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(54) Title: MATERIALS AND METHODS FOR IDENTIFYING AND TREATING CANCER PATIENTS

(57) Abstract: The present disclosure relates to materials and methods for treating cancers associated with expression of tumor antigens, in particular, NY-ES 0-1 -specific cancers, including biomarkers and methods for identifying subjects that will benefit from treatment with tumor antigen-specific cancer therapy.

MATERIALS AND METHODS FOR IDENTIFYING AND TREATING CANCER PATIENTS

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

5 Incorporated by reference in its entirety is a computer-readable amino acid/nucleotide sequence listing submitted concurrently herewith and identified as follows: Filename: 51650A_Seqlisting.txt; Size: 9,722 bytes; Created: February 5, 2018.

BACKGROUND

10 Immunization with NY-ESO-1 has been used to induce antibody, CD4 + T-cell and CD8+ CTL responses, although moderate, effect on cancer progression was observed in these studies (Jager et al., Proc Natl Acad Sci USA. 2000;97:12198, Odunsi et al., Proc Natl Acad Sci 2012; 109:5797-802). Adoptive immunotherapy, the transfer of lymphocytes with high antitumor activity, can mediate the regression of large established tumors, but the generation of HLA-matched, reactive lymphocytes is difficult, expensive, and labor intensive
15 (Rosenberg et al., N Engl J Med. 1988;319:1676; Walter et al., N Engl J Med. 1995;333:1038; Mackinnon et al., Blood. 1995; 86:1261; Papadopoulos et al., N Engl J Med. 1994;330:1185; Dudley et al., J Immunother. 2003; 26:332; Dudley et al., Nat Rev Cancer. 2003; 3:666). Furthermore, a large proportion of patients have relapsed a few months after initial response. Tumor-infiltrating lymphocytes have been used in cell transfer therapies and
20 have been shown to recognize a variety of melanoma tumor-associated antigens (TAA). The most commonly recognized TAA in melanoma is the MART-1, a melanocyte differentiation antigen, which is expressed in ~90% of melanomas, whereas NY-ESO-1 is expressed in ~34% of melanomas (Chen et al., Proc Natl Acad Sci USA. 1997;94:1914).

25 Zhao et al, (J. Immunol., 2005 Apr 1; 174(7): 4415–4423) describes the isolation of TCRs specific to the NY-ESO-1 CT antigen and their use to construct retroviral vectors, which were shown to transfer anti-NY-ESO-1 effector functions to normal primary human T cells. rTCR gene vectors, directed against common TAA, have the potential to be used to treat large numbers of cancer patients with their own transduced T cells without the need to identify antitumor T cells uniquely from each patient. However, currently all approaches to
30 develop ACT based on rTCR are HLA-dependent and thus limited to certain patient populations.

Cancer represents the phenotypic end-point of multiple genetic lesions that endow

cells with a full range of biological properties required for tumorigenesis. Indeed, a hallmark genomic feature of many cancers is the presence of numerous complex chromosome structural aberrations, including translocations, intra-chromosomal inversions, point mutations, deletions, gene copy number changes, gene expression level changes, and germline mutations, among others. Whether a cancer will respond to a particular treatment option may depend on the particular genomic features present in the cancer as well as the genetic makeup of the patient.

One approach to treating cancer patients is to genetically modify T cells to target antigens expressed on tumor cells through the expression of chimeric antigen receptors (CARs) or recombinant T-cell receptors (rTCRs) for adoptive cell therapy (ACT). CARs are antigen receptors that are designed to recognize cell surface antigens in a human leukocyte antigen-independent manner. Attempts in using genetically modified cells expressing CARs to treat certain types of cancers have met with impressive success, especially in CD19 expressing liquid tumors. See for example, Porter David L, et al. The New England journal of medicine 365(8): 725-33, Aug 2011; Kalos Michael, et al., Science translational medicine 3(95): 95ra73, Aug 2011. Likewise, autologous T cells engineered to express a recombinant T cell receptor specific to a MHC-restricted peptide derived from the cancer-testis antigen NY-ESO-1 have recently shown impressive clinical efficacy in multiple myeloma and sarcoma. See for example, Rapoport AP, et al., Nat Med. 2015 Aug;21(8):914-21; Robbins PF, et al., Clin Cancer Res. 2015 Mar 1;21(5):1019-27.).

However, setbacks have also been encountered, namely toxicities, especially in the case of rTCR with in vitro enhanced affinities (see e.g., Linette GP, et al., Blood. 2013 Aug 8;122(6):863-71.). Accordingly, notwithstanding the foregoing efforts, there remains a continuing need for more effective and safe methods for redirecting T cells to tumors or other targets of interest.

The need still exists for identifying effective treatment options for cancer. To this end, identification of features in a cancer and biomarkers in patients, specifically predictive immune markers indicating a favorable response to immunotherapies, may be an effective approach to develop compositions, methods and assays for evaluating and treating the cancer through modulation of the immune system (Angell et al., Curr Opin Immunol. 2013; 25:261-7.). However, the correlation of immune response and clinical benefit in patients who receive active immunotherapy remains controversial. Thus, there remains a continuing need to

identify those patients who are likely to respond to a particular cancer therapy and there remains an urgent need to develop subtype specific or biomarker driven therapies.

SUMMARY

5 In one aspect, the present disclosure provides a method of identifying a mammalian subject that is likely to benefit from a tumor antigen-specific cancer therapy, in particular an *in vivo* DC targeted lentiviral vector tumor antigen-specific cancer therapy, comprising detecting in one or more biological samples from the mammalian subject the presence of one, two, three or four of the following: (i) a polynucleotide encoding a TCR polypeptide
10 comprising a V β CDR3 that is specific for the tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence; (ii) a tumor antigen-specific antibody; (iii) a tumor antigen-specific CD4+ or CD8+ T cell; and (iv) the presence of the tumor antigen in > about 50% of tumor cells in a tumor sample. In certain embodiments, the presence of at least two of (i) – (iv) is indicative that the subject is likely to benefit from the tumor antigen-specific cancer therapy. As used herein, the phrase “is likely to benefit from” can be used
15 interchangeably with the phrase “a patient is suitable for.” Generally, such methods are carried out prior to starting the treatment. As described elsewhere herein, similar methods may be used to determine whether to continue a treatment that has already begun. In one particular embodiment, the present disclosure provides a method of identifying a mammalian
20 subject that is likely to benefit from an *in vivo* DC targeted lentiviral vector tumor antigen cancer therapy comprising detecting in one or more biological samples from the mammalian subject the presence of the following: (i)) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for the tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence; (ii) the presence of the tumor antigen in >
25 about 50% of tumor cells in a tumor sample; wherein the presence of (i) and (ii) is indicative that the subject is likely to benefit from the tumor antigen-specific cancer therapy.

In one aspect, the present disclosure provides a method of identifying a mammalian subject that is likely to benefit from an NY-ESO-1 cancer therapy, in particular an *in vivo* DC targeted lentiviral vector NY-ESO-1 cancer therapy, comprising detecting in one or more
30 biological samples from the mammalian subject the presence of one, two, three or four of the following: (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of

CASSLNDRXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both, or a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNDRXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both; (ii) an NY-ESO-1 specific antibody; (iii) an NY-ESO-1 specific CD4+ or CD8+ T cell; and (iv) the presence of NY-ESO-1 in > about 50% of tumor cells in a tumor sample. In certain embodiments, the presence of at least two of (i) – (iv) is indicative that the subject is likely to benefit from the NY-ESO-1 cancer therapy. Generally, such methods are carried out prior to starting the treatment. As described elsewhere herein, similar methods may be used to determine whether to continue a treatment that has already begun. In one particular embodiment, the present disclosure provides a method of identifying a mammalian subject that is likely to benefit from an *in vivo* DC targeted lentiviral vector NY-ESO-1 cancer therapy comprising detecting in one or more biological samples from the mammalian subject the presence of the following: (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises an amino acid sequence selected from the group consisting of CASSLNDRDYGTYF (SEQ ID NO: 2), CASSLNDRDQPQHF (SEQ ID NO: 3) and CASRLAGQETQYF (SEQ ID NO: 4), or any combination of one or more of the foregoing.; and (ii) the presence of NY-ESO-1 in > about 50% of tumor cells in a tumor sample; wherein the presence of (i) and (ii) is indicative that the subject is likely to benefit from the NY-ESO-1 cancer therapy. In one embodiment of the methods herein, the V β CDR3 comprises an amino acid sequence selected from the group consisting of CASSLNDRDYGTYF (SEQ ID NO: 2), CASSLNDRDQPQHF (SEQ ID NO: 3) and CASRLAGQETQYF (SEQ ID NO: 4), or any combination of one or more of the foregoing.

Another aspect of the present disclosure provides a method of treating cancer comprising first identifying a subject that is likely to benefit from a tumor antigen-specific cancer therapy, such as an *in vivo* DC targeted lentiviral vector tumor antigen cancer therapy, comprising detecting in one or more biological samples from the mammalian subject the presence of at least two of the following: (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for the tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence; (ii) a tumor antigen-specific antibody; (iii) a tumor antigen-specific CD4+ or CD8+ T cell; and (iv) the presence of the tumor antigen in > about 50% of tumor cells in a tumor sample; wherein the presence of at least two of (i) – (iv) is indicative that the subject is likely to benefit from the NY-ESO-1 cancer therapy; and then

administering a tumor antigen-specific cancer therapy.

Another aspect of the present disclosure provides a method of treating cancer comprising first identifying a subject that is likely to benefit from an NY-ESO-1 cancer therapy, such as an in vivo DC targeted lentiviral vector NY-ESO-1 cancer therapy, comprising detecting in one or more biological samples from the mammalian subject the presence of at least two of the following: (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNDRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both, or a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNDRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both; (ii) an NY-ESO-1 specific antibody; (iii) an NY-ESO-1 specific CD4+ or CD8+ T cell; and (iv) the presence of NY-ESO-1 in > about 50% of tumor cells in a tumor sample; wherein the presence of at least two of (i) – (iv) is indicative that the subject is likely to benefit from the NY-ESO-1 cancer therapy; and then administering a NY-ESO-1 cancer therapy.

The present disclosure provides in one embodiment a method of treating cancer in a patient where the cancer is a locally advanced unresectable or metastatic synovial sarcoma. In another embodiment, the patient has no evidence of progression after first-line chemotherapy. Thus, the present disclosure provides in one embodiment a method of treating cancer where the cancer is a locally advanced unresectable or metastatic synovial sarcoma in a patient who has no evidence of progression after first-line chemotherapy. In certain embodiments, the method of treating cancer where the cancer is a locally advanced unresectable or metastatic synovial sarcoma in a patient who has no evidence of progression after first-line chemotherapy, comprises first identifying whether the patient is likely to benefit from an NY-ESO-1 cancer therapy, such as an in vivo DC targeted lentiviral vector NY-ESO-1 cancer therapy, comprising detecting in one or more biological samples from the patient the presence of at least two of the biomarkers disclosed herein, and then administering to the patient the in vivo DC targeted lentiviral vector NY-ESO-1 cancer therapy.

In certain embodiments, the tumor antigen-specific cancer therapy contemplated herein comprises administering to the subject a therapeutic composition, said composition comprising one or more therapeutic agents selected from (a) an isolated cell, wherein said isolated cell is a T cell comprising a nucleic acid, or expression vector, wherein said nucleic

acid comprises a polynucleotide sequence that encodes a chimeric heterodimeric TCR; wherein the expression vector is a retroviral vector comprising a polynucleotide sequence that encodes a chimeric heterodimeric TCR; (b) an isolated cell, wherein said isolated cell is a T cell comprising a nucleic acid or expression vector, wherein said nucleic acid comprises a polynucleotide sequence that encodes a single chain TCR; wherein the expression vector is a retroviral vector comprising a polynucleotide sequence that encodes a single chain TCR; (c) an *in vivo* DC targeted retroviral vector wherein the retroviral vector expresses the tumor antigen; wherein the therapeutic composition is administered in an amount effective to treat the cancer in the subject. In one embodiment of the method, the chimeric heterodimeric TCR comprises: a. a first polypeptide comprising a TCR beta chain variable region, a TCR beta chain constant region, and optionally a transmembrane domain and a cytoplasmic signaling domain; b. a second polypeptide comprising a TCR alpha chain variable region, a TCR alpha chain constant region, and optionally a transmembrane domain and a cytoplasmic signaling domain; wherein the heterodimeric TCR specifically binds to the tumor antigen/ MHC complex (e.g., an NY-ESO-1/MHC complex), wherein the TCR beta chain variable region comprises a public TCR V β CDR3; wherein the TCR alpha chain variable region comprises the cognate TCR alpha chain variable region amino acid sequence; and wherein there is at least one disulfide bond between the first polypeptide and the second polypeptide. In one embodiment, the TCR beta chain variable region comprises the TCR beta chain variable region amino acid sequence set forth in SEQ ID NO:9; wherein the TCR alpha chain variable region comprises the cognate TCR alpha chain variable region amino acid sequence set forth in SEQ ID NO:8; and wherein there is at least one disulfide bond between the first polypeptide and the second polypeptide. In another embodiment of the method, the TCR beta chain variable region CDR3 comprises the amino acid sequence CASRLAGQETQYF (SEQ ID NO: 4). In a further embodiment of the method, the chimeric heterodimeric TCR is soluble, and wherein the first polypeptide and the second polypeptide do not comprise the transmembrane domain and the cytoplasmic signaling domain. In certain embodiments, the single chain TCR comprises a beta chain variable region comprising a TCR beta chain variable region amino acid sequence set forth in SEQ ID NO:9; and a TCR alpha chain variable region comprising the cognate TCR alpha chain variable region amino acid sequence as set forth in SEQ ID NO:8. In another embodiment, the single chain TCR is a soluble single chain TCR and wherein the single chain TCR does not comprise the transmembrane domain and the cytoplasmic signaling domain.

In certain embodiments of any of the methods described herein, the tumor antigen-specific cancer therapy comprises administering a vector encoding a tumor antigen polypeptide. In this regard, the vector may comprise a dendritic cell targeting retroviral vector and in particular, a lentiviral vector. In some embodiments of the methods described

5 herein, the method may comprise administering an adjuvant to the subject in addition to a retroviral vector. In some embodiments, the adjuvant is a TH1 inducing adjuvant, such as glucopyranosyl lipid A (GLA). In this regard, the GLA may be formulated in a stable oil-in-water emulsion. In other embodiments, the GLA composition may be in an aqueous formulation.

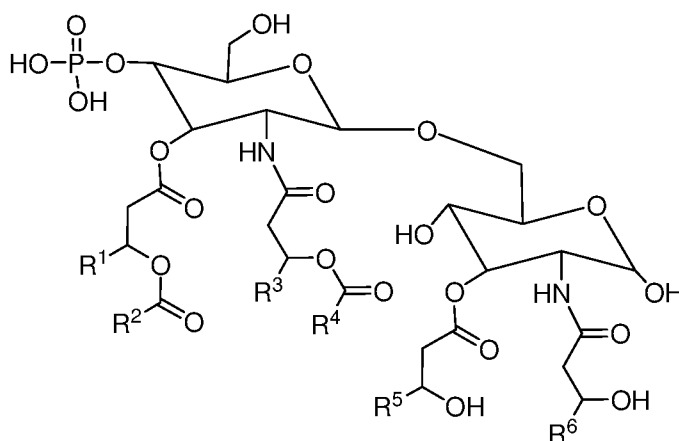
10 In certain embodiments of any of the methods described herein, the NY-ESO-1 cancer therapy comprises administering a vector encoding an NY-ESO-1 polypeptide. In this regard, the vector may comprise a dendritic cell targeting retroviral vector and in particular, a lentiviral vector. In some embodiments of the methods described herein, the method may comprise administering an adjuvant to the subject in addition to a retroviral vector. In some

15 embodiments, the adjuvant is glucopyranosyl lipid A (GLA). In this regard, the GLA may be formulated in a stable oil-in-water emulsion. In other embodiments, the GLA composition may be in an aqueous formulation.

In certain embodiments of the methods described herein, the vector is a lentiviral vector. In another embodiment of any of the methods described herein, the NY-ESO-1

20 cancer therapy comprises administering to the subject a composition comprising GLA, said composition comprising:

(a) GLA of the formula:



wherein:

R1, R3, R5 and R6 are C11-C20 alkyl; and

R2 and R4 are C12-C20 alkyl; and

(b) a pharmaceutically acceptable carrier or excipient.

5 In certain embodiments, the composition comprising GLA does not comprise antigen. In certain embodiments, R1, R3, R5 and R6 are undecyl and R2 and R4 are tridecyl. In certain embodiments, the composition is an aqueous formulation or may be in the form of an oil-in-water emulsion, a water-in-oil emulsion, liposome, micellar formulation, or a microparticle. In certain embodiments, the composition is administered by subcutaneous, intradermal, intramuscular, intratumoral, or intravenous injection. In certain embodiments of the methods herein, the composition is administered in conjunction with one or more additional therapeutic agents or treatments. In certain embodiments, the therapeutic agent is an immune checkpoint inhibitor or an antibody that activates a costimulatory pathway, including but not limited to an anti-CD40 antibody. In some embodiments, the one or more additional therapeutic agents or treatments is a cancer therapeutic agent. Such cancer therapeutic agents may be selected from the group consisting of taxotere, carboplatin, trastuzumab, epirubicin, cyclophosphamide, cisplatin, docetaxel, doxorubicin, etoposide, 5-FU, gemcitabine, methotrexate, and paclitaxel, mitoxantrone, epothilone B, epidermal-growth factor receptor (EGFR)-targeting monoclonal antibody 7A7.27, vorinostat, romidepsin, docosahexaenoic acid, bortezomib, shikonin and an oncolytic virus. In certain embodiments, the one or more additional therapeutic treatments is radiation therapy.

In any of the methods described herein, the mammal may be a human.

25 In certain embodiments of any of the methods described herein, the cancer comprises a solid tumor. Cancers contemplated herein may be selected from the group consisting of a sarcoma (including without limitation osteosarcoma, chondrosarcoma, liposarcoma, leiomyosarcoma, soft tissue sarcoma, synovial sarcoma, myxoid round cell liposarcoma, and spindle cell sarcoma), prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, oesophageal cancer, non-small-cell lung cancer, non-Hodgkin's lymphoma, multiple myeloma, melanoma, hepatocellular carcinoma, head and neck cancer, gastric cancer, endometrial cancer, colorectal cancer, cholangiocarcinoma,

breast cancer, bladder cancer, myeloid leukemia and acute lymphoblastic leukemia.

Another aspect of the present disclosure provides a method of identifying a mammalian subject undergoing a tumor antigen-specific cancer therapy that is likely to benefit from an extended tumor antigen-specific cancer therapy comprising, detecting in a sample from the mammalian subject the presence of one or more, or at least two of the following as compared to before the cancer therapy: (i) induction of a tumor antigen-specific CD8 T cell response; (ii) induction of a tumor antigen-specific immune response comprising each of a tumor antigen-specific CD4 T cell response, a tumor antigen-specific CD8 T cell response, and tumor antigen-specific antibody production; (iii) antigen spreading; (iv) increased T cell clonality; and (v) increase in the presence of (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for the tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence. In certain embodiments, the presence of one or more, or at least two of the foregoing indicates that the subject is likely to benefit from an extended tumor antigen-specific cancer therapy. In certain embodiments, the presence of at least three of the foregoing indicates that the subject is likely to benefit from an extended tumor antigen-specific cancer therapy. In certain embodiments, the presence of induction of T cells expressing a polynucleotide encoding a tumor-antigen-specific public TCR indicates that the subject is likely to benefit from continuing the tumor antigen-specific cancer therapy.

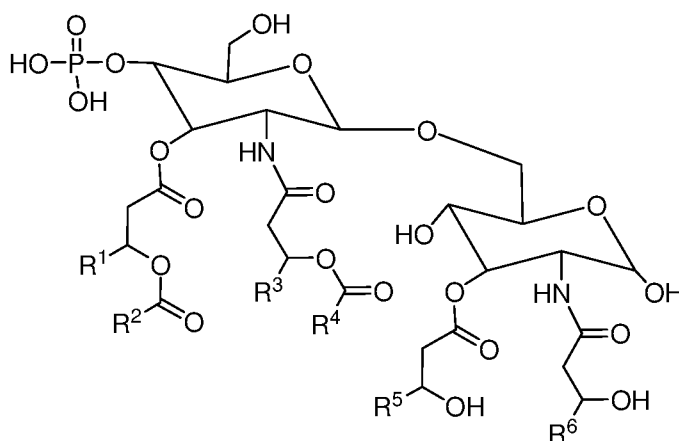
Another aspect of the present disclosure provides a method of identifying a mammalian subject undergoing an NY-ESO-1 cancer therapy that is likely to benefit from an extended NY-ESO-1 cancer therapy comprising detecting in a sample from the mammalian subject the presence of one or more, or at least two of the following as compared to before the cancer therapy: (i) induction of an NY-ESO-1 specific CD8 T cell response; (ii) induction of an immune response comprising each of an NY-ESO-1 specific CD4 T cell response, an NY-ESO-1 specific CD8 T cell response, and NY-ESO-1 antibody production; (iii) antigen spreading; (iv) increased T cell clonality; and (v) increase in the presence of (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both, or a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ

ID NO: 4), or both. In certain embodiments, the presence of at least two of the foregoing indicates that the subject is likely to benefit from an extended NY-ESO-1 cancer therapy. In certain embodiments, the presence of at least three of the foregoing indicates that the subject is likely to benefit from an extended NY-ESO-1 cancer therapy. In certain embodiments of this aspect, the V β CDR3 comprises an amino acid sequence selected from the group consisting of CASSLNRDYGYTF (SEQ ID NO: 2), CASSLNRDQPQHF (SEQ ID NO: 3) and CASRLAGQETQYF (SEQ ID NO: 4), or any combination of one or more of the foregoing.

