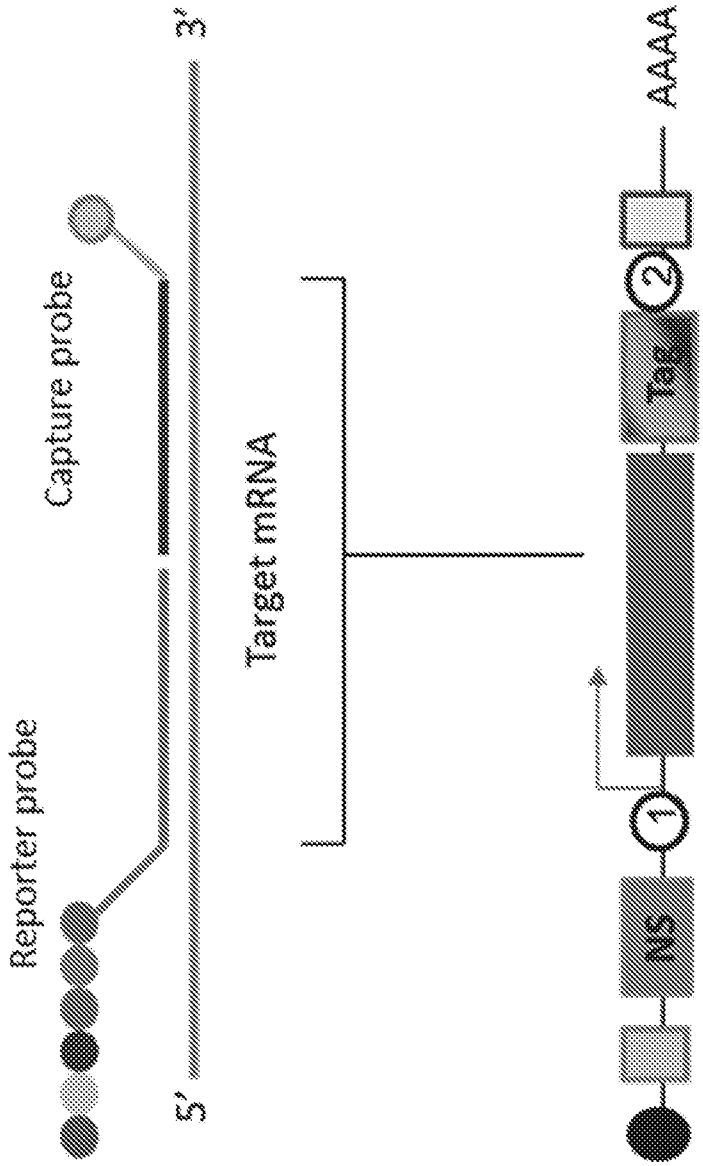

FIG. 24

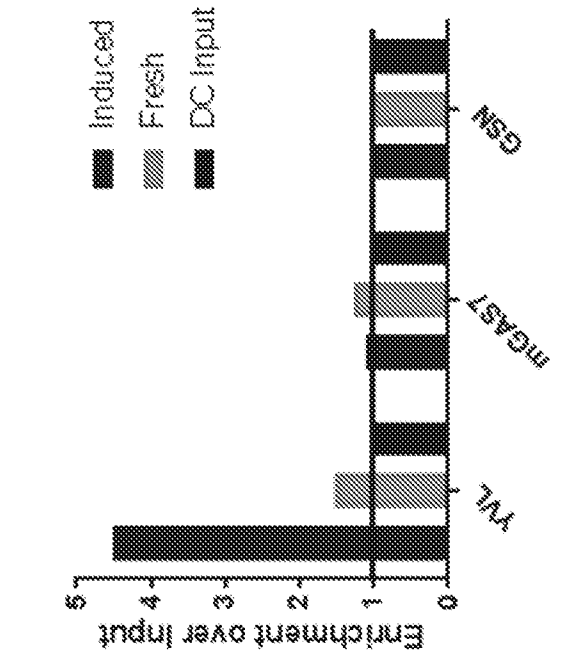

FIG. 25B

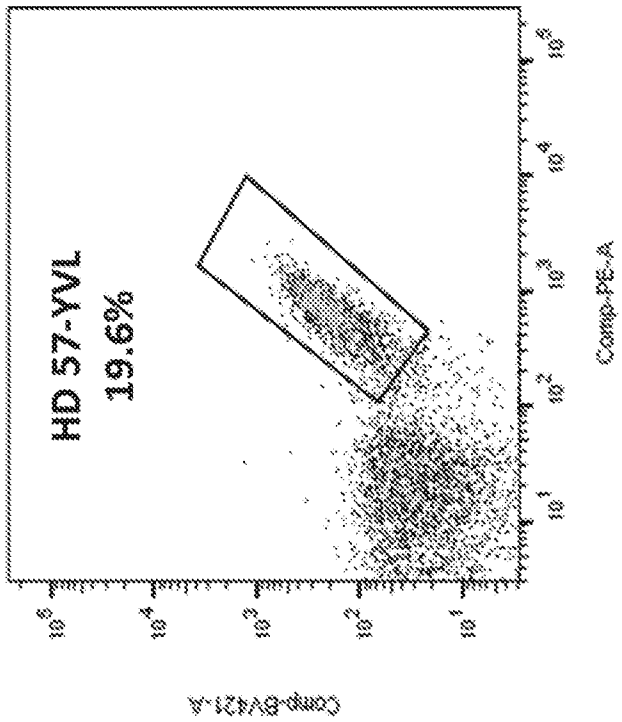

FIG. 25A