Another aspect of the present disclosure provides a method of treating cancer comprising identifying a subject as described in the previous method and then continuing the tumor antigen-specific cancer therapy. In one embodiment, the tumor antigen-specific cancer therapy is an NY-ESO-1 cancer therapy. In certain embodiments, the tumor antigen-specific cancer therapy (e.g., NY-ESO-1 cancer therapy) comprises administering a vector encoding a tumor antigen (e.g., NY-ESO-1 polypeptide). In one particular embodiment, the vector comprises a dendritic cell targeting retroviral vector and in some cases, the vector is a lentiviral vector. In some embodiments of the methods, the method further comprises administering an adjuvant to the subject. In this regard, the adjuvant may be glucopyranosyl lipid A (GLA), which may be formulated in a stable oil-in-water emulsion.

In some embodiments of the methods herein, the tumor antigen-specific (e.g., NY-ESO-1-specific) cancer therapy comprises administering to the subject a composition comprising GLA, said composition comprising:

(a) GLA of the formula:



wherein:

R1, R3, R5 and R6 are C11-C20 alkyl; and

R2 and R4 are C12-C20 alkyl; and

(b) a pharmaceutically acceptable carrier or excipient.

5 In certain embodiments, the composition comprising GLA does not comprise antigen. In certain embodiments, R1, R3, R5 and R6 are undecyl and R2 and R4 are tridecyl. In one embodiment, the composition is an aqueous formulation. In another embodiment, the composition is in the form of an oil-in-water emulsion, a water-in-oil emulsion, liposome, micellar formulation, or a microparticle.

10 In certain embodiments of the methods herein, the cancer comprises a solid tumor. The cancer may be selected from the group consisting of a sarcoma, prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, oesophageal cancer, non-small-cell lung cancer, non-Hodgkin's lymphoma, multiple myeloma, melanoma, hepatocellular carcinoma, head and neck cancer, gastric cancer,
15 endometrial cancer, colorectal cancer, cholangiocarcinoma, breast cancer, bladder cancer, myeloid leukemia and acute lymphoblastic leukemia. In one embodiment, the sarcoma is a soft tissue sarcoma, such as

 In some embodiments of the methods herein, the composition is administered by subcutaneous, intradermal, intramuscular, intratumoral, or intravenous injection. In other
20 embodiments, the composition is administered in conjunction with one or more additional therapeutic agents or treatments such as an immune checkpoint inhibitor, an antibody that activates a costimulatory pathway (e.g, an anti-CD40 antibody). In one embodiment, the therapeutic agent is a cancer therapeutic agent. The cancer therapeutic agent may be selected from the group consisting of taxotere, carboplatin, trastuzumab, epirubicin,
25 cyclophosphamide, cisplatin, docetaxel, doxorubicin, etoposide, 5-FU, gemcitabine, methotrexate, and paclitaxel, mitoxantrone, epothilone B, epidermal-growth factor receptor (EGFR)-targeting monoclonal antibody 7A7.27, vorinostat, romidepsin, docosahexaenoic acid, bortezomib, shikonin and an oncolytic virus. In one embodiment, the one or more additional therapeutic treatments is radiation therapy.

Another aspect of the present disclosure provides a method of identifying a mammalian subject that is likely to benefit from an in vivo DC targeted lentiviral vector NY-ESO-1 cancer therapy comprising detecting in one or more biological samples from the mammalian subject the presence of the following: (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises an amino acid sequence selected from the group consisting of CASSLNRDYGYTF (SEQ ID NO: 2), CASSLNRDQPQHF (SEQ ID NO: 3) and CASRLAGQETQYF (SEQ ID NO: 4), or any combination of one or more of the foregoing; and (ii) the presence of NY-ESO-1 in > about 50% of tumor cells in a tumor sample; wherein the presence of (i) and (ii) is indicative that the subject is likely to benefit from the NY-ESO-1 cancer therapy.

In any of the methods herein the mammal may be a human.

Another aspect of the present disclosure provides kits for identifying patients that are likely to benefit from a tumor antigen-specific cancer therapy. In one aspect, the present disclosure provides a kit for identifying a patient likely to benefit from a tumor antigen-specific cancer therapy comprising two or more of the following: (i) a set of polynucleotides for detecting a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for a tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence; (ii) ELISA reagents for detecting a tumor antigen-specific antibody; (iii) a sufficient amount of recombinant tumor antigen, or a set of overlapping peptides spanning the tumor antigen, suitable for stimulating tumor antigen-specific CD4⁺ or CD8⁺ T cells; and (iv) an antibody specific for the tumor antigen useful to detect the presence of the tumor antigen in a tumor sample.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Treatment with LV305 results in increased affinity of a polyclonal NY-ESO-1 specific T cell response. Cryopreserved PBMC from pre-Tx and post-Tx leukapheresis samples were thawed and used to set up the ELISPOT assay. 200,000 cells were plated into each well of the ELISPOT plate. Cells were treated with different concentrations of NY-ESO-1 peptide mix, which contains 43 of 15mer peptides that are overlapping by 11 amino acid. The concentration of peptides tested were 2.5 ug/mL (1670 nM), 0.5 ug/mL (334nM), 0.1 ug/mL (60nM), and 0.02 ug/mL (12nM). Cells were incubated with the peptides or control treatment medium for 40 hr. The spots were counted by using an

automated spot counter from C.T.L Technologies. The data symbol represents the average number of spot-forming units (SFU) per million PBMC in pre-Tx sample (empty circle) or post-Tx sample (filled square) at each of the tested concentration. The error bar represents standard deviation at each treatment condition.

5 Figure 2: Tumor antigen-specific TCR sequences are enriched in post-Tx PBMC as compared to pre-Tx PBMC. In this study, the PBMC were collected from the patient before LV305 treatment and after three vaccinations with LV305. A pre-therapy tumor sample was also collected from the patient. The PBMC and tumor samples were subjected to DNA extraction and subsequent sequencing analysis of the T cell receptor (TCR) beta chain. The sequence similarity between pre-Tx and post-Tx PBMC was analyzed using scatter plot and the TCR sequences obtained from the tumor sample were also compared to the patients pre-Tx and post-Tx PBMC for similarity. The result showed that the TCR sequence from tumor samples, which contain tumor infiltrating lymphocytes that recognize tumor antigens, are enriched in the patient's post-Tx PBMC as compared to pre-Tx PBMC.

15 Figure 3: Establishment of an oligoclonal culture from post-Tx PBMC through in vitro culture (IVS), that is highly enriched for NY-ESO-1 specific T. PBMC were collected from a patient Pt151006 after three vaccinations with LV305. The PBMC were cultured in OpTmizer T cell expansion medium (Invitrogen, Carlsbad, CA) with NY-ESO-1 overlapping peptides (0.5 ug/mL, JPT Technologies, Berlin, Germany) in the presence of IL-2 and IL-7 (10 ng/mL). After repeated stimulation, the PBMC culture was highly enriched for NY-ESO-1 specific T cells as measured by ELISPOT assay. (A) Representative ELISPOT showing the secretion of IFN- γ of the oligoclonal T cells upon stimulation with NY-ESO-1 peptide pool. The cells (10K/well) were plated in triplicate in ELISPOT plates pre-coated with anti-IFN γ capture antibody (MabTech). The cells were treated with NY-ESO-1 peptide pool (2.5 ug/mL) or control medium (No Ag) and incubated in a CO₂ incubator for 40 hr. The cells were then washed off and the plate was incubated with a HRP-conjugated secondary antibody for 2 hours before TMB substrate was added. The number of spot forming units (SFU) per well was counted by an automated plate reader. Shown is the image of three wells with No Ag treatment (top row) and three wells with NY-ESO-1 treatment (bottom row). (B) Summary graph showing the number of SFU per well in the No Ag control and NY-ESO-1 peptide mix-treated wells. The column represents the Mean $\pm$ SEM of each group. (C) Show are the top clones from the oligoclonal culture as determined by TCR β deep sequencing

analysis. Each column represents one clone with a unique TCR β CDR3 sequence. The y-axis shows the relative frequency of each clone (percentage) among all sequence reads from the oligoclonal culture. The top 6 clones account for more than 90% of all the TCRs, indicating that the culture is highly oligoclonal.

5 Figure 4: Several high-frequency TCR β CDR3 sequences in the oligoclonal culture are enriched in post-Tx PBMC as compared to pre-Tx PBMC. Shown is a log-scaled scatter plot comparing the TCR sequences in pre-Tx PBMC (x-axis) to post-Tx PBMC (y-axis) of the same patient, from whom the NY-ESO-1 specific oligoclonal culture was derived. The sequences from the oligoclonal culture was overlaid onto the PBMC sequence. There is a
10 skewing of the overlapping sequencing toward the y-axis, indicating that the NY-ESO-1 specific sequences are more frequently found in post-Tx PBMC than pre-Tx PBMC, as an indication that LV305 induced NY-ESO-1 specific T-cell responses.

 Figure 5: A TCR β CDR3 clone that has a frequency of 20.5% in PT151006 IVS3 can be detected in PT151016 post-Tx PBMC. Shown is a log-scaled scatter plot comparing the
15 TCR sequences of the oligoclonal culture from PT151006 IVS3 (y-axis) to the TCR sequences detected in post-Tx PBMC from a second patient, PT151016. The two clones that have high frequency in IVS3 are also detectable in post-Tx sample from PT151016. The box within the scatter plot shows the amino acid sequence of the CDR3 region of the TCR β and the percent of frequency in PT151016 post-Tx PBMC (0.000298) and the percent of
20 frequency in IVS3 (20.5). A similar analysis showed that this sequence is non-detectable in pre-Tx PBMC from PT151016.

 Figure 6: A TCR β CDR3 clone that has a frequency of 8.5% in PT151006 IVS3 can be detected in PT151016 post-Tx PBMC. Shown is a log-scaled scatter plot comparing the
25 TCR sequences of the oligoclonal culture from PT151006 IVS3 (y-axis) to the sequence in post-Tx PBMC from a second patient, PT151016. Two clones with a high frequency in IVS3 are also detectable in post-Tx sample from PT151016. The box within the scatter plot shows the amino acid sequence of the CDR3 region of the TCR β and the percent of frequency in PT151016 post-Tx PBMC (0.000642) and the percent of frequency in IVS3 (8.52). Similar analysis showed that this sequence is non-detectable in pre-Tx PBMC from PT151016.

30 Figure 7: A TCR β CDR3 clone that has a frequency of 26.2% in PT151006 IVS3 can be detected in PT151014 post-Tx PBMC. Shown is a log-scaled scatter plot comparing the

TCR sequences of the oligoclonal culture from PT151006 IVS3 (y-axis) to the sequence in post-Tx PBMC from PT151014. The clone that have the highest frequency in IVS3 (26.2%) is also detectable in post-Tx sample from PT151014. The box within the scatter plot shows the amino acid sequence of the CDR3 region of the TCR β and the percent of frequency in PT151014 post-Tx PBMC (0.00076) and the percent of frequency in IVS3 (26.2). Similar analysis showed that this sequence is non-detectable in pre-Tx PBMC from PT151014.

Figure 8: frequency of three public TCR β CDR3 sequences in pre-Tx and post-Tx PBMC from eight sarcoma patients. The bar graph shows the frequency of public TCR in pre-Tx (hatched bar) and post-Tx (black bar) PBMC from each of the eight tested patients. All patients have received LV305 treatment. The amino acid sequence of the relevant TCR β CDR3 is included in the top of each graph. See also Tables 1-3. 8A: SEQ ID NO:2; 8B: SEQ ID NO:3; 8C: SEQ ID NO:4.

Figure 9: Public TCRs that are shared between patients have different nucleotide sequences in each individual. Shown are the nucleotide sequences and amino acid sequences for the three public TCR from three patients. There is no complete homology at nucleotide level between different patients even though the sequences are the same at amino acid level. This is consistent with the concept of convergent recombination, which has been proposed for the generation of public TCR (Venturi et al., Nat Rev Immunol., 2008).

Figure 10: Public TCR β CDR3 sequences have a shorter length and fewer nontemplated nucleotide additions at the VJD junction region. (A) Shown is the TCR β CDR3 length distribution in IVS3 (black column), the oligoclonal NY-ESO-1 specific T-cell in vitro culture from Pt151006, and the TCR β CDR3 length distribution in the post-Tx PBMC from the same patient (hatched column). (B) The nucleotide sequence (CDR3 encoding sequence is underlined) and amino acid sequence of the CDR3 of the three public TCRs. The number of deletions and additions in the junction regions are also listed for each TCR. All three public TCR have the same CDR3 nucleotide length (n=39). These TCRs also have relatively few non-templated nucleotide additions at the junction region. One of the public TCRs we identified, CASRLAGQETQYF (SEQ ID NO: 4), had no nucleotide addition at either the N1 (VD) and N2 (DJ) insertion sites. The shorter CDR3 length and limited nt addition support the conclusion that these TCRs are public TCRs.

Figure 11: The sequence of the full TCR α (SEQ ID NO: 8) and TCR β (SEQ ID NO:

9) variable regions for one of the identified public TCR CDR3 CASRLAGQETQYF (SEQ ID NO: 4). This CDR3 sequence has a 26.2% frequency in IVS3 and is also shared in PT151014 and PT151050, as listed in Table 3. The annotation shown is standard annotation available from the IMGT database (The international ImMunoGeneTics information system; internet address at IMGT (dot) org). A BLAST search of the TCR α sequence indicates homology with a known NY-ESO-1 specific TCR α sequence.

Figure 12: An alignment of the polynucleotide sequences encoding public TCR β CDR3s identified from different cancer patients shows different nucleic acid sequences from different patients encoding the same CDR3 amino acid sequence. The nucleotide sequence SEQ ID NOs are as follows: 1st public TCR β CDR3 – PT006: SEQ ID NO: 5; PT016: SEQ ID NO: 10; PT050: SEQ ID NO: 11; 2nd public TCR β CDR3 – PT 006: SEQ ID NO: 6; PT 016: SEQ ID NO: 12; PT 050; SEQ ID NO: 13; 3rd public TCR β CDR3 – PT 006: SEQ ID NO: 7; PT 016: SEQ ID NO: 14; PT 050: SEQ ID NO: 15. (see also Figure 9)

Figure 13 shows the amino acid sequence of a TCR β chain (SEQ ID NO: 16) identified from an NY-ESO-1 expanded T cell culture (IVS3) expanded from PBMCs from a cancer patient. The sequence was isolated as described in Example 11.

Figure 14: MCC patient G2 expresses NY-ESO-1 and the expression level decreases after treatment with G100. Tumor biopsy was taken at baseline prior to intratumoral G100 treatment and at 4 weeks after treatment with G100. RNA was extracted from snap frozen biopsy tissue and 200ng of RNA was used for nanostring gene expression analysis by using the human panCancer Immune Profiling kit. Shown is the expression of CTAG1B gene, which encodes NY-ESO-1, the cancer testis antigen. The Y-axis shows the binding density, which reflects the expression level of the gene. The expression level was lower in post-G100 sample as compared to baseline.

Figure 15: T cells with public TCRs are detectable after treatment with G100 in a lymphnode biopsy of MCC patient G2. A tumor biopsy was taken at baseline prior to intratumoral G100 treatment and at 4 weeks after treatment with G100. DNA was extracted from snap frozen biopsy tissue and used for deep sequencing analysis of the CDR3 region of the TCR beta chain. Shown are two scatter plots showing the sequence overlaps between tumor biopsies from patient G2 and TCR isolated from an oligoclonal, NY-ESO-1 stimulated T-cell culture obtained from patient 151006 in the LV305 trial. Graph (A) shows the

correlation between pre-G100 sample (X-axis) and the NY-ESO-1 specific TCRs from LV305 patient 151006 (Y-axis). Graph (B) shows the correlation between post-G100 sample (X-axis) and the NY-ESO-1 specific TCRs from LV305 patient 151006 (Y-axis). Two public CDR3 sequences that were non-detectable in a pre-G100 lymph node biopsy with MCC were detectable in a biopsy of a draining lymph node post-G100 treatment.

Figure 16. The three public TCR β CDR3 sequences were detected in patients from the anti-CTLA4 clinical trial. Shown are the frequency of the 3 public CDR3 amino acid sequences in the 21 patients on the anti-CTLA4 trial. The numbers on the X axis represent patient number. There are two columns associated with each number. The column on the left indicates frequency in pre-Tx PBMC. The column on the right indicates frequency in post-Tx PBMC.

Figure 17. Patient 151006 and patient 151119 use different TCR β V-genes for the same CDR3 sequence, CASSLNRDQPQHF (SEQ ID NO:3). The majority of TCR β use the V07-07 gene. However, a small percentage of the TCR β receptors use V07-08, V07-06, V07-09, V07-02, V07-03, V07-04, or V11-02. In patient 151119, only V07-08 is used for this TCR CDR3. Both patients use the same TCR β J-gene, J01-05.

Figure 18: This figure shows that one of the public TCR β CDR3 CASSLNRDQPQHF (SEQ ID NO:3) was encoded by at least three different nucleotide sequences in patient PT151006. The polynucleotide sequences are provided in SEQ ID NOs:

Figure 19. Patients from different clinical trials have different nucleotide sequences and different TCR β V-gene usage for the same CDR3 amino acid sequences. For the first public TCR, CASSLNRDYGYTF (SEQ ID NO:2), PT151006 (from LV305 trial) and C131-001 (from C131 trial) and patient G2-C1W4B (from the G100-MCC trial) -use TCR β V07-08, TCR β V02-01, and TCR β V28-01, respectively. For the second public TCR, CASSLNRDQPQHF (SEQ ID NO:3), PT151006 use TCR β V07-07. Patient 131-013 uses three different TCR β V, V05-08, V13-01, and V05-05. For the third public TCR, CASRLAGQETQYF (SEQ ID NO:4), PT151006, C131-001, and G2-C1W4B use TCR β V28-01, TCR β V06-06, and TCR β V25-01, respectively.

Figure 20. The NY-ESO-1 specific T cell culture from PT151006 (IVS3) are CD4 T cells. Shown are side-by-side staining of IVS3 cell culture (bottom row) with PBMC from a normal donor (top row). The cells were stained with anti-CD3-Pacific blue (PB), anti-CD4-

FITC, anti-CD8-PerCP, and anti-CD56-APC. Samples were acquired on a BD LSRII flow cytometer. Data analysis was done using the FlowJo software. The lymphocytes population was first gated on the FSC/SSC plot, then CD4 T cells were gated as CD3+CD4+ lymphocytes and CD8 T cells were gated as CD3+CD8+ lymphocytes. The NK cells were gated as CD3-CD56+ lymphocytes. The control donor PBMC has the expected percentages of CD4, CD8 T cells and NK cells, as normally observed in healthy donor PBMC (top row). In contrast, the cultured cells from PT151006-IVS3 show a lack of NK cells and CD8 T cells and only contains CD4 T cells (bottom row).

Figure 21 CMB305 patient immune responses have increased clonality post treatment. Side by side box plot of Post/Pre Treatment ratio for Clonality, Entropy, and Richness of T cell receptor repertoire in patients who received CMB305 or LV305 vaccine. A=CMB305, B=LV305. The graph shows that by raw values, CMB305 shows *significant clonality increase* post/pre ($p=0.039$) and trend towards a decrease in entropy ($p=0.069$, Wilcoxon Signed Rank Test). No difference in richness was observed. CMB305 has a possible trend toward higher clonality ratio (post/pre) than LV305 ($p=0.293$, Wilcoxon Rank Sum Test). No difference in the entropy or richness ratios vs. LV305 was observed.