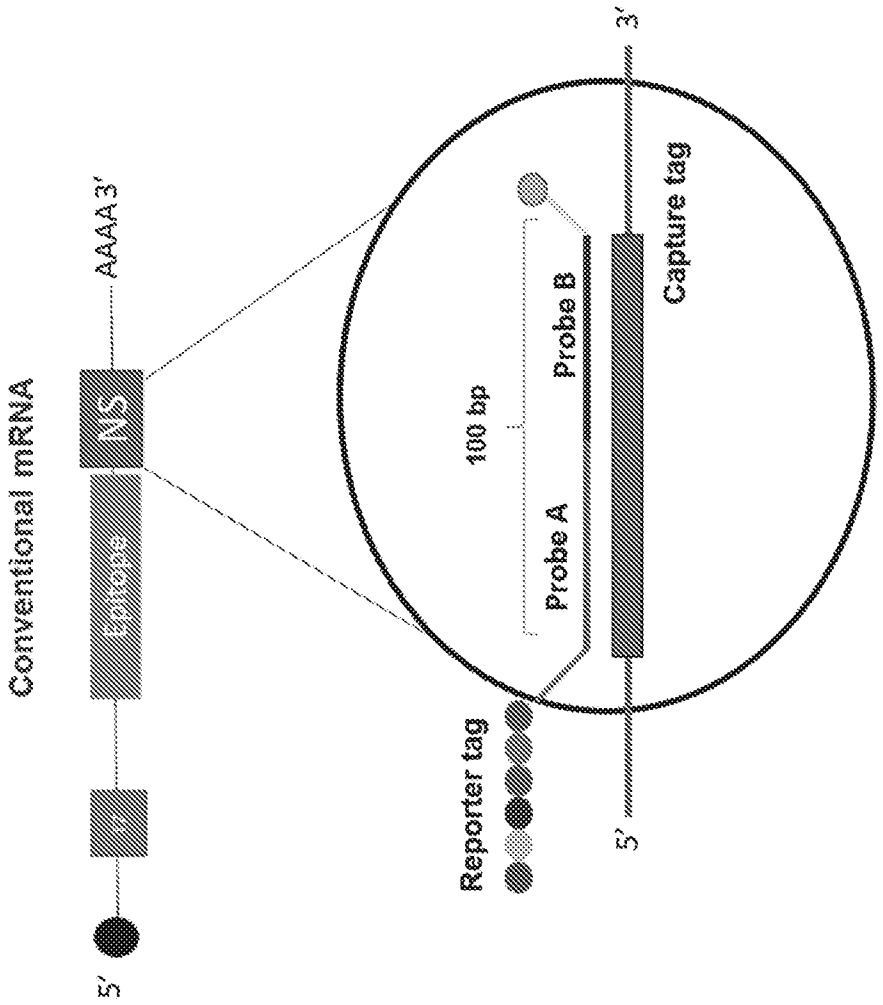
Conventional mRNA
AAAA 3'
MS
Epitope
5'
Reporter tag
100 bp
Probe A
Probe B
3'
Capture tag
5'
• Probe A is a Epitope specific Barcode

FIG. 26

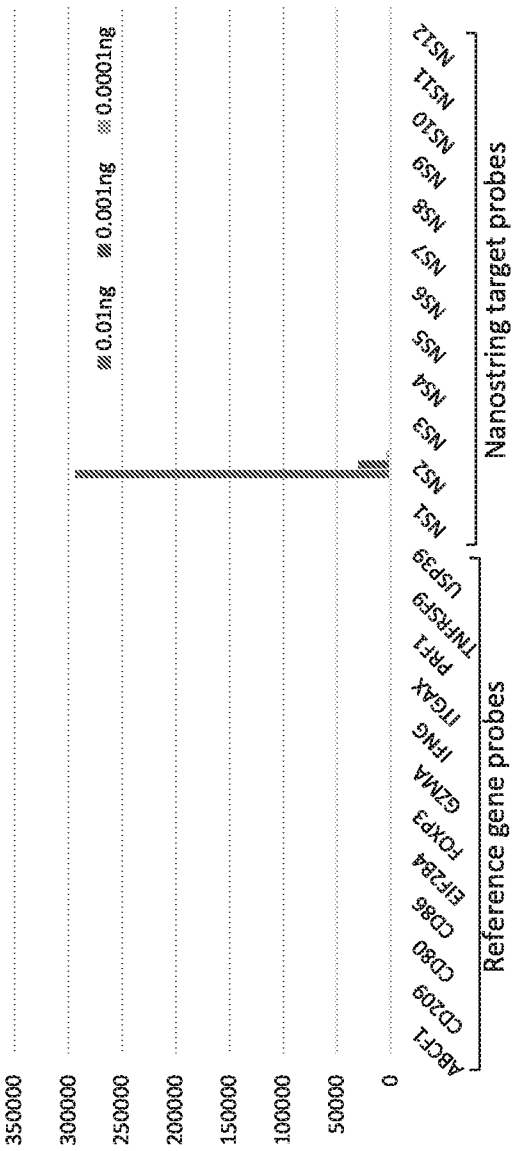

FIG. 27A

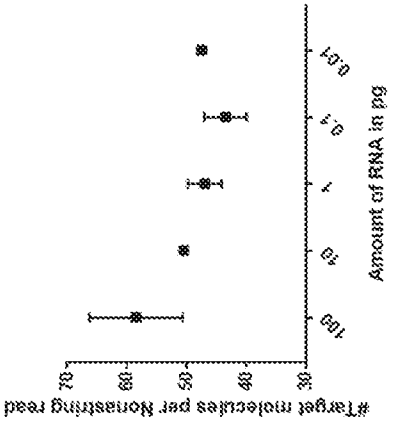

FIG. 27C

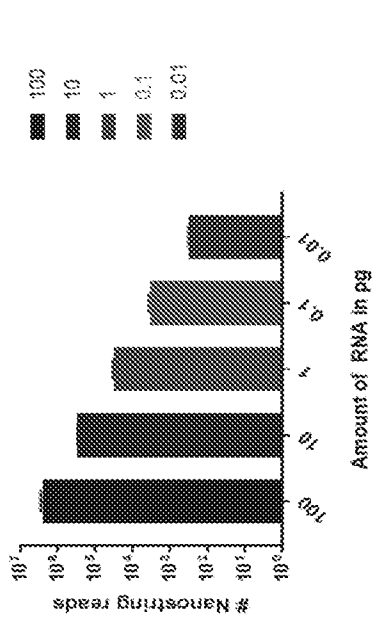

FIG. 27B