Figure 22 shows survival curves of patients in the LV305 and CMB305 clinical trials for those patients having the indicated biomarker(s). A: Overall survival (OS) for patients having CD8+T cells induced by treatment; B: OS for patients having pre-existing T cells (CD4 and/or CD8 T cells) at the time of treatment; C: OS for patients having an integrated immune response (antibody, CD4+ and CD8+T cells) induced by treatment; D: progression free survival (PFS) for patients having CD8+T cells induced by treatment; E: PFS for patients having pre-existing T cells (CD4 and/or CD8) at the time of treatment; F: PFS for patients having an integrated immune response (antibody, CD4+ and CD8+T cells) induced by treatment. PFS for patients having pre-existing T cells was highly statistically significant as compared to patients who did not have pre-existing T cells. OS in patients having an induced integrated response was statistically significant.

Figure 23 shows survival curves of patients in the LV305 and CMB305 clinical trials for those patients having the indicated biomarker(s). A: PFS for patients having any one of three public TCR (pTCR) as disclosed herein at any time point (before or after therapy). B: PFS for patients having any one of three pTCR and >50% tumor cells expressing NY-ESO-1. PFS for patients in whom the pTCR was detected, as well as for those having both pTCR

and >50% tumor cells expressing NY-ESO-1 was highly statistically significant as compared to patients who did not (see also Summary Table 11).

Figure 24 is a bar graph summarizing the immune response from patients in the LV305 and CMB305 clinical trials and shows that CMB305 induced stronger and broader immune responses than LV305 (see also Table 11). Antigen spreading was observed in 2/12 LV305 patients and 4/12 CMB305 patients. Anti-NY-ESO-1 Abs detected by ELISA against either the whole NY-ESO-1 protein or to peptides. A 4-fold rise or newly positive response was scored as positive (induced response). Anti-NY-ESO-1 CD4 and CD8 responses were determined to be positive by the following assays: IFN γ production detected by ELISPOT in separated T cell fractions; Intracellular cytokine staining for IFN γ or TNF α after stimulation with NY-ESO-1 peptides; ELISPOT >50 spots & >2-fold rise and/or ICS >2-fold over baseline were considered positive (induced response); Antigen spreading by ELISA with 24 rec. tumor associated antigens and selected ELISPOT assays.

Figure 25 shows that LV305 and CMB305 induce *de novo* immune responses and boost pre-existing immune responses.

Figure 26 shows that induction of anti-NY-ESO-1 immune response is associated with a better overall survival. All patients treated with LV305 or CMB305 (ID/IM and SQ dosing) and who had biomarker samples were analyzed (n=64). 50/64 pts (78%) of patients had an induced anti-NY-ESO-1 immune response on LV305 or CMB305 therapy (assessed by one of the antibody or T cell assays).

Figure 27A, B, C, D shows that baseline and induced immune responses are associated with survival. Biomarkers showing the strongest association with overall survival (OS) and progression free survival (PFS) were pre-existing anti-NY-ESO-1 T-cell responses and Ab, integrated IR (T cells and antibodies), and NY-ESO-1 expression level. Panels A and B show analysis for all patients (n=64) and panels C and D show analysis for soft tissue sarcoma patients (n=45) (A and C=overall survival; B and D= progression free survival).

Figure 28 shows the overall survival in soft tissue sarcoma patient (STS) patients receiving CMB305 treatment. The overall survival in soft tissue sarcoma patient (STS; including synovial sarcoma (N=14), MRCL (N=9) and spindle cell sarcoma (N=2) patients) on CMB305 (C131 Study; NCT02387125; Arm B SQ dose excluded) compares favorably to currently approved agents for recurrent soft tissue sarcoma where the survival ranges from

12.4 to 13.5 months.

Figure 29 shows association of three public NY-ESO-1 TCR (SEQ ID Nos:2-4) with overall survival (OS). Patients are stratified by presence (+) or absence (-) of any of the three pTCR pre-treatment, and induction (*i.e.* positive from negative baseline, or $\geq$ doubling of baseline frequency) of pTCR by LV305 or CMB305 treatment (+: induced, -: not induced). Patients with induction of pTCR by LV305/CMB305 from negative or positive baseline shows a trend for longer survival compared to patients with negative baseline and no induction ($p=n.s.$).

Figure 30 is a waterfall plot showing the percent target lesion change from baseline (safety population) in the C232 Phase 2 clinical trial (see Example 19). Top panel: Arm A (atezolizumab alone). Bottom panel: Arm C+A (CMB305 + Atezolizumab).

Figure 31 is a bar graph showing induction of NY-ESO-1 specific Ab and T cell responses and antigen epitope spreading in patients from the C232 clinical trial (see Example 19). Induction of NY-ESO-1 specific T cells was defined as a ≥ 2 fold increase in ELISPOT. Induction of NY-ESO-1 specific Ab was defined as ≥ 4 fold increase in ELISA. Antigen epitope spreading was defined as Ab response to tumor antigens other than NY-ESO-1.

Figure 32 is a Kaplan-Meier curve showing OS in patients with (solid lines) and without (dashed line)) induced anti-NY-ESO-1 T Cells. A: arm A. B: arm C+A.

Figure 33 is a Kaplan-Meier curve showing is a Kaplan-Meier curve showing OS in patients with (solid lines) and without (dashed line)) induced anti-NY-ESO-1 antibodies. A: arm A. B: arm C+A.

Figure 34 are bar graphs showing T cell responses in patients showing partial response on C232 study (see Example 19). T cell response was measured by ELISPOT. Cryopreserved PBMC thawed and plated at 300,000 cells/well (quadruplicate) into ELISPOT plate, incubated overnight (medium alone; negative control) or stimulated with NY-ESO-1 overlapping peptides (a mixture of 43 15mer peptides, overlapping by 11aa, from JPT Peptide Technologies, Berlin). Shown are the mean $\pm$ SEM of spots per well. Antibody response was measured by ELISA using recombinant NY-ESO-1 protein and serially diluted plasma samples. *1 patient with unconfirmed PR; NC = not collected; na – not analyzable; nd – not detectable.

BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

SEQ ID NO:1 is the amino acid sequence of a public TCR CDR3 consensus sequence CASSLNRDXXXXF.

5 SEQ ID NO:2 is the amino acid sequence of a first public TCR CDR3 CASSLNRDYGTYF.

SEQ ID NO: 3 is the amino acid sequence of a second public TCR CDR3 CASSLNRDQPQHF.

10 SEQ ID NO: 4 is the amino acid sequence of a third public TCR CDR3 CASRLAGQETQYF.

SEQ ID NO: 5 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 2.

SEQ ID NO: 6 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 3.

15 SEQ ID NO: 7 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 4.

SEQ ID NO: 8 is the amino acid sequence of the alpha chain of a public TCR as shown in Figure 11.

20 SEQ ID NO: 9 is the amino acid sequence of the beta chain variable region of a public TCR as shown in Figure 11. This public TCR has the V β CDR3 sequence as shown in SEQ ID NO: 4.

SEQ ID NO: 10 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 2 (see Figure 9).

25 SEQ ID NO: 11 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 2 (see Figure 9).

SEQ ID NO: 12 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 3 (see Figure 9).

SEQ ID NO: 13 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 3 (see Figure 9).

SEQ ID NO: 14 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 4 (see Figure 9).

5 SEQ ID NO: 15 is a polynucleotide sequence encoding the TCR V region including the CDR3 amino acid sequence set forth in SEQ ID NO: 4 (see Figure 9).

SEQ ID NO: 16 is the amino acid sequence of a TCR β chain identified from an NY-ESO-1 expanded T cell culture (IVS3) expanded from PBMCs from a cancer patient as described in Example 11. The sequence is shown in Figure 13 and annotated according to
10 methods outlined at the IMGT database website.

SEQ ID NO:17-23 are nucleotide sequences encoding public TCR β CDR3 sequences from patients C131-001, G2-C1WB4, C131-013 as shown in Figure 19.

SEQ ID NO:24-26 are nucleotide sequences from PT151006 all encoding public TCR β CDR3 sequence CASSLNRDQPQHF (SEQ ID NO:3) as shown in Figure 18.

15

DETAILED DESCRIPTION

The present disclosure is based in part on the discovery that certain biomarkers are associated with better clinical responses to immunotherapies such as tumor antigen-specific cancer treatments, in particular treatment with a prime boost regimen of DC targeting
20 lentiviral vector that expresses a tumor antigen, optionally in combination with a TLR4 agonist plus recombinant tumor antigen protein. The present disclosure is based in part on the discovery that certain biomarkers are associated with better clinical responses to NY-ESO-1 cancer treatments, in particular treatment with a prime boost regimen of DC targeting lentiviral vector that expresses NY-ESO-1, in combination with a TLR4 agonist plus
25 recombinant NY-ESO-1 protein. In particular, analysis of data from clinical trials NCT02387125 (CMB305 is a prime-boost active immunotherapy regimen, consisting of alternating dosing of LV305 (dendritic cell targeting lentiviral vector encoding NY-ESO-1) and G305 (NY-ESO-1 recombinant protein plus the TLR4 agonist, glucopyranosyl lipid A formulated in a squalene emulsion (GLA-SE)) designed to generate and expand anti-NY-

ESO-1 T cells) and NCT02122861 (a Phase 1 Study of Intradermal LV305 in Patients With Locally Advanced, Relapsed or Metastatic Cancer Expressing NY-ESO-1) indicate that patients with one or more of the following biomarkers are more likely to benefit from NY-ESO-1 cancer treatments than other patients:

- 5 (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both, or a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or
 10 CASRLAGQETQYF (SEQ ID NO: 4), or both;
- (ii) an NY-ESO-1 specific antibody;
- (iii) an NY-ESO-1 specific CD4+ or CD8+ T cell; and
- (iv) the presence of NY-ESO-1 in > about 50% of tumor cells in a tumor sample.

In certain embodiments, the presence of at least two of (i) – (iv) is indicative that a
 15 subject is likely to benefit from an NY-ESO-1 cancer therapy. Said another way, the presence of at least two of (i) – (iv) is indicative that a subject is suitable for an NY-ESO-1 cancer therapy. In particular, the presence of one or more of the public TCRs described herein in combination with the presence of NY-ESO-1 in > about 50% of tumor cells in a tumor sample indicates a patient is more likely to benefit from treatment with NY-ESO-1
 20 cancer therapy such as a prime boost regimen of a DC targeted lentiviral vector expressing NY-ESO-1 and a TLR4 agonist (e.g., GLA-SE) combined with recombinant NY-ESO-1 protein.

The present disclosure is also based in part on the discovery that patients who would benefit from continued, extended NY-ESO-1 cancer therapy exhibit particular biomarkers. In
 25 particular, the present disclosure provides a method of identifying a mammalian subject undergoing an NY-ESO-1 cancer therapy that is likely to benefit from (i.e., the subject is suitable for) an extended NY-ESO-1 cancer therapy comprising detecting in the mammalian subject the presence of one or more of the following as compared to before the cancer therapy:

(i) induction of an NY-ESO-1 specific CD8 T cell response;

(ii) induction of an immune response comprising each of an NY-ESO-1 specific CD4 T cell response, an NY-ESO-1 specific CD8 T cell response, and NY-ESO-1 antibody production;

5 (iii) antigen spreading;

(iv) increased T cell clonality; and

(v) increase in the presence of (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNDRXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both, or a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNDRXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both.

The presence of at least two, at least three, or all four of the foregoing indicates that the subject is likely to benefit from (i.e., the subject is suitable for) an extended NY-ESO-1 cancer therapy. In one embodiment, the presence of at least two of the foregoing indicates that the subject is likely to benefit from (i.e., the subject is suitable for) an extended NY-ESO-1 cancer therapy.

The foregoing can be measured in any of a variety of different biological samples including but not limited to tumor biopsies, blood samples, plasma or serum samples, tissue samples, etc. The biomarkers described herein are not necessarily detected in the same sample. In other words, more than one biological sample may be used to detect the two or more biomarkers.

The present disclosure is also based in part on the discovery of a panel of public tumor antigen-specific TCR amino acid sequences that are shared between cancer patients of different MHC class I haplotypes. The term “public TCR” as used herein refers to a TCR sequence, in particular TCR β chain variable region CDR3 (V β CDR3) amino acid sequences, shared among multiple individuals (Venturi et al., Nat Rev Immunol. 2008; 8(3):231-238). As demonstrated in the Examples, the frequency of the public TCRs identified increased after treatment with tumor antigen-specific therapeutic immunization as well as after treatment

with G100, a synthetic TLR4 agonist. See PCT Appl. No. PCT/US16/50826. In particular, , the frequency of the public TCRs identified increased after treatment with NY-ESO-1 specific therapeutic immunization as well as after treatment with G100, a synthetic TLR4 agonist. See PCT Appl. No. PCT/US16/50826.

5 **Engineered TCR**

The $\alpha\beta$ TCR is a membrane-anchored heterodimeric protein comprising a highly variable alpha (α) and beta (β) chain expressed as part of a complex with the invariant CD3 chain molecules. Janeway CA Jr, Travers P, Walport M et al. (2001). *Immunobiology: The Immune System in Health and Disease. 5th edition*. Glossary: Garland Science. Each chain
10 of the T cell receptor is comprised of two extracellular domains: Variable (V) region and a Constant (C) region. The Constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the Variable region binds to the peptide/MHC complex. The variable domain of both the TCR α -chain and β -chain each have three hypervariable or complementarity determining regions (CDRs), whereas the variable
15 region of the β -chain has an additional area of hypervariability (HV4) that does not normally contact antigen and, therefore, is not considered a CDR. The TCR is membrane bound, however, it is not able to mediate signal transduction itself due to its short cytoplasmic tail, so the TCR requires CD3 and zeta to carry out the signal transduction.

The present invention provides for engineered T cell receptors specific for a tumor
20 antigen/MHC complex and isolated cells expressing the engineered T cell receptors described herein. In one embodiment, the present invention provides for engineered T cell receptors specific for NY-ESO-1/MHC complex and isolated cells expressing the engineered T cell receptors described herein. In certain embodiments described herein, the engineered TCR comprises the alpha chain variable region and the beta chain variable region as provided in
25 SEQ ID NO:8 and 9 respectively. In other embodiments described herein, the engineered TCR comprises the beta chain variable region as provided in SEQ ID NO: 9 and an alpha chain pair such that the alpha/beta pair form a functional TCR that recognizes an NY-ESO-1 epitope in the context of MHC. In particular embodiments, the V β CDR3 of the engineered TCRs described herein comprises an amino acid sequence comprising CASSLNDRDXXXXF,
30 wherein X is any amino acid (SEQ ID NO: 1). In some embodiments, the V β CDR3 comprises an amino acid sequence selected from the group consisting of CASSLNDRDYGYTF (SEQ ID NO: 2) and CASSLNDRDQPQHF (SEQ ID NO: 3), each of

which are examples of sequences that fall within the consensus sequence of SEQ ID NO: 1. In another embodiment, the V β CDR3 comprises an amino acid sequence set forth in SEQ ID NO: 4.

In one aspect of the present invention, the engineered TCR is a heterodimeric TCR. A heterodimeric TCR comprises two polypeptides connected by at least one disulfide bond. One polypeptide in the heterodimeric TCR comprises an alpha chain variable region and an alpha chain constant region. In one embodiment, the alpha chain polypeptide optionally includes a transmembrane domain. In certain embodiments, the alpha chain polypeptide optionally includes a transmembrane domain and a cytoplasmic domain (e.g., intracellular signaling domain such as CD3 zeta chain signaling domain and optionally a costimulatory domain). The second polypeptide in the heterodimeric TCR comprises a beta chain variable region and a beta chain constant region, and optionally a transmembrane domain. In certain embodiments in the heterodimeric TCR, the second polypeptide comprises a beta chain variable region and a beta chain constant region and optionally a transmembrane domain and a cytoplasmic domain (e.g., an intracellular signaling domain and optionally a costimulatory domain). In those embodiments where the first and second polypeptides of the heterodimeric TCR do not contain the transmembrane domain and the cytoplasmic domain (e.g., intracellular signaling domain), the heterodimeric TCR is a soluble heterodimer. In one embodiment, the heterodimeric TCR may comprise one or more modifications to stabilize expression and minimize interaction with the native TCR that may be present in a host cell. In certain embodiments, the heterodimeric TCR is a chimeric heterodimeric TCR.

In one aspect of the present invention, the engineered T cell receptor is a chimeric T cell receptor (TCR). As used herein, the term "chimeric" refers to a molecule, e.g., a TCR, composed of parts of different origins. A chimeric molecule, as a whole, is non-naturally occurring, e.g., synthetic or recombinant, although the parts which comprise the chimeric molecule can be naturally occurring.

In some embodiments, the engineered TCR disclosed herein is a chimeric heterodimeric TCR. A chimeric heterodimeric TCR comprises two polypeptides connected by at least one disulfide bond. One polypeptide in the heterodimeric TCR comprises an alpha chain variable region and an alpha chain constant region. The alpha chain polypeptide optionally includes a transmembrane domain, or optionally a transmembrane domain and a cytoplasmic domain (e.g., an intracellular signaling domain such as a CD3 zeta chain

signaling domain, with or without a costimulatory domain). The second polypeptide in the chimeric heterodimeric TCR comprises a beta chain variable region and a beta chain constant region, and optionally a transmembrane domain, or optionally a transmembrane domain and a cytoplasmic domain (e.g., an intracellular signaling domain with or without a costimulatory domain). In those embodiments where the first and second polypeptides of the chimeric heterodimer TCR do not contain the transmembrane domain and the cytoplasmic domain, the chimeric heterodimeric TCR is a soluble chimeric heterodimer.

Polypeptide chains of TCRs are known in the art.

The engineered TCRs described herein comprise an antigen binding domain generally composed of at least a portion of a beta chain variable region and at least a portion of an alpha chain variable region, wherein the antigen binding domain is specific for or specifically binds to NY-ESO-1/MHC. The term "specifically binds," as used herein refers to the ability of the TCR to recognize a specific antigen in the context of an MHC, but that does not substantially recognize or bind irrelevant antigen/MHC in a sample. In some instances, the terms "specific binding" or "specifically binding," can be used in reference to the interaction of a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

Single chain TCRs (see, e.g., US20100113300) are also contemplated. Briefly stated, a single chain ("sc-") TCR molecule includes an alpha chain variable region ($V\alpha$) and a beta chain variable region ($V\beta$) covalently linked through a suitable peptide linker sequence. For example, the $V\alpha$ can be covalently linked to the $V\beta$ through a suitable peptide linker sequence fused to the C-terminus of the $V\alpha$ and the N-terminus of the $V\beta$. The scTCR of the present invention may have a structure $V\alpha - L - V\beta$ or may be in the other orientation, e.g. $V\beta - L - V\alpha$. In certain embodiments, the scTCR further comprises a constant domain (also referred to as constant region). In a further embodiment, the scTCR further comprises a constant domain, a transmembrane domain and a cytoplasmic domain. In one embodiment, the cytoplasmic domain comprises an intracellular signaling domain with or without a

costimulatory domain. The V α and V β of the sc-TCR fusion protein are generally about 200 to 400 amino acids in length, or about 300 to 350 amino acids in length, and will be at least 90% identical, and preferably 100% identical to the V α and V β of a naturally-occurring TCR, such as the V α and V β amino acid sequences provided herein that are specific for NY-ESO-1/MHC complex. By the term "identical" is meant that the amino acids of the V α or V β are 100% identical to the corresponding naturally-occurring TCR V β or V α .

In some embodiments, the engineered TCR described herein comprises an intracellular signaling domain (e.g., CD3 zeta chain signaling domain). The intracellular signaling domain, which may also be referred to as the cytoplasmic signaling domain, of an engineered TCR described herein is responsible for activation of at least one of the normal effector functions of the immune cell that expresses the engineered TCR. The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus the term "intracellular signaling domain" or "cytoplasmic signaling domain" refers to the portion of a protein that transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire signaling domain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact full length signaling domain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

Examples of intracellular signaling domains for use in the engineered TCRs described herein include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. Also contemplated herein are NK signaling molecules. Thus, contemplated for use herein as intracellular signaling domains are the polypeptides constituting the CD3 complex which are involved in the signal transduction, e.g., the γ , δ , ϵ , ζ , and η , CD3 chains. Among the polypeptides of the TCR/CD3 (the principal signaling receptor complex of T cells), especially promising are the zeta and its eta isoform chain, which appear as either homo- or hetero-S-S-linked dimers, and are responsible for mediating

at least a fraction of the cellular activation programs triggered by the TCR recognition of ligand (Weissman, A. et al. EMBO J. 8:3651-3656 (1989); Bauer, A. et al. Proc. Natl. Acad. Sci. USA 88:3842-3846 (1991)). Further examples of intracellular signaling domains for use herein include the MB1 chain (CD79A), B29, Fc RIII and Fc RI and the like. Intracellular signaling portions of other members of the families of activating proteins can also be used, such as Fc γ RIII and Fc ϵ RI. See Gross et al., FASEB J 6:3370, 1992; Stancovski, I. et al., J.Immunol. 151:6577, 1993; Moritz, D. et al., Proc.Natl.Acad.Sci.U.S.A. 91:4318, 1994; Hwu et al., Cancer Res. 55:3369, 1995; Weijtens, M. E. et al., J.Immunol. 157:836, 1996; and Hekele, A. et al., Int.J.Cancer 68:232, 1996; for disclosures of various alternative transmembrane and intracellular domains contemplated for use herein. Additional examples include the intracellular signaling domains of any one of the IL-2 receptor (IL-2R) p55 (.alpha.) or p75 (.beta.) or .gamma. chains, especially the p75 and .gamma. subunits which are responsible for signaling T cell and NK proliferation.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or costimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic signaling sequences).

Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use as intracellular signaling domains herein include those derived from TCR ζ , FcR γ , FcR β , CD3 γ , CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. In certain particular embodiments, the cytoplasmic signaling domain in the engineered TCR used herein comprises a cytoplasmic signaling sequence derived from CD3 zeta. The zeta chain portion sequence useful herein includes the intracellular domain. This domain, which spans amino acid residues 52-163 of the human CD3 zeta chain, can be amplified using standard molecular biology techniques.

In some embodiments, the cytoplasmic domain of the engineered TCR can be designed to comprise the CD3-zeta signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of the TCRs for use herein. The “costimulatory signaling region” or “costimulatory domain” refers to a portion of the engineered TCR comprising the intracellular domain, or a functional fragment thereof, of a costimulatory molecule. Thus, the cytoplasmic domain of the engineered TCRs described herein may comprise an intracellular signaling domain and a costimulatory domain.

"Costimulatory ligand," includes a molecule on an antigen presenting cell (e.g., an aAPC, dendritic cell, B cell, and the like) that specifically binds a cognate costimulatory molecule on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A costimulatory ligand can include, but is not limited to, CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A costimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a costimulatory molecule present on a T cell, such as, but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83.

A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40L, PD-1, DAP-10, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, and the like. Particularly illustrative costimulatory domains contemplated for use with the TCRs described herein are derived from 4-1BB and CD28 however, other costimulatory domains derived from other costimulatory molecules are within the scope of the invention.

The intracellular signaling sequences and costimulatory sequences within the cytoplasmic domain of the engineered TCRs disclosed herein may be linked to each other in

any order in which each portion functions to signal properly. In certain embodiments, the costimulatory region, when present, is just on the cytoplasmic side of the TM domain, followed by a signaling domain (e.g. the signaling domain of CD3 zeta). In other embodiments, the order is reversed, with the signaling portion just next to the TM domain, followed by a costimulatory domain, when present. Optionally, a short oligo- or polypeptide linker, in certain embodiments, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in length may form the linkage. A glycine-serine doublet provides a particularly suitable linker. Any of a variety of linkers can be used and are known to the skilled person.

As noted herein, the engineered TCRs also comprise, in some embodiments, a transmembrane (TM) domain to anchor them to the surface of the host cell (e.g., T cell, NK cell). The TM can be derived from a TCR alpha chain, a TCR beta chain, from the CD3 zeta chain or can be derived from another transmembrane molecule, such as CD28 or CD4. As would be recognized by the skilled person, any TM domain that functions properly to anchor the chimeric receptor to the membrane can be used. With respect to the transmembrane domain, the chimeric TCR can be designed to comprise a transmembrane domain that is fused to the extracellular domain of the chimeric TCR. In one embodiment, the transmembrane domain that naturally is associated with one of the domains in the chimeric TCR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention may be derived from (i.e. comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. Alternatively the transmembrane domain may be synthetic, in which case it may comprise predominantly hydrophobic residues such as leucine and valine. In certain embodiments, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short linker, in certain embodiments between 1 or 2 and about 10, 11, 12, 13, 14, or 15 amino acids in length may form the linkage between the transmembrane

domain and the cytoplasmic signaling domain of the chimeric TCR. A glycine-serine doublet provides a particularly suitable linker.

In certain embodiments, the transmembrane domain in the chimeric TCR is the CD8 transmembrane domain. In some instances, the transmembrane domain of the chimeric TCR for use herein comprises the CD8 hinge domain. In certain embodiments, the transmembrane domain is the CD28 transmembrane domain, in particular in the embodiment where the costimulatory region is derived from CD28 since it is largely as a matter of convenience to minimize the number of amplification/cloning steps that need to be performed. Thus, in certain embodiments, the TM domain may be derived from the same molecule as a costimulatory or intracellular signaling domain of the chimeric TCR. However, this is not necessary and the TM domain may be derived from any suitable transmembrane protein, including, but not limited to, the CD8 and CD3 zeta transmembrane domains.

TCR constant domains: the constant domains for use in the engineered TCRs of the present invention may be derived from the TCR alpha chain or the TCR beta chain. Such constant domains are known in the art and available from public sequence databases.

In certain embodiments one or more disulfide bonds may link amino acid residues of the constant domain sequences included in the engineered TCRs of the present invention. In one embodiment, the disulfide bond is between cysteine residues corresponding to amino acid residues whose beta carbon atoms are less than 0.6 nm apart in native TCRs. For example, the disulfide bond may be between cysteine residues substituted for Thr 48 of exon 1 of TRAC*01 and Ser 57 of exon 1 of TRBC1*01 or TRBC2*01 or the non-human equivalent thereof. Other sites where cysteines can be introduced to form the disulfide bond are the following residues in exon 1 of TRAC*01 for the TCR .alpha. chain and TRBC1*01 or TRBC2*01 for the TCR β chain:

TCR alpha chain	TCR beta chain	native beta carbon separation (nm)
Thr 45	Ser 77	0.533
Tyr 10	Ser 17	0.359
Thr 45	Asp 59	0.560

Ser 15	Glu 15	0.59
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In addition to the non-native disulfide bond referred to above, the dimeric TCR or scTCR form of the TCRs of the invention may include a disulfide bond between residues corresponding to those linked by a disulfide bond in native TCRs.

5 Soluble TCR

In some embodiments, the engineered TCR is a soluble TCR. Generally, "soluble TCRs" comprise TCR chains which have been truncated to remove the transmembrane regions thereof. For example, WO 03/020763 describes the production and testing of soluble TCRs having a non-native disulfide interchain bond to facilitate the association of the truncated TCR chains. Details of other potentially suitable soluble TCR designs can be found in WO 99/60120 which described the production of non-disulfide linked truncated TCR chains which utilize heterologous leucine zippers fused to the C-termini thereof to facilitate chain association, and WO 99/18129 which describe the production of single-chain soluble TCRs comprising a TCR V α chain covalently linked to a TCR V β chain via a peptide linker. Boulter et al. also describe methods for producing soluble functional and stable TCR heterodimers (see Boulter et al., Protein Eng 2003, 16:707-711). In further embodiments, the soluble TCRs described herein can be chimeric, e.g., fused to a heterologous protein, such as IL-2 or other cytokines, Fc domain of an antibody, and the like. Illustrative soluble TCR fusion proteins are described for example in Cancer Immunol Immunother. 2004 Apr;53(4):345-57; J Immunol. 2005 Apr 1;174(7):4381-8; Clin Immunol. 2006 Oct;121(1):29-39.

Functional Variants and Portions of Engineered TCR

Functional variants of the TCRs described herein are also contemplated. The term "functional variant" as used herein refers to a TCR having substantial or significant sequence identity or similarity to a parent TCR, which functional variant retains the biological activity of the TCR of which it is a variant. Functional variants encompass, for example, those variants of the TCR described herein that retain the ability to specifically bind to a tumor antigen polypeptide/MHC complex, such as an NY-ESO-1 polypeptide/MHC complex, for which the parent TCR has antigenic specificity or to which the parent polypeptide or protein specifically binds, to a similar extent, the same extent, or to a higher extent, as the parent

TCR. In some embodiments, the functional variant comprises a beta chain variable domain comprising an amino acid sequence that is at least 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the beta chain variable domain amino acid sequence of the parent TCR, such as the beta chain variable domain amino acid sequences set forth in the sequence listing provided herein. In some embodiments, the functional variant comprises a V β CDR3 amino acid sequence that is at least 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the V β CDR3 amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

In some embodiments, the functional variant comprises an alpha chain variable domain comprising an amino acid sequence that is at least 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the alpha chain variable domain amino acid sequence of the parent TCR, such as the alpha chain variable domain amino acid sequences set forth in the sequence listing provided herein.

In some embodiments, the amino acid sequence of the functional variant can comprise, for example, the amino acid sequence of the parent TCR with at least one conservative amino acid substitution. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same chemical or physical properties. For example, the conservative amino acid substitution can be an acidic amino acid substituted for another acidic amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (e.g., Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Val, etc.), a basic amino acid substituted for another basic amino acid (Lys, Arg, etc.), an amino acid with a polar side chain substituted for another amino acid with a polar side chain (Asn, Cys, Gln, Ser, Thr, Tyr, etc.), etc.

Alternatively or additionally, the functional variants can comprise the amino acid sequence of the parent TCR with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution not to interfere with or inhibit the biological activity of the functional variant. Preferably, the non-conservative amino acid substitution enhances the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent TCR,

polypeptide, or protein.

The amino acid substitution(s) of the amino acid sequence of the functional variant can be within any region of the amino acid sequence. For example, in some embodiments, the amino acid substitution(s) is located within the region of the amino acid sequence which encodes the variable region or the constant region of the functional variant. In the instance that the amino acid substitution(s) is/are located within the region of the amino acid sequence which encodes the variable region (e.g., a V β CDR3 amino acid sequence, such as SEQ ID NO: 1), it is understood that the amino acid substitution(s) do not significantly decrease the ability of the functional variant to bind to the peptide – MHC complex for which the parent TCR has antigenic specificity.

Functional portions of the engineered TCRs are also provided. In some embodiments, the functional portions can comprise any portion comprising contiguous amino acids of the parent TCR, provided that the functional portion comprises a portion of the V β chain comprising the amino acid sequence set forth in SEQ ID NO: 1. The term “functional portion” when used in reference to a TCR refers to any part or fragment of the TCR of the invention, which part or fragment retains the biological activity of the TCR of which it is a part (the parent TCR). Functional portions encompass, for example, those parts of a TCR that retain the ability to, e.g., specifically bind to NY-ESO-1 peptide – MHC complex, as the parent TCR.

In some embodiments, the functional portion comprises additional amino acids at the amino- or carboxy-terminus of the portion, or at both termini, that do not interfere with the biological function of the TCR portion.

The TCRs (including functional portions and functional variants) described herein optionally comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, .alpha.-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β -phenylserine .beta.-hydroxyphenylalanine, phenylglycine, α -naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-

dibenzyl-lysine, 6-hydroxylysine, ornithine, α -aminocyclopentane carboxylic acid, α -aminocyclohexane carboxylic acid, α -aminocycloheptane carboxylic acid, α -(2-amino-2-norbornane)-carboxylic acid, α,γ -diaminobutyric acid, α,β -diaminopropionic acid, homophenylalanine, and α -tert-butylglycine.

- 5 The TCRs (including functional portions and functional variants) described herein can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

Nucleic Acids, Vectors and Cells

- 10 Nucleic acids comprising a nucleotide sequence encoding any of the tumor antigens or engineered TCRs (or functional portion and functional variant thereof) described herein are also contemplated.

By “nucleic acid” as used herein includes “polynucleotide,” “oligonucleotide,” and “nucleic acid molecule,” and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

Preferably, the nucleic acids described herein are recombinant. As used herein, the term “recombinant” refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be in vitro replication or in vivo replication.

The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art or commercially available (e.g., from Genscript, Thermo Fisher and similar companies). See, for example, Sambrook et al., supra,

and Ausubel et al., supra. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides).

5 Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl)uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, Colo.) and Synthegen (Houston, Tex.).

20 The nucleic acid can comprise any nucleotide sequence which encodes any of the tumor antigens or engineered TCRs, polypeptides, or proteins, or functional portions or functional variants thereof.

The present disclosure also provides variants of the isolated or purified nucleic acids wherein the variant nucleic acids comprise a nucleotide sequence that has at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence encoding the parent tumor antigen or parent TCR. In certain embodiments, the present disclosure provides isolated or purified nucleic acids comprising a nucleotide sequence that is at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence provided in the sequence listing herein, wherein such variant nucleotide sequence encodes a functional TCR that specifically recognizes its cognate MHC-peptide complex (e.g., NY-ESO-1 peptide/MHC complex) at least as well as the parent TCR or wherein such variant nucleotide sequence encodes a tumor antigen that induces an

immune response equivalent to or greater to an immune response induced by the parent tumor antigen.

The disclosure also provides an isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

The nucleotide sequence which hybridizes under stringent conditions preferably hybridizes under high stringency conditions. By “high stringency conditions” is meant that the nucleotide sequence specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-10 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70° C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the TCRs described herein. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

In certain embodiments, the nucleic acids described herein can be incorporated into any of a variety of different types of vectors. In this regard, the disclosure provides one or more recombinant expression vectors comprising any one or more of the nucleic acids described herein. For purposes herein, the term “recombinant expression vector” means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors described herein are not naturally-occurring

as a whole. However, parts of the vectors can be naturally-occurring. The recombinant expression vectors described herein can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring, non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages does not hinder the transcription or replication of the vector.

The recombinant expression vector described herein can be any suitable recombinant expression vector, and can be used to transform, transfect or transduce any suitable host. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.) and other commercially available plasmid vectors. Bacteriophage vectors, such as λ G10, λ GT11, λ ZapII (Stratagene), λ EMBL4, and λ NM1149, also can be used. Examples of plant expression vectors include pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech).

In some embodiments, a vector for use herein is a viral vector, e.g., a retroviral vector, such as a lentiviral vector, an adenoviral vector, a poxvirus vector, and a vaccinia virus vector. "Lentivirus" refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells. Several examples of lentiviruses include HIV (human immunodeficiency virus: including HIV type 1, and HIV type 2); equine infectious anemia virus; feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

Exemplary lentiviral vectors include, but are not limited to, vectors derived from HIV-1, HIV-2, FIV, equine infectious anemia virus, SIV, and maedi/visna virus. Methods of using viral vectors, retroviral and lentiviral viral vectors and packaging cells for transducing mammalian target cells with viral particles containing TCRs transgenes are well known in the art and have been previously described, for example, in U.S. Pat. No. 8,119,772; Walchli et al., 2011, PLoS One 6:327930; Zhao et al., J. Immunol., 2005, 174:4415-4423; Engels et al.,

2003, Hum. Gene Ther. 14:1155-68; Frecha et al., 2010, Mol. Ther. 18:1748-57; Verhoeven et al., 2009, Methods Mol. Biol. 506:97-114. Retroviral and lentiviral vector constructs and expression systems are also commercially available.

The recombinant expression vectors can be prepared using standard recombinant DNA techniques described in, for example, Current Protocols in Molecular Biology (2015 John Wiley & Sons, Inc) or Sambrook et al., *supra*, and Ausubel et al., *supra*. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColEI, 2 μ plasmid, λ , SV40, bovine papilloma virus, and the like.

In some embodiments, the recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based.

The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

The recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the tumor antigen or engineered TCR, polypeptide, or protein (including functional portions and functional variants thereof), or to the variant nucleotide sequence encoding an immunogenic tumor antigen or functional TCR, or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the modified TCR, polypeptide, or protein. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV

promoter, an EF1 α promoter, a ubiquitin promoter, an MHC Class I or II promoter, a T cell specific promoter, a cytokine promoter, or a promoter found in the long-terminal repeat of the murine stem cell virus. In certain embodiments, the promoter is a synthetic promoter.

As discussed herein, in those embodiments where viral vectors are used, the viral
5 vector genome comprises a sequence of interest that is desirable to express in target cells. With regard to retroviral vectors, typically, the sequence of interest (e.g., a nucleic acid encoding a tumor antigen of interest or an engineered TCR as described herein) is located between the 5' LTR and 3' LTR sequences (or partial 5' or 3' LTR sequence as may be used in certain embodiments). In certain embodiments, the sequence of interest is in a functional
10 relationship with other genetic elements, for example transcription regulatory sequences including promoters or enhancers, to regulate expression of the sequence of interest in a particular manner. In certain instances, the useful transcriptional regulatory sequences are those that are highly regulated with respect to activity, both temporally and spatially. Expression control elements that may be used for regulating the expression of the
15 components are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, enhancers and other regulatory elements.

The sequence of interest and any other expressible sequence is typically in a functional relationship with internal promoter/enhancer regulatory sequences. An "internal" promoter/enhancer is one that is located between the 5' LTR and the 3' LTR sequences (or
20 partial sequences thereof) in the viral vector construct and is operably linked to the sequence of interest. The internal promoter/enhancer may be any promoter, enhancer or promoter/enhancer combination known to increase expression of a nucleic acid with which it is in a functional relationship. A "functional relationship" and "operably linked" mean, without limitation, that the sequence is in the correct location and orientation with respect to
25 the promoter and/or enhancer that the sequence of interest will be expressed when the promoter and/or enhancer is contacted with the appropriate molecules.

The choice of an internal promoter/enhancer is based on the desired expression pattern of the sequence of interest and the specific properties of known promoters/enhancers. Thus, the internal promoter may be constitutively active. Non-limiting examples of
30 constitutive promoters that may be used include the promoter for ubiquitin (US Patent No. 5,510,474; WO 98/32869,), GAPDH, CMV (Thomsen et al., PNAS 81:659, 1984; US Patent No. 5,168,062,), beta-actin (Gunning et al. 1989 Proc. Natl. Acad. Sci. USA 84:4831-4835

and pgk (see, for example, Adra et al. 1987 Gene 60:65-74; Singer-Sam et al. 1984 Gene 32:409-417; and Dobson et al. 1982 Nucleic Acids Res. 10:2635-2637. In some embodiments, the promoter used to control expression of the sequence of interest (e.g., a tumor antigen or the engineered TCRs described herein) encoded by a vector (e.g., a pseudotyped retroviral vector genome) is an intron-deficient promoter. In some
5 embodiments, the human Ubiquitin-C (UbiC) promoter is used to control expression of the antigen or TCRs encoded by the viral vector genome. In various embodiments, the UbiC promoter has been modified to remove introns, i.e., the promoter is intron deficient. The full-length UbiC promoter is 1250 nucleotides. The intron begins at 412 and goes all the way to
10 the end (412-1250). This region can be deleted for the purpose of minimizing heterogeneous viral genomic transcripts. The HIV viral genome has a native intron within it. Thus, a lentivirus comprising a UbiC promoter would have a total of 2 introns in the lentivirus genome. The UbiC intron can exist in both spliced and unspliced forms. Deletion of the UbiC intron eliminates the possibility of heterogeneous viral transcripts and ensures
15 homogeneity in the delivered pseudotyped lentiviral particles.

Alternatively, the promoter may be a tissue specific promoter. In some preferred embodiments, the promoter is a target cell-specific promoter. For example, the promoter can be from any product expressed by dendritic cells, T cells, NK cells, including but not limited to, IL-2, IL-2R, interferon γ , MHC class I, MHC class II, CD3, CD11c, CD103, TLRs, DC-
20 SIGN, BDCA-3, DEC-205, DCIR2, mannose receptor, Dectin-1, Clec9A. In addition, promoters may be selected to allow for inducible expression of the sequence of interest. A number of systems for inducible expression are known in the art, including the tetracycline responsive system, the lac operator-repressor system, as well as promoters responsive to a variety of environmental or physiological changes, including heat shock, metal ions, such as
25 metallothionein promoter, interferons, hypoxia, steroids, such as progesterone or glucocorticoid receptor promoter, radiation, such as VEGF promoter. A combination of promoters may also be used to obtain the desired expression of the gene of interest. The artisan of ordinary skill will be able to select a promoter based on the desired expression pattern of the gene in the organism or the target cell of interest.

30 The viral genome may comprise at least one RNA Polymerase II or III responsive promoter. This promoter can be operably linked to the sequence of interest and can also be linked to a termination sequence. In addition, more than one RNA Polymerase II or III

promoters may be incorporated. RNA polymerase II and III promoters are well known to one of skill in the art. A suitable range of RNA polymerase III promoters can be found, for example, in Paule and White, *Nucleic Acids Research.*, Vol. 28, pp 1283-1298 (2000). RNA polymerase II or III promoters also include any synthetic or engineered DNA fragment that can direct RNA polymerase II or III to transcribe downstream RNA coding sequences. Further, the RNA polymerase II or III (Pol II or III) promoter or promoters used as part of the viral vector genome can be inducible. Any suitable inducible Pol II or III promoter can be used with the methods of the disclosure. Particularly suited Pol II or III promoters include the tetracycline responsive promoters provided in Ohkawa and Taira, *Human Gene Therapy*, Vol. 11, pp 577-585 (2000) and in Meissner et al. *Nucleic Acids Research*, Vol. 29, pp 1672-1682 (2001), .