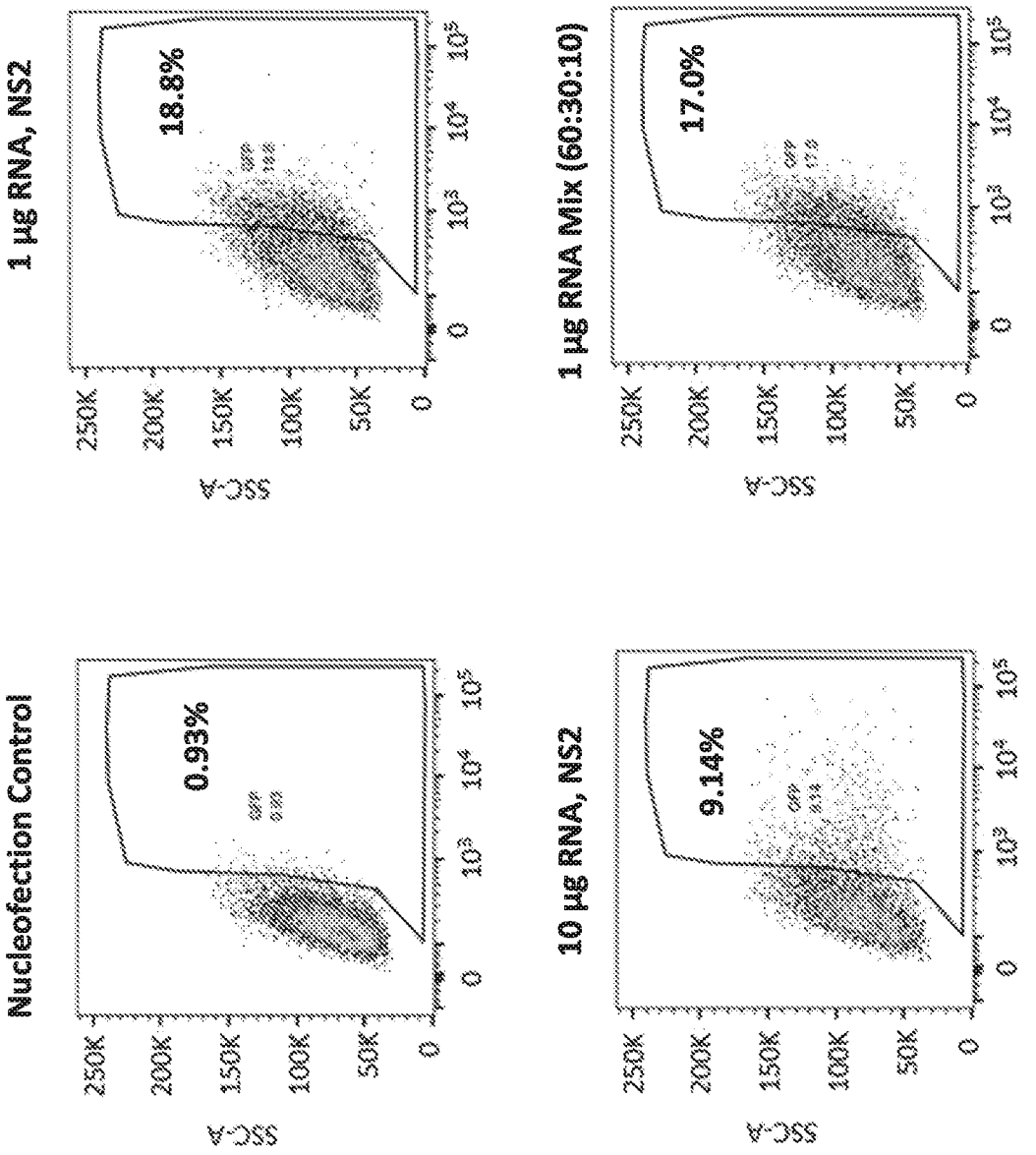
1 µg RNA, NS2
18.8%
1 µg RNA Mix (60:30:10)
17.0%
Nucleofection Control
0.93%
10 µg RNA, NS2
9.14%
SSC-A
250K
200K
150K
100K
50K
0
10^3
10^4
10^5