An internal enhancer may also be present in the viral construct to increase expression of the gene of interest. For example, the CMV enhancer (Boshart et al. *Cell*, 41:521, 1985) may be used. Many enhancers in viral genomes, such as HIV, CMV, and in mammalian genomes have been identified and characterized (see GenBank). An enhancer can be used in combination with a heterologous promoter. One of ordinary skill in the art will be able to select the appropriate enhancer based on the desired expression pattern.

The viral vector genome may also contain additional genetic elements. The types of elements that may be included in the construct are not limited in any way and may be chosen to achieve a particular result. For example, a signal that facilitates nuclear entry of the viral genome in the target cell may be included. An example of such a signal is the HIV-1 cPPT/CTS. Further, elements may be included that facilitate the characterization of the provirus integration site in the target cell. For example, a tRNA amber suppressor sequence may be included in the construct. An insulator sequence from e.g., chicken β -globin may also be included in the viral genome construct. This element reduces the chance of silencing an integrated provirus in the target cell due to methylation and heterochromatinization effects. In addition, the insulator may shield the internal enhancer, promoter and exogenous gene from positive or negative positional effects from surrounding DNA at the integration site on the chromosome. In addition, the vector genome may contain one or more genetic elements designed to enhance expression of the gene of interest. For example, a woodchuck hepatitis virus responsive element (WRE) may be placed into the construct (Zufferey et al. 1999. *J. Virol.* 74:3668-3681; Deglon et al. 2000. *Hum. Gene Ther.* 11:179-190,).

The viral vector genome is typically constructed in a plasmid form that may be transfected into a packaging or producer cell line. The plasmid generally comprises sequences useful for replication of the plasmid in bacteria. Such plasmids are well known in the art. In addition, vectors that include a prokaryotic origin of replication may also include a gene whose expression confers a detectable or selectable marker such as a drug resistance. Typical bacterial drug resistance products are those that confer resistance to ampicillin or tetracycline.

The inventive recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term “suicide gene” refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art (see, for example, Suicide Gene Therapy: Methods and Reviews, Springer, Caroline J. (Cancer Research UK Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, UK), Humana Press, 2004) and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, and nitroreductase.

Also provided is a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term “host cell” refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5 α E. coli cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, 293F, 293T cells, and the like. In particular, for the purposes of producing viral particles for delivery of the TCRs described herein, 293F and 293T host cells may be used. For purposes of amplifying or replicating the recombinant expression vector, the host cell may be a prokaryotic cell, e.g., a DH5 α cell. For purposes of producing a recombinant tumor antigen protein or recombinant modified TCR, polypeptide, or protein, the host cell may be a

mammalian cell. In certain embodiments, the host cell is a human cell. The host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage. In certain embodiments, the host cell is a dendritic cell or peripheral blood lymphocyte (PBL). In certain embodiments, the host cell is a T cell.

5 In some embodiments, a vector described herein encodes a tumor antigen (or functional variant or portion thereof) described herein. In certain embodiments, the vector is a viral vector such as a DC targeted lentiviral vector for in vivo targeting of DCs and expression of the tumor antigen in vivo. Exemplary vectors are described for example in US Patent Nos. 8329162; 8372390; 8273345; 8187872; 8323662 and published PCT application
10 WO2013/149167.

 In some embodiments, a vector described herein encodes an engineered TCR (or functional variant or portion) described herein. In this regard, in those embodiments where the TCR is a heterodimeric TCR, both the TCR beta chain and the TCR alpha chain may be expressed from the same vector or may be expressed from different vectors within the same
15 host cell such that a functional dimeric TCR is expressed at the surface of the cell. In other embodiments where the TCR is a single chain TCR, a vector described herein comprises a nucleic acid encoding the single chain TCR or other forms of the TCRs described herein. .

 In additional embodiments, a vector described herein may encode more than one product. In this regard, the sequence to be delivered can comprise a nucleic acid encoding a
20 TCR as described herein in addition to other nucleic acids of interest, encoding multiple genes encoding at least one protein (e.g., a tumor antigen), at least one siRNA, at least one microRNA, at least one dsRNA or at least one anti-sense RNA molecule or any combinations thereof. For example, the sequence to be delivered can include one or more genes that encode one or more TCRs. The one or more TCRs can be associated with a single disease or
25 disorder, or they can be associated with multiple diseases and/or disorders. In some instances, a gene encoding an immune regulatory protein can be included along with a gene encoding a TCR as described herein, and the combination can elicit and regulate the immune response to the desired direction and magnitude. In other instances, a sequence encoding an siRNA, microRNA, dsRNA or anti-sense RNA molecule can be constructed with a gene
30 encoding a TCR as described herein, and the combination can regulate the scope of the immune response. The products may be produced as an initial fusion product in which the encoding sequence is in functional relationship with one promoter. Alternatively, the

products may be separately encoded and each encoding sequence in functional relationship with a promoter. The promoters may be the same or different.

In some embodiments, vectors contain polynucleotide sequences that encode immunomodulatory molecules. Exemplary immunomodulatory molecules include GM-CSF, IL-2, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, interferon gamma, TNF α , B7.1, B7.2, 4-1BB, CD40, CD40 ligand (CD40L), drug-inducible CD40 (iCD40), and the like, or ligands, or single chain antibodies that bind thereto. These polynucleotides are typically under the control of one or more regulatory elements that direct the expression of the coding sequences in host cells.

In certain embodiments, the vectors described herein may express a checkpoint inhibitor. The checkpoint inhibitor may be expressed from the same vector as the TCRs described herein or from a separate vector. Immune checkpoints refer to a variety of inhibitory pathways of the immune system that are crucial for maintaining self-tolerance and for modulating the duration and amplitude of an immune responses. Tumors use certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T cells that are specific for tumor antigens. (see., e.g., Pardoll, 2012 Nature 12:252; Chen and Mellman 2013 Immunity 39:1). The present disclosure provides immune checkpoint inhibitors that can be expressed from the expression vectors described herein in combination with the TCRs described herein. Illustrative checkpoint inhibitors include antibodies, or antigen-binding fragments thereof, that bind to and block or inhibit immune checkpoint receptors or antibodies, or antigen-binding fragments thereof that bind to and block or inhibit immune checkpoint receptor ligands. Illustrative immune checkpoint molecules that may be targeted for blocking or inhibition include, but are not limited to, CTLA-4, 4-1BB (CD137), 4-1BBL (CD137L), PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, TIM3, B7H3, B7H4, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, $\gamma\delta$, and memory CD8⁺ ($\alpha\beta$) T cells), CD160 (also referred to as BY55) and CGEN-15049. Immune checkpoint inhibitors include antibodies, or antigen binding fragments thereof, or other binding proteins, that bind to and block or inhibit the activity of one or more of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, TIM3, B7H3, B7H4, VISTA, KIR, 2B4, CD160 and CGEN-15049. Illustrative immune checkpoint inhibitors include Tremelimumab (CTLA-4 blocking antibody), anti-OX40, PD-L1 monoclonal Antibody (Anti-B7-H1; MEDI4736),

MK-3475 (PD-1 blocker), Nivolumab (anti-PD1 antibody), CT-011 (anti-PD1 antibody), BY55 monoclonal antibody, AMP224 (anti-PDL1 antibody), BMS-936559 (anti-PDL1 antibody), MPLDL3280A (atezolizumab; TECENTRIQ; anti-PDL1 antibody), MSB0010718C (anti-PDL1 antibody) and Yervoy/ipilimumab (anti-CTLA-4 checkpoint inhibitor).

The expression vectors (e.g., retroviral vectors or lentiviral vectors) for expressing the tumor antigens and TCRs herein can be engineered to express more than one, e.g., two, three, or four, sequences of interest at a time. Several methods are known in the art for simultaneously expressing more than one sequences from a single vector. For example, the vectors can comprise multiple promoters fused to a coding sequence's open reading frames (ORFs), insertion of splicing signals between coding sequences, fusion of sequences of interest whose expressions are driven by a single promoter, insertion of proteolytic cleavage sites between coding sequences, insertion of internal ribosomal entry sites (IRESs) between coding sequences, insertion of bi-directional promoters between coding sequences, and/or "self-cleaving" 2A peptides. Each component to be expressed in a multicistronic expression vector may be separated, for example, by an internal ribosome entry site (IRES) element or a viral 2A element, to allow for separate expression of the various proteins from the same promoter. IRES elements and 2A elements are known in the art (U.S. Pat. No. 4,937,190; de Felipe et al. 2004. *Traffic* 5: 616-626,). In one embodiment, oligonucleotides encoding furin cleavage site sequences (RAKR) (Fang et al. 2005. *Nat. Biotech* 23: 584-590) linked with 2A-like sequences from foot-and-mouth diseases virus (FMDV; F2A), porcine teschovirus-1 (P2A), equine rhinitis A virus (ERAV; E2A), and thosea asigna virus (TaV; T2A) (Szymczak et al. 2004. *Nat. Biotechnol.* 22: 589-594) are used to separate genetic elements in a multicistronic vector. The efficacy of a particular multicistronic vector can readily be tested by detecting expression of each of the genes using standard protocols.

Expression of two or more sequences of interest (e.g. sequences encoding a TCR alpha chain and a TCR beta chain; a single chain TCR and an immunomodulatory molecule; and/or a tumor antigen) can also be accomplished using Internal Ribosome Entry Sites (IRES). IRES enable eukaryotic ribosomes to enter and scan an mRNA at a position other than the 5' m⁷ G-cap structure. If positioned internally, e.g., 3' of a first coding region (or cistron), an IRES will enable translation of a second coding region within the same transcript. The second coding region is identified by the first ATG encountered after the IRES.

Exemplary IRES elements include viral IRES such as the picornavirus IRES and the
cardiovirus IRES (see, e.g., U.S. Pat. No. 4,937,190) and non-viral IRES elements found in 5'
UTRs (e.g., those elements of transcripts encoding immunoglobulin heavy chain binding
protein (BiP) (Macejak et al., *Nature*, 353:90-4, 1991); *Drosophila Antennapedia* (Oh et al.,
5 *Genes Dev.* 6:1643-53, 1992) and *Ultrabithorax* (Ye et al., *Mol. Cell Biol.*, 17:1714-21,
1997); fibroblast growth factor 2 (Vagner et al., *Mol. Cell Biol.*, 15:35-44, 1995); initiation
factor eIF4G (Gan et al., *J. Biol. Chem.* 273:5006-12, 1998); proto-oncogene c-myc (Nanbru
et al., *J. Biol. Chem.*, 272:32061-6, 1995; Stoneley, *Oncogene*, 16:423-8, 1998); and vascular
endothelial growth factor (VEGF) (Stein et al., *Mol. Cell Biol.*, 18:3112-9, 1998).

10 Expression of two or more sequences of interest can also be accomplished using
bidirectional promoters, i.e., a promoter region or two back-to-back cloned promoters whose
reading directions point away from each other, and from which two open reading frames
flanking the promoter region are transcribed. Examples of such promoters include the
PDGF-A, neurotropic JC virus, BRCA1, transcobalamin II, and dipeptidylpeptidase IV
15 promoters.

Production of lentiviral particles

In certain embodiments, retroviral vectors are used to deliver a tumor antigen of
interest *in vivo*, in particular to dendritic cells, in particular in patients who have been
identified using the biomarkers and methods herein as being likely to benefit from (i.e., the
20 subject is suitable for) tumor antigen-specific cancer therapy. Production and delivery of
such retroviral vectors is described for example in US Patent Nos. 8329162; 8372390;
8273345; 8187872; 8323662 and published PCT application WO2013/149167.

In other embodiments, retroviral vectors are used to transduce T cells to modify the T
cells to express a public TCR specific for a tumor antigen, such as the TCRs of the present
25 disclosure, and other sequences of interest as described herein. Any of a variety of methods
already known in the art may be used to produce infectious viral, e.g., retroviral and
lentiviral, particles whose genome comprises an RNA copy of the viral vector genome. In
one method, the viral vector genome is introduced into a packaging cell line that contains all
the components necessary to package viral genomic RNA, transcribed from the viral vector
30 genome, into viral particles. Alternatively, the viral vector genome may comprise one or
more genes encoding viral components in addition to the one or more sequences of interest.
In order to prevent replication of the genome in the target cell, however, endogenous viral

genes required for replication will usually be removed and provided separately in the packaging cell line.

In general, the retroviral vector particles are produced by a cell line that is transfected with one or more plasmid vectors containing the components necessary to generate the particles. These retroviral vector particles are typically not replication-competent, i.e., they are only capable of a single round of infection. Most often, multiple plasmid vectors are utilized to separate the various genetic components that generate the vector particles, mainly to reduce the chance of recombination events that might otherwise generate replication competent viruses. A single plasmid vector having all of the retroviral components can be used if desired, however. As one example of a system that employs multiple plasmid vectors, a cell line is transfected with at least one plasmid containing the viral vector genome (i.e., the vector genome plasmid), including the LTRs, the cis-acting packaging sequence, and the sequence(s) of interest, which are often operably linked to a heterologous promoter, at least one plasmid encoding the virus enzymatic and structural components (i.e., the packaging plasmid that encodes components such as, Gag and Pol), and at least one envelope plasmid encoding an envelope glycoprotein (e.g., an envelope protein derived from a retrovirus or other suitable envelope glycoproteins such as VSV G, Sindbis envelope, measles virus envelope, and the like). Additional plasmids can be used to enhance retrovirus particle production, e.g., Rev-expression plasmids, as described herein and known in the art. Viral particles bud through the cell membrane and comprise a core that includes a genome containing the sequence of interest and an envelope glycoprotein.

Transfection of packaging cells with plasmid vectors of the present disclosure can be accomplished by well-known methods, and the method to be used is not limited in any way. A number of non-viral delivery systems are known in the art, including for example, electroporation, lipid-based delivery systems including liposomes, delivery of "naked" DNA, and delivery using polycyclodextrin compounds, such as those described in Schatzlein AG. (2001. *Non-Viral Vectors in Cancer Gene Therapy: Principles and Progresses*. Anticancer Drugs). Cationic lipid or salt treatment methods are typically employed, see, for example, Graham et al. (1973. *Viol.* 52:456; Wigler et al. (1979. *Proc. Natl. Acad. Sci. USA* 76:1373-76). The calcium phosphate precipitation method is most often used. However, other methods for introducing the vector into cells may also be used, including nuclear microinjection and bacterial protoplast fusion.

The packaging cell line provides the components, including viral regulatory and structural proteins, that are required in trans for the packaging of the viral genomic RNA into retroviral (e.g., lentiviral) vector particles. The packaging cell line may be any cell line that is capable of expressing lentiviral proteins and producing functional lentiviral vector particles. Some suitable packaging cell lines include 293 (ATCC CCL X), 293T, HeLa (ATCC CCL 2), D17 (ATCC CCL 183), MDCK (ATCC CCL 34), BHK (ATCC CCL-10) and Cf2Th (ATCC CRL 1430) cells. The packaging cell line may stably express the necessary viral proteins. Such a packaging cell line is described, for example, in U.S. Pat. No. 6,218,181. Alternatively, a packaging cell line may be transiently transfected with nucleic acid molecules encoding one or more necessary viral proteins along with the viral vector genome. The resulting viral particles are collected and used to infect a target cell. The gene(s) encoding envelope glycoprotein(s) is usually cloned into an expression vector, such as pcDNA3 (Invitrogen, CA USA). Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Packaging cells, such as 293T cells are then co-transfected with the viral vector genome encoding a sequence of interest (typically encoding an antigen), at least one plasmid encoding virus packing components, and a vector for expression of the targeting molecule. The envelope is expressed on the membrane of the packaging cell and incorporated into the viral vector.

For purposes provided herein, the TCRs herein can be expressed in T cells or NK cells or other suitable cells of the immune system. The T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, peripheral blood mononuclear cells (PBMCs), peripheral blood leukocytes (PBLs), apheresis sample, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. In some embodiments, the T cell is a human T cell. In some embodiments, the T cell is a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4+/CD8+ double positive T cells, CD4+ helper T cells, e.g., Th1 and Th2 cells, CD8+ T cells (e.g., cytotoxic T cells), tumor infiltrating cells (TILs), memory T cells, naïve T cells, and the like.

Also provided by the disclosure is a population of cells comprising at least one cell described herein. The population of cells can be a heterogeneous population comprising the

host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, NK cells, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cells, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly of cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising cells comprising a recombinant expression vector as described herein.

Ex Vivo Genetic Modification of T cells

As noted above, in certain embodiments, it may be desirable to use the viral vectors disclosed herein to genetically modify T cells *ex vivo*. In this regard, the sources of T cells, culture and expansion of the T cells is described.

Prior to expansion and genetic modification of the T cells, a source of T cells is obtained from a subject. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available in the art, may be used. In certain embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation. In one embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains 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 one embodiment of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. Again, surprisingly, initial activation steps in the absence of calcium lead to magnified

activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca^{2+} -free, Mg^{2+} -free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

In another embodiment, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as CD3^+ , CD28^+ , CD4^+ , CD8^+ , CD45RA^+ , and CD45RO^+ T cells, can be further isolated by positive or negative selection techniques. For example, in one embodiment, T cells are isolated by incubation with anti-CD3/anti-CD28 (i.e., 3X28)-conjugated beads, such as DYNABEADS™ M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one embodiment, the time period is about 30 minutes. In a further embodiment, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred embodiment, the time period is 10 to 24 hours. In one preferred embodiment, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immune-compromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8^+ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In certain embodiments,

it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

5 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. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD 14, CD20,
10 CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express CD4+, CD25+, CD62Lhi, GITR+, and FoxP3+. Alternatively, in certain embodiments, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar method of selection.

For isolation of a desired population of cells by positive or negative selection, the
15 concentration of cells and surface (e.g., particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further
20 embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell
25 expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (i.e., leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of CD8+ T cells
30 that normally have weaker CD28 expression.

In certain embodiments, specific sub-types of T cells may be isolated and genetically modified with the lentiviral vector particles as described herein, using methods such as those

described for example, in WO 2012/129514.

In a related embodiment, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4+ T cells express higher levels of CD28 and are more efficiently captured than CD8+ T cells in dilute concentrations. In one embodiment, the concentration of cells used is 5×10^6 /ml. In other embodiments, the concentration used can be from about 1×10^5 /ml to 1×10^6 /ml, and any integer value in between.

In other embodiments, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10°C or at room temperature.

T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80C. at a rate of 1o per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20C. or in liquid nitrogen.

In certain embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present invention.

Also contemplated in the context of the invention is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen

for later use in T cell therapy for any number of diseases or conditions that would benefit from T cell therapy, such as those described herein. In one embodiment a blood sample or an apheresis is taken from a generally healthy subject. In certain embodiments, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain embodiments, the T cells may be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further embodiment, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-773, 1993). In a further embodiment, the cells are isolated for a patient and frozen for later use in conjunction with (e.g., before, simultaneously or following) bone marrow or stem cell transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cells are isolated prior to and can be frozen for later use for treatment following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan.

In a further embodiment, T cells are obtained from a patient directly following treatment. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand *ex vivo*. Likewise, following *ex vivo* manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and *in vivo* expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic

cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

Whether prior to or after genetic modification of the T cells *ex vivo* to express a sequence of interest, the T cells can be activated and expanded generally using methods known in the art. Illustrative methods for activating and expanding T cells are as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and published application Nos. WO2012/129514 and US20060121005.

In certain embodiments, T cells may be expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either CD4⁺ T cells or CD8⁺ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diacclone, Besancon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):1319-1328, 1999; Garland et al., J. Immunol. Meth. 227(1-2):53-63, 1999).

In certain embodiments, the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the costimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts.

In certain embodiments the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further embodiments the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted
5 above, however certain preferred values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one preferred ratio being at least 1:1 particles per T cell. In one embodiment, a ratio of particles to cells of 1:1 or less is used. In one particular embodiment, a preferred particle: cell ratio is 1:5. In further embodiments, the ratio of particles to cells can be varied depending on the day of
10 stimulation.

Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15,
15 TGF. β , and TNF- α . or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α -MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or
20 supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37°C.)
25 and atmosphere (e.g., air plus 5% CO₂).

T cells that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (TH, CD4+) that is greater than the cytotoxic or suppressor T cell population (Tc, CD8+). Ex vivo expansion of T cells by stimulating CD3
30 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of TH cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of TC cells. Accordingly, depending on the purpose of

treatment, infusing a subject with a T cell population comprising predominately of TH cells may be advantageous. Similarly, if an antigen-specific subset of TC cells has been isolated it may be beneficial to expand this subset to a greater degree.

Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

In certain embodiments, the present disclosure contemplates the use of T cells genetically modified to stably express a TCR as described herein. T cells expressing and engineered TCR, are referred to herein as chimeric TCR modified T cells. Preferably, the cell can be genetically modified to stably express an antibody binding domain on its surface, conferring novel antigen specificity that is MHC independent.

Compositions Comprising and Administration of Modified T Cells

In certain embodiments, the present disclosure provides compositions comprising T cells that have been modified using the lentiviral vector particles described herein to express a transgene of interest, such as the engineered TCRs described herein. Such compositions can be administered to subjects in the methods of the present disclosure as described further herein.

Compositions comprising the modified T cells as described herein can be utilized in methods and compositions for adoptive immunotherapy in accordance with known techniques, or variations thereof that will be apparent to those skilled in the art based on the instant disclosure. See, e.g., US Patent Application Publication No. 2003/0170238 to Gruenberg et al; see also US Patent No. 4,690,915 to Rosenberg.

In some embodiments, the cells are formulated by first harvesting them from their culture medium, and then washing and concentrating the cells in a medium and container system suitable for administration (a "pharmaceutically acceptable" carrier) in a treatment-effective amount. Suitable infusion medium can be any isotonic medium formulation, typically normal saline, Normosol R (Abbott) or Plasma-Lyte A (Baxter), but also 5% dextrose in water or Ringer's lactate can be utilized. The infusion medium can be supplemented with human serum albumin.

A treatment-effective amount of cells in the composition is typically greater than 10^2 cells, and up to 10^6 , up to and including 10^8 or 10^9 cells and can be more than 10^{10} cells. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein. For example, if cells that are specific for a particular antigen are desired, then the population will contain greater than 70%, generally greater than 80%, 85% and 90-95% of such cells. For uses provided herein, the cells are generally in a volume of a liter or less, can be 500 mls or less, even 250 mls or 100 mls or less. Hence the density of the desired cells is typically greater than 10^6 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^9 , 10^{10} or 10^{11} cells or the appropriate number of immune cells as determined by a clinician skilled in the art.

Methods of Diagnosis and Treatment

Provided herein are methods for identifying subjects likely to benefit from (i.e., the subject is suitable for) tumor antigen-specific cancer therapy. Also provided herein are methods of treatment and compositions useful for such treatment. In this regard, for those patients identified as likely to benefit from (i.e., the subject is suitable for) tumor antigen cancer therapy using the biomarkers and methods herein, any such therapy is contemplated herein, such as a cancer vaccine (e.g., a protein vaccine, an RNA vaccine, a viral vector vaccine, a retroviral vector vaccine, an adenoviral vector vaccine, a lentiviral vector vaccine, or other such vaccine) that induces an immune response against the tumor antigen. It should be understood that such immunotherapies may induce an immune response against one or more tumor antigens. Such tumor antigens are known in the art and are described elsewhere herein. In certain embodiments, the tumor antigen cancer therapy comprises adoptive immunotherapy with cells expressing a tumor antigen specific TCR. Combination therapies may also be used.

Provided herein are compositions comprising tumor antigens, nucleic acids encoding tumor antigens (DNA, RNA), and viral vectors (e.g., retroviral vectors, lentiviral vectors, adenoviral vectors and the like) expressing the tumor antigens or TCRs described herein.

Provided herein are compositions comprising engineered TCRs (e.g., soluble TCRs described herein, and fusion proteins or chimeric proteins thereof); compositions comprising viral vector particles comprising a sequence encoding a tumor antigen or an engineered TCR;

or compositions comprising cells, in particular T cells, expressing the engineered TCRs described herein for use in methods of treating cancer (e.g., a tumor antigen associated cancer such as a NY-ESO-1 cancer) or for use in inhibiting proliferation of a cancer cell that expresses a tumor antigen, such as NY-ESO-1.

5 In this regard, described herein is a method of treating a cancer associated with expression of a particular tumor antigen, such as a cancer testis antigen like NY-ESO-1, in a mammalian subject comprising administering to the subject a therapeutic composition, said composition comprising one or more therapeutic agents selected from the group consisting of (a) an engineered TCR as described herein; (b) an isolated cell comprising a polynucleotide
10 encoding an engineered TCR polypeptide disclosed herein; (c) a soluble TCR, or a chimeric or fusion polypeptide comprising the soluble TCR that is specific for the tumor antigen (e.g., NY-ESO-1) in the context of a MHC molecule; (d) a polynucleotide encoding an engineered TCR polypeptide; (e) a polynucleotide encoding a soluble TCR that is specific for a tumor antigen (e.g., NY-ESO-1) in the context of a MHC molecule; (f) a viral vector comprising a
15 polynucleotide encoding an engineered TCR polypeptide herein that is specific for a tumor antigen /MHC complex (e.g., NY-ESO-1/MHC complex); wherein the therapeutic composition is administered to the subject in an amount effective to treat the cancer in the subject. Illustrative TCR sequences for use in the engineered TCRs described herein for use in the methods of treatment and methods for inhibiting the proliferation of cancer described
20 herein are provided in the sequence listing and include the beta chain variable region provided in SEQ ID NO:9 and the alpha chain variable region provided in SEQ ID NO:8.

Described herein is a method of treating a cancer associated with tumor antigen expression in a mammalian subject comprising administering to the subject a therapeutic composition, said composition comprising one or more therapeutic agents selected from the
25 group consisting of (a) an isolated cell comprising a polynucleotide encoding an engineered TCR polypeptide comprising a V β CDR3 that is specific for the tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence; and a cognate alpha chain variable region; (b) a soluble TCR, or a chimeric or fusion polypeptide comprising the soluble TCR, comprising a V β chain CDR3 that is specific for the tumor antigen in the
30 context of a MHC molecule, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence; (c) a polynucleotide encoding a chimeric TCR polypeptide comprising a V β chain CDR3 that is specific for the tumor antigen, wherein the V β CDR3 comprises a public

TCR V β CDR3 amino acid sequence; (d) a polynucleotide encoding a soluble TCR comprising a V β chain CDR3 that is specific for the tumor antigen in the context of a MHC molecule, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence; (e) a vector comprising a polynucleotide encoding a chimeric TCR polypeptide comprising a V β chain CDR3 that is specific for the tumor antigen; and (f) a vector comprising a V β chain CDR3 that is specific for the tumor antigen in the context of a MHC molecule, wherein the therapeutic composition is administered to the subject in an amount effective to treat the cancer in the subject.

In this regard, described herein are methods for treating a cancer associated with tumor antigen expression wherein the tumor antigen is any one or more tumor antigens known in the art, such as described at [cvc\(dot\)dfci\(dot\)harvard\(dot\)edu/cvccgi/tadb/nomenclature\(dot\)pl](http://cvc(dot)dfci(dot)harvard(dot)edu/cvccgi/tadb/nomenclature(dot)pl). In particular, tumor antigens include any one or more cancer testis antigens (see e.g., those listed at CTDatabase available at [www\(dot\)cta\(dot\)lncc\(dot\)br](http://www(dot)cta(dot)lncc(dot)br)). In certain embodiments, the tumor antigens include, but are not limited to, Her2, Survivin, CEA, BING4, BAGE1, TRAG-3, TRP-2, GAGE1, GAGE2, GAGE3, GAGE4, GAGE5, GAGE6, GAGE7, GAGE8, IL13RA2, MAGEA1, MAGEA2, MAGEA3, MAGEA4, MAGEA6, MAGEA9, MAGEA10, MAGEA12, MAGEB1, MAGEB2, MAGEC2, TYR, SAGE, SSX2, SSX4, KRAS, PRAME, alpha actinin 4, CASP8, CDC27, CDK4, FN1, HSP70, KIAA0205 (LPGAT1), Malic enzyme, MART2, MUMw, MUM3, OS9, PTPRK, alpha-fetoprotein, annexin II, ART4, CLCA2, CPSF1, cyclophilin B, EphA3, FGF5, CA9, TERT, GNT-V, TEL1, Livin/ML-IAP, MCSF, MUC1, MUM1, NY-ESO-1, MRPL28 (melanoma antigen p15), PSMA, PSA, RAGE, renal ubiquitous protein 1, RU2AS (KAAG1), SART1, TSPYL1, SART3, SOX10, TRG, WT1, TACSTD1 (Ep-CAM), SILV (gp100), SCGB2A2 (mammaglobin A), Mart-1, CASP5, CDKN2A, COA-1, EFTUD2, cyclin D1, CYP1B1, MDM2, STEAP1, TGFBR2, PXDNL, AKAP13, ACPP, LCK, RPS2, SCL45A3, BCL2L1, DKK1, ENAH, CSPG4, BCR, BCR-ABL, DEK, DEK-CAN, FLT3, ABL1, AML1, Mesothelin, UBE2V1, BCL-2, MCL1, cathepsin H, 5T4 oncofetal antigen, XAGE antigen, RPSA, calreticulin 2, ErbB3-binding protein 1, FMNL1, HPSE, CDC2, SEPT2, STAT1, LFP1, ADAM17, JUP, DDR1, HMOX1, PAGE4, PAK2, CDKN1A, SOX2, SOX11, PGK1, SOX4, TOR3A, SLBP, EGFR, TTK, LY6K, IGF2BP3, GPC3, SLC35A4, ALDH1A1, MMP14, SDCBP, PARP12, c-MET, cyclin B1, PAX3, FOXO1, XBP1, ETV5, HERC1, SLC46A1, BCL11A, MAP1A, NFATC2, NCBP3, PPP1CA, SIK1, HMGN1, MAP3K11, GFI1, CCDC88B, TPX2, SRSF7, and

SETD2. In one embodiment, the present disclosure provides methods for treating a cancer associated with expression of NY-ESO-1, MAGEA1, MAGEA3, MAGEA4, MAGEA10, or any combination of the foregoing tumor antigens.

Described herein is a method of treating a cancer associated with NY-ESO-1 expression in a mammalian subject comprising administering to the subject a therapeutic composition, said composition comprising one or more therapeutic agents selected from the group consisting of (a) an isolated cell comprising a polynucleotide encoding an engineered TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID NOs: 2 – 4; or where in the beta chain variable region is as provided in SEQ ID NO:9 and the alpha chain variable region is as provided in SEQ ID NO:8; (b) a soluble TCR, or a chimeric or fusion polypeptide comprising the soluble TCR, comprising a V β chain CDR3 that is specific for NY-ESO-1 in the context of a MHC molecule, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; (c) a polynucleotide encoding a chimeric TCR polypeptide comprising a V β chain CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises the amino acid sequence as set forth in SEQ ID Nos: 2 – 4; (d) a polynucleotide encoding a soluble TCR comprising a V β chain CDR3 that is specific for NY-ESO-1 in the context of a MHC molecule, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1 or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; (e) a vector comprising a polynucleotide encoding a chimeric TCR polypeptide comprising a V β chain CDR3 that is specific for NY-ESO-1; and (f) a vector comprising a V β chain CDR3 that is specific for NY-ESO-1 in the context of a MHC molecule, wherein the therapeutic composition is administered to the subject in an amount effective to treat the cancer in the subject. In certain of the embodiments described herein, the engineered TCR comprises the alpha chain variable region and the beta chain variable region as provided in SEQ ID NO:8 and 9 respectively.

The terms "tumor antigen-associated cancer" and "cancer cell that expresses a tumor antigen" as used herein refer to a tumor comprising cells that express one or more tumor

antigens. Such cancers are known in the art and expression of tumor antigens in a particular cancer can be determined by a person of ordinary skill in the art. Tumor antigen expression can be determined using a variety of assays known in the art, such as but not limited to, immunohistochemistry, quantitative PCR or RT-PCR whole cell ELISA (see *e.g.*, J Immunol Methods. 2001 Dec 1;258(1-2):47-53), flow cytometry, RNASCOPE® (Advanced Cell Diagnostics, Inc., Newark, CA) or other commercially available test (*e.g.*, from Nanostring, Seattle, WA), FISH, and Western blot.

In some embodiments, the tumor is a solid tumor. Exemplary tumor antigen-associated cancers include, but are not limited to, sarcoma (*e.g.* soft tissue sarcoma, synovial sarcoma, myxoid round cell liposarcoma, spindle cell sarcoma), melanoma, lymphoma, prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, oesophageal cancer, non-small-cell lung cancer, non-Hodgkin's lymphoma, NHL (DLCL), multiple myeloma, hepatocellular carcinoma, head and neck cancer, gastric cancer, endometrial cancer, renal cancer, colorectal cancer, cholangiocarcinoma, breast cancer, bladder cancer, neuroblastoma, myeloid leukemia and acute lymphoblastic leukemia.

Any tumor antigen known in the art is relevant to the present methods of treatment and methods for identifying patients likely to benefit from treatment. In particular, tumor antigens include any one or more cancer testis antigen (CTA) (see *e.g.*, those listed at CTDatabase available at [www\(dot\)cta\(dot\)lncc\(dot\)br](http://www.cta.lncc.br)). In certain embodiments, the tumor antigens include, but are not limited to, Her2, Survivin, CEA, BING4, BAGE1, TRAG-3, TRP-2, GAGE1, GAGE2, GAGE3, GAGE4, GAGE5, GAGE6, GAGE7, GAGE8, IL13RA2, MAGEA1, MAGEA2, MAGEA3, MAGEA4, MAGEA6, MAGEA9, MAGEA10, MAGEA12, MAGEB1, MAGEB2, MAGEC2, TYR, SAGE, SSX2, SSX4, KRAS, PRAME, alpha actinin 4, CASP8, CDC27, CDK4, FN1, HSP70, KIAA0205 (LPGAT1), Malic enzyme, MART2, MUMw, MUM3, OS9, PTPRK, alpha-fetoprotein, annexin II, ART4, CLCA2, CPSF1, cyclophilin B, EphA3, FGF5, CA9, TERT, GNT-V, TEL1, Livin/ML-IAP, MCSF, MUC1, MUM1, NY-ESO-1, MRPL28 (melanoma antigen p15), PSMA, PSA, RAGE, renal ubiquitous protein 1, RU2AS (KAAG1), SART1, TSPYL1, SART3, SOX10, TRG, WT1, TACSTD1 (Ep-CAM), SILV (gp100), SCGB2A2 (mammaglobin A), Mart-1, CASP5, CDKN2A, COA-1, EFTUD2, cyclin D1, CYP1B1, MDM2, STEAP1, TGFBR2, PXDNL, AKAP13, ACPP, LCK, RPS2, SCL45A3, BCL2L1, DKK1, ENAH, CSPG4, BCR,

BCR-ABL, DEK, DEK-CAN, FLT3, ABL1, AML1, Mesothelin, UBE2V1, BCL-2, MCL1, cathepsin H, 5T4 oncofetal antigen, XAGE antigen, RPSA, calreticulin 2, ErbB3-binding protein 1, FMNL1, HPSE, CDC2, SEPT2, STAT1, LFP1, ADAM17, JUP, DDR1, HMOX1, PAGE4, PAK2, CDKN1A, SOX2, SOX11, PGK1, SOX4, TOR3A, SLBP, EGFR, TTK, LY6K, IGF2BP3, GPC3, SLC35A4, ALDH1A1, MMP14, SDCBP, PARP12, c-MET, cyclin B1, PAX3, FOXO1, XBP1, ETV5, HERC1, SLC46A1, BCL11A, MAP1A, NFATC2, NCBP3, PPP1CA, SIK1, HMGN1, MAP3K11, GFI1, CCDC88B, TPX2, SRSF7, and SETD2. In one embodiment, the present disclosure provides methods for treating a cancer associated with expression of NY-ESO-1, MAGEA1, MAGEA3, MAGEA4, MAGEA10, or any combination of the foregoing tumor antigens.

The terms "NY-ESO-1 cancer" and "cancer cell that expresses NY-ESO-1" as used herein refer to a tumor comprising cells that express the NY-ESO-1 tumor antigen. Such cancers are known in the art and expression of NY-ESO-1 in a particular cancer can be determined by a person of ordinary skill in the art. In some embodiments, the tumor is a solid tumor. Exemplary NY-ESO-1 cancers include, but are not limited to, sarcoma (e.g. soft tissue sarcoma), melanoma, lymphoma, prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, oesophageal cancer, non-small-cell lung cancer, non-Hodgkin's lymphoma, NHL (DLCL), multiple myeloma, hepatocellular carcinoma, head and neck cancer, gastric cancer, endometrial cancer, renal cancer, colorectal cancer, cholangiocarcinoma, breast cancer, bladder cancer, neuroblastoma, myeloid leukemia and acute lymphoblastic leukemia.

Also described herein is a method of inhibiting proliferation of a cancer cell that expresses one or more tumor antigens, such as NY-ESO-1, in a mammalian subject comprising administering to the subject a therapeutic composition comprising one or more therapeutic agents selected from the group consisting of (a) an isolated cell comprising a polynucleotide encoding a chimeric TCR polypeptide comprising a V β chain complementarity determining region 3 (CDR3) that is specific for the tumor antigen (e.g., NY-ESO-1), wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence or wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; (b) a soluble TCR comprising a V β chain CDR3 that is specific for the tumor antigen (e.g., NY-ESO-1) in the context of a MHC molecule, wherein the V β CDR3

comprises a public TCR V β CDR3 amino acid sequence or comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; (c) a polynucleotide encoding a chimeric TCR polypeptide comprising a V β chain CDR3 that is specific for the tumor antigen (e.g., NY-ESO-1), wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence or comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; (d) a polynucleotide encoding a soluble TCR comprising a V β chain CDR3 that is specific for a tumor antigen (e.g., NY-ESO-1) in the context of a MHC molecule, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence or comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; (e) a vector comprising a polynucleotide encoding a chimeric TCR polypeptide comprising a V β chain CDR3 that is specific for the tumor antigen (e.g., NY-ESO-1); and (f) a vector comprising a V β chain CDR3 that is specific for the tumor antigen (e.g., NY-ESO-1) in the context of a MHC molecule, wherein the therapeutic composition is administered to the subject in an amount effective to inhibit proliferation of the cancer cell in the subject. In certain of the embodiments described herein, the engineered TCR comprises the alpha chain variable region and the beta chain variable region as provided in SEQ ID NO:8 and 9 respectively.

A "therapeutically effective amount" or "effective amount" as used herein, means an amount which provides a therapeutic or prophylactic benefit.

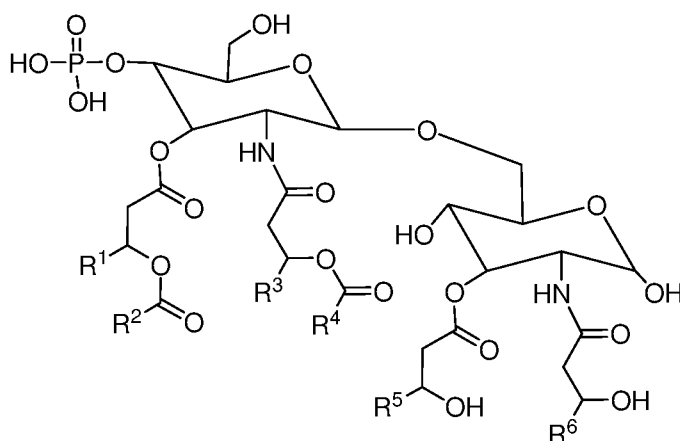
The methods of treatment contemplated herein include any tumor antigen-specific cancer therapy, in particular immunotherapies. In one embodiment, the treatment compositions and methods useful for patients expressing the immune biomarkers as described herein are such as those described in US Patent No. 9,044,420; USSN 15/439,324; 8329162; 8372390; 8273345; 8187872; 8323662 and published PCT application WO2013/149167. In some embodiments, a tumor antigen-specific cancer therapy comprises administering a vector comprising a polynucleotide encoding a tumor antigen polypeptide to the subject. In some embodiments, the vector is a lentiviral vector. In some embodiments, the vector comprises a polynucleotide encoding a tumor antigen polypeptide. In other embodiments, the tumor antigen-specific cancer therapy comprises administering DNA or RNA encoding the tumor antigen(s). Other therapies that can be used for treatment of the patients identified as likely

to benefit from (i.e., the subject is suitable for) such treatment are known to the skilled person.

The methods of treatment contemplated herein include any NY-ESO-1 specific cancer therapy, in particular immunotherapies. In one embodiment, the treatment methods useful for patients expressing the public TCRs as described herein are such as those described in US Patent No. 9,044,420. In some embodiments, the NY-ESO-1 cancer therapy comprises administering a vector comprising a polynucleotide encoding an NY-ESO-1 polypeptide to the subject. In some embodiments, the vector is a lentiviral vector. In some embodiments, the vector comprises a polynucleotide encoding an NY-ESO-1 polypeptide.