FIG. 28A

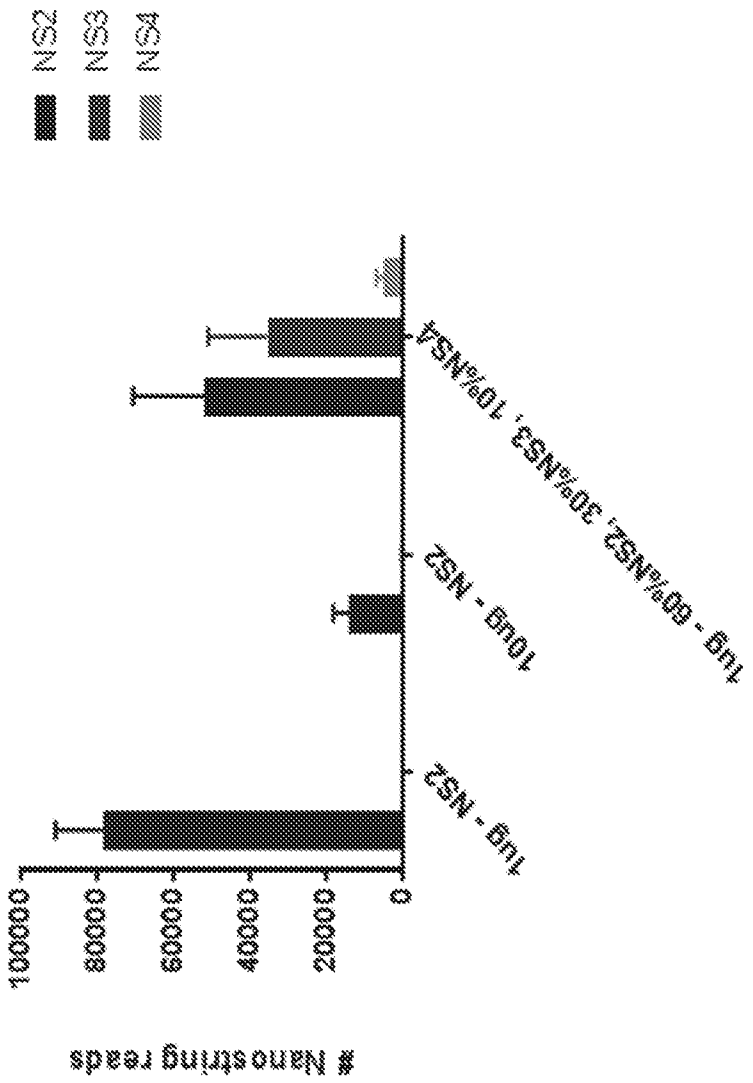
NS2
NS3
NS4
100000
80000
60000
40000
20000
0
Nanostring reads
1ug - 6n1
2SN - NS2
10ug - 6n1
2SN - NS2
1ug - 60%NS2, 30%NS3, 10%NS4
2SN - 6n1
2SN - NS2
2SN - NS3
2SN - NS4

FIG. 28B

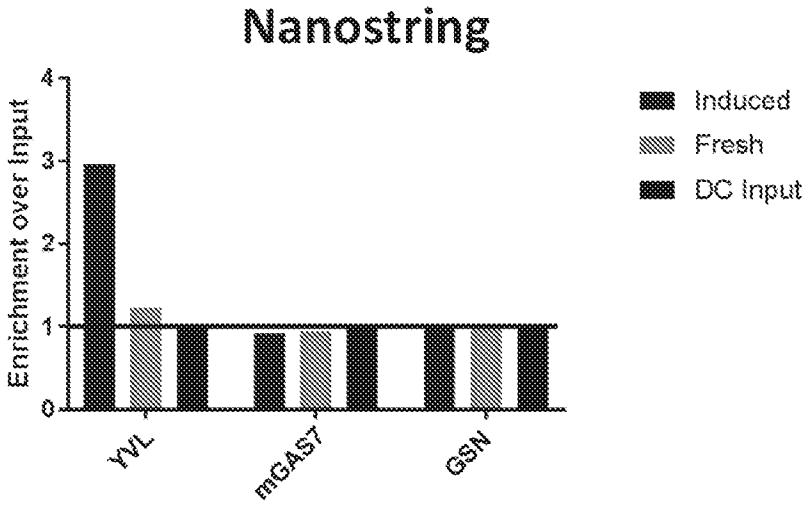

FIG. 29A

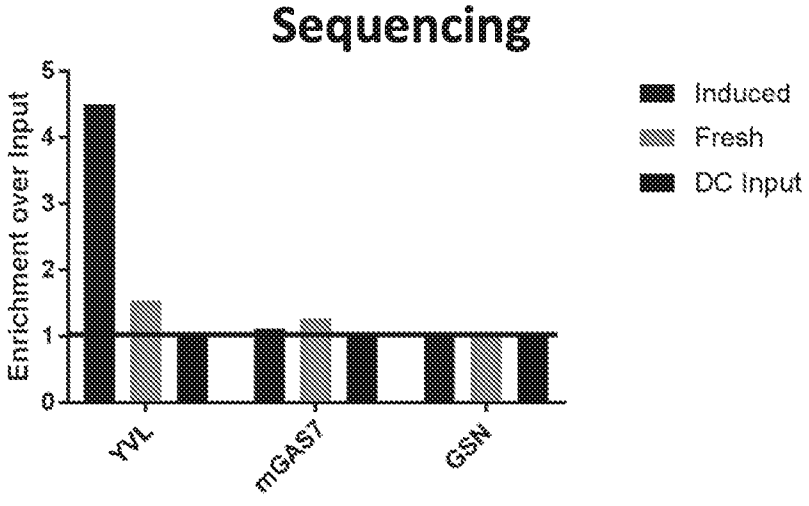

FIG. 29B

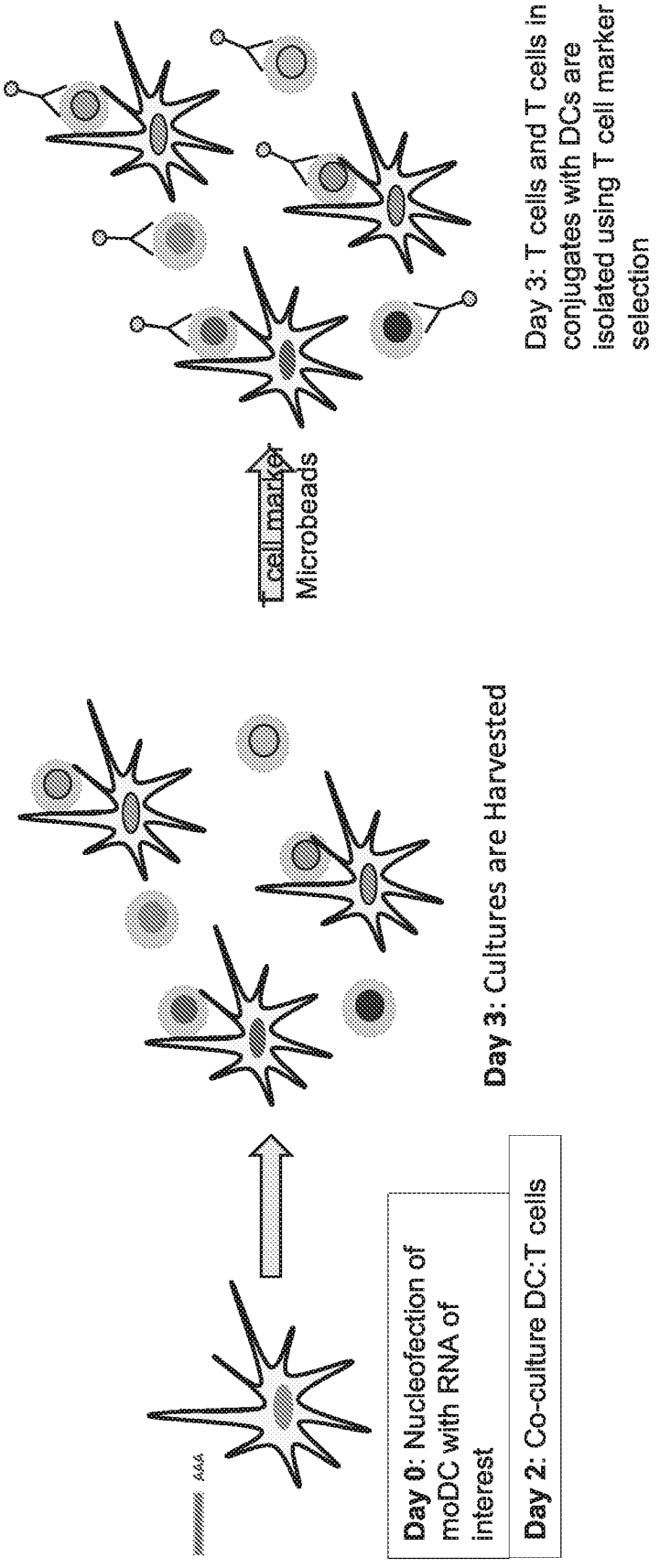
Day 0: Nucleofection of moDC with RNA of interest
Day 2: Co-culture DC:T cells
Day 3: Cultures are Harvested
T cell marker Microbeads
Day 3: T cells and T cells in conjugates with DCs are isolated using T cell marker selection