In some embodiments, the NY-ESO-1 cancer therapy comprises administering to the subject an effective amount of a composition comprising GLA, said composition comprising:

(a) GLA of formula (I):



wherein: R¹, R³, R⁵ and R⁶ are C₁₁-C₂₀ alkyl; and R² and R⁴ are C₁₂-C₂₀ alkyl; and

(b) a pharmaceutically acceptable carrier or excipient. In certain embodiments, the composition comprising GLA does not comprise antigen. In one embodiment of the methods described herein, R¹, R³, R⁵ and R⁶ are undecyl and R² and R⁴ are tridecyl. In another embodiment of the methods described herein, the mammal is human. In yet a further embodiment, the composition is an aqueous formulation, and in certain embodiments, the composition is in the form of an oil-in-water emulsion, a water-in-oil emulsion, liposome, micellar formulation, or a microparticle.

U.S. Patent Publication No. 2008/0131466 that provides formulations, such as

aqueous formulation (AF) and stable emulsion formulations (SE) for GLA compounds, wherein these formulations may be used for any of the compounds of formula (I).

GLA as described herein is present in a composition in an amount of 0.1-10 µg/dose, or 0.1-20 µg/dose, 0.1-30 µg/dose, 0.1-40 µg/dose, or 0.1-50 µg/dose, or 1-20 µg/dose, or 1-30 µg/dose, or 1-40 µg/dose, or 1-50 µg/dose, or 0.2-5 µg/dose, or in an amount of 0.5-2.5 µg/dose, or in an amount of 0.5-8 µg/dose or 0.5-15 µg/dose. Doses may be, for example, 0.5 µg/dose, 0.6 µg/dose, 0.7 µg/dose, 0.8 µg/dose, 0.9 µg/dose, 1.0 µg/dose, 2.0 µg/dose, 3.0 µg/dose, 3.5 µg/dose, 4.0 µg/dose, 4.5 µg/dose, 5.0 µg/dose, 5.5 µg/dose, 6.0 µg/dose, 6.5 µg/dose, 7.0 µg/dose, 7.5 µg/dose, 8.0 µg/dose, 9.0 µg/dose, 10.0 µg/dose, 11.0 µg/dose, 12.0 µg/dose, 13.0 µg/dose, 14.0 µg/dose, or 15.0 µg/dose. Doses may be adjusted depending upon the body mass, body area, weight, blood volume of the subject, or route of delivery. In one embodiment, 2 µg, 3 µg, 4 µg, 5 µg, 6 µg, 7 µg, 8 µg, 9 µg, 10 µg, 11 µg, or 12 µg of GLA in 1 ml is administered intratumorally. In this regard, the 1 mL dose of GLA may be injected in equal amounts in multiple zones of the tumor. In certain embodiments, about 0.01 µg/kg to about 100 mg/kg body weight of GLA will be administered, typically by the intradermal, intratumoral, subcutaneous, intramuscular or intravenous route, or by other routes. In certain embodiments, GLA is administered by any of the routes described herein in combination with recombinant protein. In one embodiment, GLA is administered intramuscularly or subcutaneously in combination with recombinant protein. In one embodiment, DC targeted lentiviral vector particles (e.g., LV305) as described herein are administered intradermally and the GLA/recombinant protein (e.g., G305) is administered intramuscularly. In certain embodiments, the dosage of GLA is about 0.1 µg/kg to about 1 mg/kg, and in certain embodiments, ranges from about 0.1 µg/kg, 0.2 µg/kg, 0.3 µg/kg, 0.4 µg/kg, 0.5 µg/kg, 0.6 µg/kg, 0.7 µg/kg, 0.8 µg/kg, 0.9 µg/kg, 1 µg/kg, 2 µg/kg, 3 µg/kg, 4 µg/kg, 5 µg/kg, 6 µg/kg, 7 µg/kg, 8 µg/kg, 9 µg/kg, 10 µg/kg to about 200 µg/kg. It will be evident to those skilled in the art that the number and frequency of administration will be dependent upon the response of the host. As described herein, the appropriate dose may also depend upon the patient's (e.g., human) condition, that is, stage of the disease, general health status, as well as age, gender, and weight, and other factors familiar to a person skilled in the medical art. In certain embodiments, the GLA compositions described herein do not include antigen.

Identifying Subjects likely to benefit from (i.e., the subject is suitable for) tumor antigen-specific cancer therapy

In some embodiments, it is desirable to identify the presence of a TCR comprising a CDR3 sequence described herein that is specific for a tumor antigen/MHC complex. In one embodiment, the TCR is a public TCR specific for a tumor antigen/MHC complex. In one embodiment, it is desirable to identify the presence of a TCR comprising a CDR3 sequence described herein that is specific for NY-ESO-1/MHC complex. Methods for identifying the presence of the TCR include deep sequencing strategies such as IMMUNOSEQTM which is commercially available from Adaptive Biotechnologies (Seattle, WA). See also Nature, 515,568–571 (27 November 2014); Carreno et al., 2015 Science 348:803-808). IMMUNOSEQ was used in Examples 2 – 7 to discover NY-ESO-1-specific TCR CDR3 sequences herein, but methods for detecting the presence of tumor antigen-specific CDR3 sequences, including those described herein, are not limited to this method. Any technology that detects the presence or absence of specific nucleotide sequences encoding the TCR CDR3 sequences are contemplated for use herein. Additionally, the public TCRs having the V β CDR3 amino acid sequences described herein might be detected directly by immunoassay with monoclonal antibodies or RNA-aptamers developed for this purpose. The immunoassays which can be used include, but are not limited to, competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA, "sandwich" immunoassays, immunoprecipitation assays, precipitin assays, gel diffusion precipitin assays, immunoradiometric assays, fluorescent immunoassays, protein A, immunoassays, and complement-fixation assays. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994 Current Protocols in Molecular Biology, Vol. 1, John Wiley & sons, Inc., New York). Additionally, routine cross-blocking assays such as those described in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane, 1988), can be performed. Either nucleic acid based assays or protein based detection assays can be used to determine in an individual the presence or absence of T cells having TCRs comprising a V β CDR3 amino acid sequence described herein.

Methods of identifying subjects likely to benefit from treatment with a therapeutic composition, such as the compositions described herein are also contemplated. In this regard, the method comprises (a) identifying a mammalian subject as likely to benefit from a tumor antigen-specific cancer therapy comprising determining in a sample from the mammalian subject the presence of (i) a polynucleotide encoding a TCR polypeptide comprising a V β chain CDR3 that is specific for the tumor antigen, wherein the V β chain comprises a public TCR V β CDR3 amino acid sequence; or (ii) a TCR polypeptide comprising a V β chain CDR3

that is specific for the tumor antigen, wherein the V β chain comprises a public TCR V β CDR3 amino acid sequence; wherein the presence of (i) and/or (ii) is indicative that the subject will likely benefit from a tumor antigen-specific cancer therapy.

Methods of identifying subjects likely to benefit from (i.e., the subject is suitable for) treatment with a therapeutic composition, such as the compositions described herein are also contemplated. In this regard, the method comprises (a) identifying a mammalian subject as likely to benefit from a NY-ESO-1 cancer therapy comprising determining in a sample from the mammalian subject the presence of (i) a polynucleotide encoding a TCR polypeptide comprising a V β chain CDR3 that is specific for NY-ESO-1, wherein the V β chain comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; or (ii) a TCR polypeptide comprising a V β chain CDR3 that is specific for NY-ESO-1, wherein the V β chain comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; wherein the presence of (i) and/or (ii) is indicative that the subject will likely benefit from a NY-ESO-1 cancer therapy.

In a further embodiment, a method of treating a subject that has been identified as a subject that is likely to benefit from (i.e., the subject is suitable for) the treatment is also contemplated herein. In this regard, the method of treatment comprises (a) identifying a mammalian subject as likely to benefit from a tumor antigen-specific cancer therapy comprising determining in a sample from the mammalian subject the presence of (i) a polynucleotide encoding a TCR polypeptide comprising a V β chain CDR3 that is specific for the tumor antigen, wherein the V β chain comprises a public TCR V β CDR3 amino acid sequence; or (ii) a TCR polypeptide comprising a V β chain CDR3 that is specific for the tumor antigen, wherein the V β chain comprises a public TCR V β CDR3 amino acid sequence; wherein the presence of (i) and/or (ii) is indicative that the subject is likely to benefit from a tumor antigen-specific cancer therapy; and (b) administering the tumor antigen-specific cancer therapy to the mammalian subject.

The present disclosure provides in one embodiment a method of treating cancer in a patient where the cancer is a locally advanced unresectable or metastatic synovial sarcoma. In another embodiment, the patient has no evidence of progression after first-line chemotherapy. Thus, the present disclosure provides in one embodiment a method of treating

cancer where the cancer is a locally advanced unresectable or metastatic synovial sarcoma in a patient who has no evidence of progression after first-line chemotherapy. In certain embodiments, the method of treating cancer where the cancer is a locally advanced unresectable or metastatic synovial sarcoma in a patient who has no evidence of progression after first-line chemotherapy, comprises first identifying whether the patient is likely to benefit from an antigen-specific cancer therapy (e.g., NY-ESO-1, MAGE or other antigen-specific therapy), such as an in vivo DC targeted lentiviral vector cancer therapy, comprising detecting in one or more biological samples from the patient the presence of at least two of the biomarkers disclosed herein, and then administering to the patient the in vivo DC targeted lentiviral vector cancer therapy. In some embodiments, the lentiviral vector cancer therapy is a lentiviral vector cancer therapy that induces an antigen-specific immune response against NY-ESO-1.

In a further embodiment, a method of treating a subject that has been identified as a subject that is likely to benefit from (i.e., the subject is suitable for) the treatment is also contemplated herein. In this regard, the method of treatment comprises (a) identifying a mammalian subject as likely to benefit from a NY-ESO-1 cancer therapy comprising determining in a sample from the mammalian subject the presence of (i) a polynucleotide encoding a TCR polypeptide comprising a V β chain CDR3 that is specific for NY-ESO-1, wherein the V β chain comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; or (ii) a TCR polypeptide comprising a V β chain CDR3 that is specific for NY-ESO-1, wherein the V β chain comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or wherein the V beta CDR3 comprises an amino acid sequence as set forth in SEQ ID Nos: 2 – 4; wherein the presence of (i) and/or (ii) is indicative that the subject is likely to benefit from a NY-ESO-1 cancer therapy; and (b) administering the NY-ESO-1 cancer therapy to the mammalian subject.

Other methods, including multiplex PCR, and other technologies known in the art that detect the presence or absence of specific nucleotide sequences can also be utilized. Additionally, the TCR polypeptides may be detected directly by immunoassay with either the appropriate tetramer or monoclonal antibodies developed for this purpose. The immunoassays which can be used include, but are not limited to, competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA, "sandwich"

immunoassays, immunoprecipitation assays, precipitin assays, gel diffusion precipitin assays, immunoradiometric assays, fluorescent immunoassays, protein A, immunoassays, plasmon surface resonance, and complement-fixation assays, and the like. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 2015 Current Protocols in Molecular Biology, Vol. 1, John Wiley & sons, Inc., New York). Additionally, routine cross-blocking assays such as those described in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane, 1988), can be performed. Either nucleic acid based or protein based assays can distinguish individuals carrying a TCR comprising a V β chain having the CDR3 amino acid sequence described herein at high frequency from individuals not carrying the diagnostic clonotype.

In certain embodiments, patients likely to benefit from (i.e., the subject is suitable for) one or more tumor antigen-specific cancer therapeutics (e.g., treatment with a DC targeted lentiviral vector expressing the tumor antigen in combination with a TLR4 agonist / recombinant tumor antigen protein in a prime boost regimen; optionally in combination with a checkpoint inhibitor; or other tumor antigen-specific cancer treatment) include those patients with two or more of the following biomarkers: 1) Pre-existing tumor antigen-specific antibodies; 2) pre-existing tumor antigen specific T cells (CD4+ and/or CD8+ T cells); 3) a tumor antigen-specific public TCR; 4) a tumor in which at least 50% of tumor cells express the tumor antigen; 5) a) a tumor antigen-specific public TCR and b) a tumor in which at least 50% of tumor cells express the tumor antigen. In one embodiment, patients likely to benefit from treatment with a tumor antigen-specific cancer therapeutic (e.g., in particular treatment with a DC targeted lentiviral vector expressing the tumor antigen in combination with a TLR4 agonist / recombinant tumor antigen protein in a prime boost regimen, optionally in combination with a checkpoint inhibitor) include those patients who have tumor antigen-specific public TCR and a tumor in which at least 50% of tumor cells express the tumor antigen. In certain embodiments, patients with synovial sarcoma or myxoid/round cell liposarcoma having two or more of the aforementioned biomarkers are more likely to benefit from one or more tumor antigen-specific cancer therapeutics as described herein as compared to patients lacking these biomarkers.

In certain embodiments, patients likely to benefit from (i.e., the subject is suitable for) treatment with a tumor antigen-specific cancer therapeutic include those patients who have a tumor in which at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%,

85%, 90%, 95% or more of tumor cells express the tumor antigen. Methods for detecting tumor antigens (either at the protein level or nucleic acid level) in biological samples are known in the art and include, for example, immunohistochemistry, whole cell ELISA, quantitative PCR or RT-PCR, flow cytometry, RNASCOPE® (Advanced Cell Diagnostics, Inc., Newark, CA) or other commercially available test (e.g., from Nanostring, Seattle, WA), FISH, and Western blot.

In another embodiment, patients likely to benefit from (i.e., the subject is suitable for) treatment with a tumor antigen-specific cancer therapeutic include those patients who have a tumor in which the expression level of a particular tumor antigen is at least 2 fold higher than in a corresponding normal tissue (or a pre-determined cut-off value). In this regard, this expression level may be independent of the percentage of cells within the tumor that express the antigen or may be in combination with the percentage of cells within the tumor that express the antigen. For example, in one embodiment, a patient likely to respond to a therapy as described herein is a patient who has a tumor in which at least 50% of the tumor cells express the tumor antigen and the expression level is at least at a certain level (e.g., at least 2-fold, 3-fold, 4-fold, 5-fold higher than a pre-determined cut-off level, or at least at a high, medium, or low level). In certain embodiments, patients likely to benefit from treatment with a tumor antigen-specific cancer therapeutic include those patients who have a tumor in which the expression level of a particular tumor antigen is at least two fold, three fold, four fold, and in certain embodiments, at least five fold, greater than a pre-determined cut-off level, or greater than the level of expression in normal tissues, as determined using a representative assay known in the art and provided herein.

A predetermined cut-off value used in the methods described herein for determining the expression of a particular tumor antigen can be readily identified using well-known techniques. For example, in one illustrative embodiment, the predetermined cut-off value for the detection of a tumor antigen (e.g., NY-ESO-1) is the average mean signal obtained when the relevant method is performed on suitable negative control samples, e.g., samples from patients without cancer. In another illustrative embodiment, a sample generating a signal that is at least two or three standard deviations above the predetermined cut-off value is considered positive. In another embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, Little Brown and Co., 1985, p. 106-7. Briefly, in this

embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for expression of a particular tumor antigen.

As noted elsewhere herein, tumor antigens include but are not limited to any one or more cancer testis antigen (see e.g., those listed at CTDatabase available at [www\(dot\)cta\(dot\)lncc\(dot\)br](http://www(dot)cta(dot)lncc(dot)br)). In certain embodiments, the tumor antigens include, but are not limited to, Her2, Survivin, CEA, BING4, BAGE1, TRAG-3, TRP-2, GAGE1, GAGE2, GAGE3, GAGE4, GAGE5, GAGE6, GAGE7, GAGE8, IL13RA2, MAGEA1, MAGEA2, MAGEA3, MAGEA4, MAGEA6, MAGEA9, MAGEA10, MAGEA12, MAGEB1, MAGEB2, MAGEC2, TYR, SAGE, SSX2, SSX4, KRAS, PRAME, alpha actinin 4, CASP8, CDC27, CDK4, FN1, HSP70, KIAA0205 (LPGAT1), Malic enzyme, MART2, MUMw, MUM3, OS9, PTPRK, alpha-fetoprotein, annexin II, ART4, CLCA2, CPSF1, cyclophilin B, EphA3, FGF5, CA9, TERT, GNT-V, TEL1, Livin/ML-IAP, MCSF, MUC1, MUM1, NY-ESO-1, MRPL28 (melanoma antigen p15), PSMA, PSA, RAGE, renal ubiquitous protein 1, RU2AS (KAAG1), SART1, TSPYL1, SART3, SOX10, TRG, WT1, TACSTD1 (Ep-CAM), SILV (gp100), SCGB2A2 (mammaglobin A), Mart-1, CASP5, CDKN2A, COA-1, EFTUD2, cyclin D1, CYP1B1, MDM2, STEAP1, TGFBR2, PXDNL, AKAP13, ACPP, LCK, RPS2, SCL45A3, BCL2L1, DKK1, ENAH, CSPG4, BCR, BCR-ABL, DEK, DEK-CAN, FLT3, ABL1, AML1, Mesothelin, UBE2V1, BCL-2, MCL1, cathepsin H, 5T4 oncofetal antigen, XAGE antigen, RPSA, calreticulin 2, ErbB3-binding protein 1, FMNL1, HPSE, CDC2, SEPT2, STAT1, LFP1, ADAM17, JUP, DDR1, HMOX1, PAGE4, PAK2, CDKN1A, SOX2, SOX11, PGK1, SOX4, TOR3A, SLBP, EGFR, TTK, LY6K, IGF2BP3, GPC3, SLC35A4, ALDH1A1, MMP14, SDCBP, PARP12, c-MET, cyclin B1, PAX3, FOXO1, XBP1, ETV5, HERC1, SLC46A1, BCL11A, MAP1A, NFATC2, NCBP3, PPP1CA, SIK1, HMGN1, MAP3K11, GFI1, CCDC88B, TPX2, SRSF7, and SETD2. In one embodiment, the present disclosure provides methods for detecting the disclosed immune biomarkers that are specific

for the aforementioned tumor antigens and also for treating a cancer associated with expression of such antigens. In one embodiment, the present disclosure provides methods for detecting the disclosed immune biomarkers that are specific for NY-ESO-1, MAGEA1, MAGEA3, MAGEA4, or MAGEA10 and also for treating a cancer associated with expression of NY-ESO-1, MAGEA1, MAGEA3, MAGEA4, MAGEA10, or any combination of the foregoing tumor antigens.

In certain embodiments, patients likely to benefit from (i.e., the subject is suitable for) one or more NY-ESO-1 cancer therapeutics (e.g., treatment with a DC targeted lentiviral vector expressing NY-ESO-1 in combination with a TLR4 agonist / recombinant NY-ESO-1 protein in a prime boost regimen) include those patients with two or more of the following biomarkers: 1) Pre-existing NY-ESO-1 antibodies; 2) pre-existing NY-ESO-1 specific T cells (CD4+ and/or CD8+ T cells); 3) an NY-ESO-1 specific public TCR as described herein 4) a) an NY-ESO-1 specific public TCR as described herein and b) a tumor in which at least 50% of tumor cells express NY-ESO-1. In one embodiment, patients likely to benefit from treatment with an NY-ESO-1 cancer therapeutic (e.g., in particular treatment with a DC targeted lentiviral vector expressing NY-ESO-1 in combination with a TLR4 agonist / recombinant NY-ESO-1 protein in a prime boost regimen) include those patients who have NY-ESO-1 specific public TCR as described herein and a tumor in which at least 50% of tumor cells express NY-ESO-1. In certain embodiments, patients with synovial sarcoma or myxoid/round cell liposarcoma having two or more of the aforementioned biomarkers are more likely to benefit from one or more NY-ESO-1 cancer therapeutics as described herein as compared to patients lacking these biomarkers.

Thus, in certain embodiments of the present disclosure, it may be desirable to identify patients having tumor antigen-specific antibodies prior to and/or after initiating treatment. In this regard, methods for detecting tumor antigen-specific antibodies are known in the art and are described further herein. Such methods include for example, ELISA. In certain embodiments, it may be desirable to identify patients having tumor antigen-specific antibodies and also having pre-existing tumor antigen-specific T cells (CD4+ and/or CD8+ T cells). In one embodiment, the present disclosure provides methods for identifying patients, particularly patients with soft tissue sarcomas (in particular, synovial sarcomas), who have two or more of the following biomarkers: 1) Pre-existing tumor antigen-specific antibodies; 2) pre-existing tumor antigen-specific T cells (CD4+ and/or CD8+ T cells); 3) a tumor antigen -

specific public TCR; 4) a) a tumor antigen-specific public TCR and b) a tumor in which at least 50% of tumor cells express the tumor antigen.

Thus, in certain embodiments of the present disclosure, it may be desirable to identify patients having NY-ESO-1-specific antibodies prior to and/or after initiating treatment. In this regard, methods for detecting NY-ESO-1 antibodies are known in the art and are described further herein. Such methods include for example, ELISA. In certain embodiments, it may be desirable to identify patients having NY-ESO-1-specific antibodies and also having pre-existing NY-ESO-1 antigen-specific T cells (CD4+ and/or CD8+ T cells). In one embodiment, the present disclosure provides methods for identifying patients, particularly patients with soft tissue sarcomas (in particular, synovial sarcomas), who have two or more of the following biomarkers: 1) Pre-existing NY-ESO-1 antibodies; 2) pre-existing NY-ESO-1 specific T cells (CD4+ and/or CD8+ T cells); 3) an NY-ESO-1 specific public TCR as described herein 4) a tumor in which at least 50% of tumor cells express the tumor antigen; 5) a) an NY-ESO-1 specific public TCR as described herein and b) a tumor in which at least 50% of tumor cells express NY-ESO-1.