FIG. 30

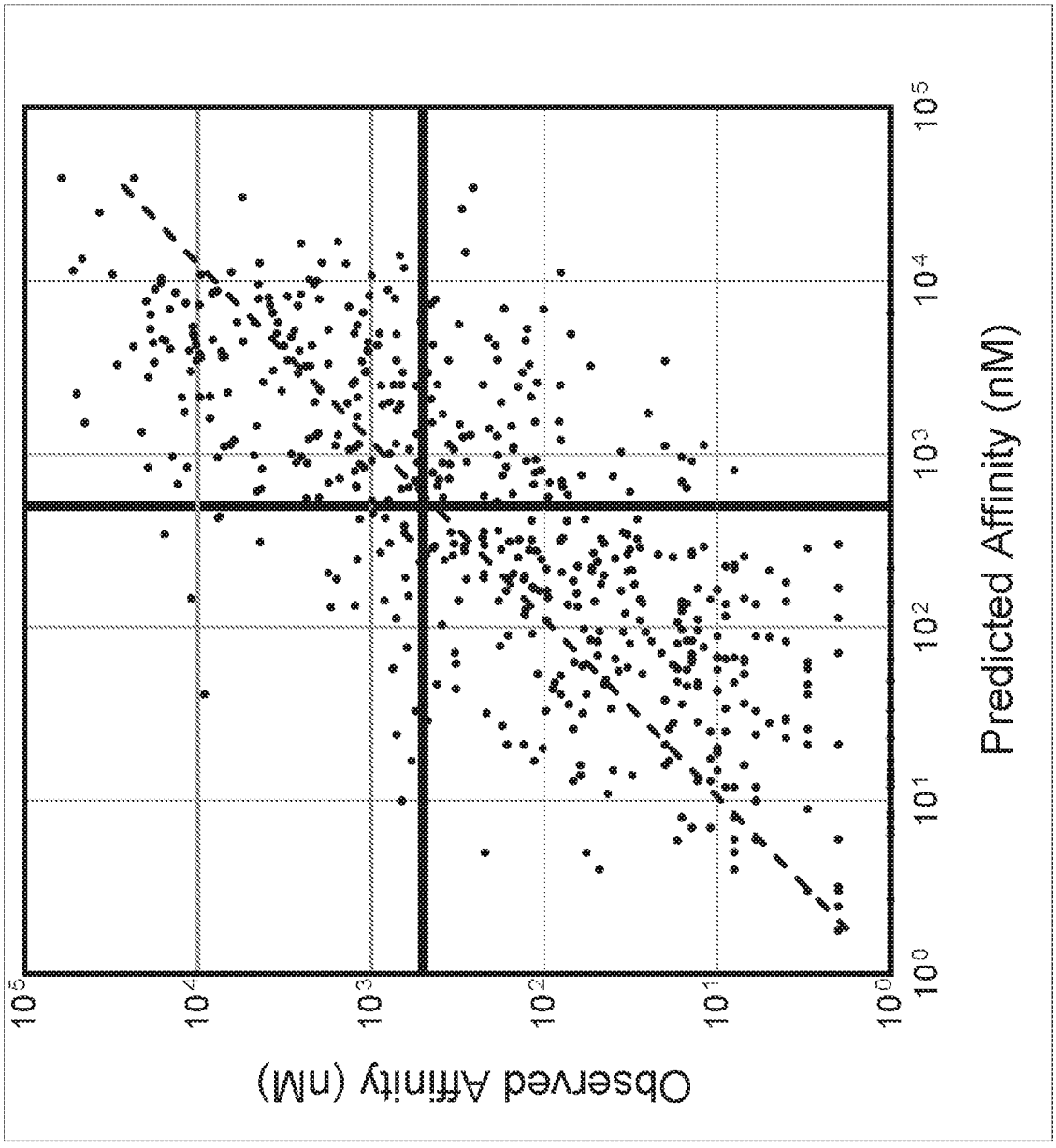
Observed Affinity (nM)
Predicted Affinity (nM)
10^0
10^1
10^2
10^3
10^4
10^5

FIG. 31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/033539

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

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

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13fer1 (a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☑ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13fer1 (a)).

      ☐ on paper or in the form of an image file (Rule 13fer1 (b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/033539

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 C12N5/0783 C12N5/0784
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/017081 Al (UNIV MUENCHEN L MAXIMI LIANS [DE] ; DORNMAI R KLAUS [DE] ; HOHLFELD REINHA) 9 February 2012 (2012-02-09)	2-72
Y	whol e document, especi al l y Fi gure 1; cl aims 1-2 ; Exampl e 1.2.2-1 .2.4	1
X	W0 2016/040900 Al (UNIV WASHINGTON [US]) 17 March 2016 (2016-03-17)	48-55 , 59-63 , 69-72
Y	whol e document, especi al l y Fi gures 1-2 , 28; Exampl es 3-4, 16	1-47

-/- .

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 August 2018	28/08/2018

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

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

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