In another embodiment of the present disclosure, it may be desirable to identify patients having pre-existing tumor antigen-specific T cells, *e.g.*, tumor antigen-specific CD4+ and/or CD8+ T cells. Such patients have been identified as being more likely to respond to treatment.

In another embodiment of the present disclosure, it may be desirable to identify patients having pre-existing NY-ESO-1 specific T cells, *e.g.*, NY-ESO-1 antigen-specific CD4+ and/or CD8+ T cells. Such patients have been identified as being more likely to respond to treatment.

In another embodiment of the present disclosure, it may be desirable to identify patients having a tumor antigen-specific public TCR, such as an NY-ESO-1 specific public TCR as described herein. In this regard, a patient may have one or more public TCRs. In one embodiment, a patient may have one or more of the public TCRs disclosed herein. In certain embodiments, it may be desirable to identify patients having a) a tumor antigen-specific public TCR, such as an NY-ESO-1 specific public TCR as described herein; and b) a tumor in which at least 50% of tumor cells express the tumor antigen, such as NY-ESO-1. Such patients have been identified herein as being more likely to benefit to the tumor antigen-

specific (e.g., NY-ESO-1) therapeutic treatments described herein (e.g., in particular treatment with a DC targeted lentiviral vector expressing NY-ESO-1 in combination with a TLR4 agonist / recombinant NY-ESO-1 protein in a prime boost regimen).

One embodiment of the present disclosure provides methods for identifying patients who would benefit from extended tumor antigen-specific treatments such as those described herein (e.g., treatment with a DC targeted lentiviral vector expressing a tumor antigen, in combination with a TLR4 agonist / recombinant tumor antigen protein in a prime boost regimen; or other tumor antigen specific immunotherapy). In particular, in one embodiment, it may be desirable to identify those patients who have induction of an integrated immune response following treatment with a tumor antigen cancer treatment (e.g., a treatment such as a prime boost regimen of a DC targeted lentiviral vector expressing tumor antigen in combination with a TLR4 agonist / recombinant NY-ESO-1 protein). In this regard, an integrated immune response refers to a tumor antigen-specific CD4+ T cell response, tumor antigen-specific CD8+ T cell response and a tumor antigen-specific antibody response. Tumor antigen-specific T cell and antibody responses can be detected using methods as described herein and then compared to the immune responses determined before treatment or as compared to a predetermined cut-off value. Patients who have an integrated immune response induced by the treatment have been shown to have a better clinical response and would benefit from continued, extended treatment. In certain embodiments, it may be desirable to identify those patients who induce a tumor antigen-specific CD8+ T cell response following treatment with a tumor antigen-specific treatment, such as but not limited to, a prime boost regimen of a DC targeted lentiviral vector expressing the tumor antigen in combination with a TLR4 agonist / recombinant tumor antigen protein. Such patients have been shown to have a better clinical response and would benefit from extended treatment.

One embodiment of the present disclosure provides methods for identifying patients who would benefit from extended NY-ESO-1 treatments such as those described herein (e.g., in particular treatment with a DC targeted lentiviral vector expressing NY-ESO-1 in combination with a TLR4 agonist / recombinant NY-ESO-1 protein in a prime boost regimen). In particular, in one embodiment, it may be desirable to identify those patients who have induction of an integrated immune response following treatment with an NY-ESO-1 cancer treatment (e.g., a treatment such as a prime boost regimen of a DC targeted lentiviral vector expressing NY-ESO-1 in combination with a TLR4 agonist / recombinant NY-ESO-1

protein). In this regard, an integrated immune response refers to an NY-ESO-1 specific CD4+ T cell response, NY-ESO-1 specific CD8+ T cell response and an NY-ESO-1 specific antibody response. NY-ESO-1 specific T cell and antibody responses can be detected using methods as described herein and then compared to the immune responses determined before treatment or as compared to a predetermined cut-off value. Patients who have an integrated immune response induced by the treatment have been shown to have a better clinical response and would benefit from continued, extended treatment. In certain embodiments, it may be desirable to identify those patients who induce an NY-ESO-1 specific CD8+ T cell response following treatment with an NY-ESO-1 treatment, such as a prime boost regimen of a DC targeted lentiviral vector expressing NY-ESO-1 in combination with a TLR4 agonist / recombinant NY-ESO-1 protein. Such patients have been shown to have a better clinical response and would benefit from extended treatment.

In another embodiment, the present disclosure provides methods for identifying patients who should receive additional or extended treatment. In this regard, such patients include patients who have developed antigen spreading following treatment with a tumor antigen-specific cancer treatment, such as an NY-ESO-1 cancer treatment (e.g., a prime boost regimen of a DC targeted lentiviral vector expressing a tumor antigen such as NY-ESO-1 in combination with a TLR4 agonist / recombinant tumor antigen protein, such as NY-ESO-1 protein). In this regard, Epitope or antigen spreading refers to a process whereby epitopes distinct from and non-cross-reactive with an inducing epitope (e.g., NY-ESO-1 epitope) become targets of an ongoing immune response (see e.g., Immunol Rev. 1998 Aug;164:63-72). For example, a patient who has received an anti-NY-ESO-1 prime-boost regimen or other treatment as described elsewhere herein may develop reactivity against new epitopes of NY-ESO-1 than initially recognized. In some embodiments, patients may develop reactivity against one or more other tumor associated antigens, such as other cancer testis antigens like any one or more of the MAGE proteins.

In certain embodiments, patients with soft tissue sarcomas, ovarian cancer, melanomas and non small cell lung carcinoma (NSCLC) may benefit from the methods described herein for identifying particular biomarkers.

In certain embodiments, the present disclosure provides methods of treating cancer comprising (a) identifying a mammalian subject as likely to benefit from (i.e., the subject is suitable for) a tumor antigen-specific cancer therapy comprising determining in a sample

from the mammalian subject the presence of two or more of the following biomarkers: 1)Pre-existing tumor antigen-specific antibodies; 2) pre-existing tumor antigen-specific T cells (CD4+ and/or CD8+ T cells); 3) a tumor antigen-specific public TCR; 4) a tumor in which at least 50% of tumor cells express the tumor antigen; 5) a) a tumor antigen-specific public TCR and b) a tumor in which at least 50% of tumor cells express the tumor antigen; wherein two or more of the foregoing is indicative that the subject is likely to benefit from the tumor antigen-specific cancer therapy; and (b) administering the tumor antigen-specific cancer therapy to the mammalian subject. In this regard, the tumor antigen-specific cancer therapy may be any one or more immunotherapies. In certain embodiments, the tumor antigen-specific cancer therapy may be as described herein, such as treatment with a DC targeted lentiviral vector expressing the tumor antigen in combination with a TLR4 agonist / recombinant tumor antigen protein in a prime boost regimen, optionally in combination with an immune checkpoint inhibitor.

In certain embodiments, the present disclosure provides methods of treating cancer comprising (a) identifying a mammalian subject as likely to benefit from (i.e., the subject is suitable for) a NY-ESO-1 cancer therapy comprising determining in a sample from the mammalian subject the presence of two or more of the following biomarkers: 1)Pre-existing NY-ESO-1 antibodies; 2) pre-existing NY-ESO-1 specific T cells (CD4+ and/or CD8+ T cells); 3) an NY-ESO-1 specific public TCR as described herein 4) a) an NY-ESO-1 specific public TCR as described herein and b) a tumor in which at least 50% of tumor cells express NY-ESO-1; wherein two or more of the foregoing is indicative that the subject is likely to benefit from the NY-ESO-1 cancer therapy; and (b) administering the NY-ESO-1 cancer therapy to the mammalian subject. In this regard, the NY-ESO-1 cancer therapy may be any one or more as described herein, such as treatment with a DC targeted lentiviral vector expressing NY-ESO-1 in combination with a TLR4 agonist / recombinant NY-ESO-1 protein in a prime boost regimen.

As described herein, in any of the methods, clinical benefit may be defined as increases in overall survival (OS), progression free survival (PFS), stable disease (SD) or other clinical measurements known to the skilled clinician, such as objective responses (OR), time-to-next treatment, and quality of life. In certain embodiments, other Response Evaluation Criteria In Solid Tumors (RECIST) criteria may be used.

Cells of the immune system that are involved in an immune response are referred to, generally, as immune cells and include a lymphocyte and a non-lymphoid cell such as

accessory cell. Lymphocytes are cells that specifically recognize and respond to foreign antigens, and accessory cells are those that are not specific for certain antigens but are involved in the cognitive and activation phases of immune responses. For example, mononuclear phagocytes (macrophages), other leukocytes (*e.g.*, granulocytes, including neutrophils, eosinophils, basophils), and dendritic cells function as accessory cells in the induction of an immune response. The activation of lymphocytes by a foreign antigen leads to induction or elicitation of numerous effector mechanisms that function to eliminate the antigen. Accessory cells such as mononuclear phagocytes that affect or are involved with the effector mechanisms are also called effector cells.

Major classes of lymphocytes include B lymphocytes (B cells), T lymphocytes (T cells), and natural killer (NK) cells, which are large granular lymphocytes. B cells are capable of producing antibodies. T lymphocytes are further subdivided into helper T cells (CD4+ (also referred to herein and in the art as CD4)) and cytolytic or cytotoxic T cells (CD8+ (also referred to herein and in the art as CD8)). Helper cells secrete cytokines that promote proliferation and differentiation of the T cells and other cells, including B cells and macrophages, and recruit and activate inflammatory leukocytes. Another subgroup of T cells, called regulatory T cells or suppressor T cells actively suppress activation of the immune system and prevent pathological self-reactivity, that is, autoimmune disease.

In a cell mediated responses, the various types of T lymphocytes act to eliminate infected or cancerous cells or antigen by a number of mechanisms. For example, helper T cells that are capable of recognizing specific antigens may respond by releasing soluble mediators such as cytokines to recruit additional cells of the immune system to participate in an immune response. Also, cytotoxic T cells are capable of specifically recognizing an antigen and may respond by binding to and destroying or damaging an antigen-bearing cell or particle. The methods described herein for inducing an immune response may also induce a humoral response, also called a B cell response herein and in the art. A humoral response includes production of antibodies that specifically bind to an antigen (or immunogen). Antibodies are produced by differentiated B lymphocytes known as plasma cells.

Whether an immune response is induced and the type of immune response induced in a host or subject (*e.g.*, a human cancer patient) may be determined by any number of well-known immunological methods described herein and with which those having ordinary skill in the art will be familiar. As described herein, methods and techniques for determining the

presence and level of an immune response include, for example, fluorescence resonance energy transfer, fluorescence polarization, time-resolved fluorescence resonance energy transfer, scintillation proximity assays, reporter gene assays, fluorescence quenched enzyme substrate, chromogenic enzyme substrate and electrochemiluminescence, immunoassays, (such as enzyme-linked immunosorbant assays (ELISA), radioimmunoassay, immunoblotting, immunohistochemistry, and the like), surface plasmon resonance, cell-based assays such as those that use reporter genes, and functional assays (e.g., assays that measure immune function and immunoresponsiveness).

Such assays include, but need not be limited to, in vivo or in vitro determination of the presence and level of soluble antibodies, soluble mediators such as cytokines (e.g., IFN- γ , IL-2, IL-4, IL-10, IL-12, IL-6, IL-23, TNF- α , and TGF- β), lymphokines, chemokines, hormones, growth factors, and the like, as well as other soluble small peptide, carbohydrate, nucleotide and/or lipid mediators. Immunoassays also include determining cellular activation state changes by analyzing altered functional or structural properties of cells of the immune system, for example, cell proliferation, altered motility, induction of specialized activities such as specific gene expression or cytolytic behavior; cell maturation, such as maturation of dendritic cells in response to a stimulus; alteration in relationship between a Th1 response and a Th2 response; cellular differentiation by cells of the immune system, including altered surface antigen expression profiles or the onset of apoptosis (programmed cell death). Procedures for performing these and similar assays are may be found, for example, in Lefkovits (Immunology Methods Manual: The Comprehensive Sourcebook of Techniques, 1998). See also Current Protocols in Immunology; Weir, Handbook of Experimental Immunology, Blackwell Scientific, Boston, MA (1986); Mishell and Shigii (eds.) Selected Methods in Cellular Immunology, Freeman Publishing, San Francisco, CA (1979); Green and Reed, Science 281:1309 (1998) and references cited therein).

Determining the presence and/or level of antibodies that specifically bind to an immunogen and the respective designated antigen of interest may be determined using any one of several immunoassays routinely practiced in the art, including but not limited to, ELISAs, immunoprecipitation, immunoblotting, countercurrent immunoelectrophoresis, radioimmunoassays, dot blot assays, inhibition or competition assays, and the like (see, e.g., U.S. Patent Nos. 4,376,110 and 4,486,530; Harlow et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory (1988)). Immunoassays may also be performed to determine

the class and isotype of an antibody that specifically binds to an immunogen. Antibodies (polyclonal and/or monoclonal or antigen-binding fragments thereof), which specifically bind to an immunogen and which may be used as controls in immunoassays detecting an antibody-specific immune response in an immunized subject, may generally be prepared by any of a variety of techniques known to persons having ordinary skill in the art. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988); Peterson, *ILAR J.* 46:314-19 (2005); (Kohler et al., *Nature*, 256:495-97 (1976); Kohler et al., *Eur. J. Immunol.* 6:511-19 (1975); Coligan et al. (eds.), *Current Protocols in Immunology*, 1:2.5.1-2.6.7 (John Wiley & Sons 1991); U.S. Patent Nos. 4,902,614, 4,543,439, and 4,411,993; Monoclonal Antibodies, *Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett et al. (eds.) (1980); *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press (1988); see also, e.g., Brand et al., *Planta Med.* 70:986-92 (2004); Pasqualini et al., *Proc. Natl. Acad. Sci. USA* 101:257-59 (2004). The immunogen, or immunogenic fragments thereof, or a cell or particle bearing the immunogen or immunogenic fragment thereof may be used for immunizing an animal for production of either polyclonal antibodies or monoclonal antibodies.

In certain embodiments, a tumor antigen-specific T cell response, such as a T cell response to NY-ESO-1, are measured by both *ex vivo* ELISPOT or ELISPOT after *in vitro* stimulation (IVS). In *ex vivo* ELISPOT assay, which can be performed from leukapheresis samples, an appropriate number of PBMC are plated in each well in triplicate in pre-coated human IFN γ ELISPOT plates (MabTech, Cincinnati, OH). Serum-free medium (OpTmizer, Life Technologies, Carlsbad, CA) is generally used for the PBMC culture. NY-ESO-1 peptide pool (a panel of 43 15mer peptides overlapping by 11, synthesized commercially) can be used to stimulate the PBMC. The PBMC are cultured for about 48hr in ELISPOT plates before the plates are developed using TMB substrate. The number of spots per well is counted using an automatic counter. In some experiments, the PBMC may be stimulated with each of the 43 overlapping peptides individually at 1 μ g/mL to determine the potential NY-ESO-1 epitope that stimulates the T cell response after expansion *in vitro*.

In certain embodiments, for example for *in vitro* stimulation (IVS) experiments (see e.g., Sabbatini P, et al., *Clin Cancer Res.* 2012; 18:6497-508), CD8 and CD4 T cells are sequentially positively selected from PBMCs using magnetic beads (Life Technologies, Carlsbad, CA) in medium (e.g., X-VIVO-15, Lonza, Basel, Switzerland). Remaining cells

are used as APCs pulsed overnight with overlapping peptides spanning the length of the tumor antigen. In certain embodiments, a NY-ESO-1 peptide pool (seventeen 20mers overlapping by 10, available from commercial sources), irradiated, washed, and placed in 96-well plates containing RPMI + 10% serum AB culture with 500,000 CD8 and CD4 T cells, separately, at each collection time point. IL-2 (10 U/ml, Roche) and IL-7 (20ng/ml, R&D Systems) may be added after 24h and twice a week thereafter, until testing by IFN- γ ELISPOT at d10 for CD8 against peptide-pulsed autologous EBV-transformed B cells (B-EBV) and d20 for CD4 against peptide-pulsed PHA-expanded CD4 T cells (T-APC).

In certain embodiments, patient plasma samples are analyzed by ELISA for seroreactivity against recombinant tumor antigen protein (e.g., NY-ESO-1 protein), as well as overlapping long peptides (20-mers) covering the length of the tumor antigen sequences, are such as, as previously described (Sabatini et al., 2012, Supra). Synthetic long peptides may be used to confirm specificity for the tumor antigen plasma antibodies, such as NY-ESO-1 plasma antibodies, and SSX2 protein and p53 peptide pools may be used as additional specificity controls. A reciprocal titer can be calculated for each plasma sample as the maximal dilution still significantly reacting to a specific antigen. This value is then extrapolated by determining the intersection of a linear trend regression with a cutoff value. The cutoff can be defined as ten times the average of OD values from the first four dilutions of a negative control pool comprising three healthy donor sera. In each assay, sera of patients with known presence or absence of specific reactivity may be used as controls. Titers >100 are generally considered reactive, and specificity determined by comparing reactivity to control antigens and to the tumor antigen (e.g., NY-ESO-1) peptides.

In certain embodiments, DNA extraction of PBMC and FFPE preserved solid tumor curls and TCR β immunosequencing are performed at Adaptive Biotechnologies (Seattle, WA, USA). In brief, DNA is extracted and TCR- β CDR3 regions amplified using a multiplexed PCR method with 54 forward primers specific to TCR V β gene segments and 13 reverse primers specific to TCR J β gene segments. The Illumina HiSeq platform is then used to sequence the resulting amplicons. Housekeeping genes are also amplified, and their template counts quantitated in order to determine the amount of DNA usable for TCRB sequencing. The number of total cells and T cells, the T-cell fraction, the number of unique rearrangements, and clonality are calculated for each sample.

Levels of cytokines may be determined according to methods described herein and

practiced in the art, including for example, ELISA, ELISPOT, intracellular cytokine staining, and flow cytometry and combinations thereof (e.g., intracellular cytokine staining and flow cytometry). Immune cell proliferation and clonal expansion resulting from an antigen-specific elicitation or stimulation of an immune response may be determined by isolating lymphocytes, such as spleen cells or cells from lymph nodes or tumors (tumor infiltrating lymphocytes (TIL)), stimulating the cells with antigen, and measuring cytokine production, cell proliferation and/or cell viability, such as by incorporation of tritiated thymidine or non-radioactive assays, such as MTT assays and the like. The effect of an immunogen described herein on the balance between a Th1 immune response and a Th2 immune response may be examined, for example, by determining levels of Th1 cytokines, such as IFN- γ , IL-12, IL-2, and TNF- β , and Type 2 cytokines, such as IL-4, IL-5, IL-9, IL-10, and IL-13.

The level of a CTL immune response and the level of a memory CD4 T cell response may be determined by any one of numerous immunological methods described herein and routinely practiced in the art. The level of a CTL immune response may be determined prior to administration of any one of the compositions, vectors, or vector particles described herein and then used for comparison with the level of CTL immune response at an appropriate time point after one or more administrations of the compositions, vectors, or vector particles that provide memory CD4 T cell help. Cytotoxicity assays for determining CTL activity may be performed using any one of several techniques and methods routinely practiced in the art (see, e.g., Henkart et al., "Cytotoxic T-Lymphocytes" in *Fundamental Immunology*, Paul (ed.) (2003 Lippincott Williams & Wilkins, Philadelphia, PA), pages 1127-50, and references cited therein).

In certain embodiments, T cell and antibody responses are determined using well validated protocols known to those of skill in the art. Illustrative methods are such as those described in Yuan et al., 2011 PNAS 108:16723-16728; Yuan et al., 2008 PNAS 105:20410-20415; Gnjjatic et al., 2009 *Methods Mol Biol* 520:11-19; Lin et al., 2009 *Cytotherapy* 11:912-922. In certain embodiments, polyfunctional T cells may be assessed using single-cell multiplex cytokine profiling according to methods described in *Journal of Clinical Oncology*, (2017) 35 (9), Suppl. 7S Abstract 49; *Nature Medicine*, 17, 738-743 (2011); *Cancer Discovery*, 3, 418 (2013). In certain embodiments, antibody titers are stratified as follows: NY-ESO-1Ab titer: -, negative; +, 100~1,000; ++, 1,000~10,000; +++, >10,000. In certain embodiments, NY-ESO-1 polyfunctional T-cell responses are stratified as follows: -,

<0.1%; +, 0.1~0.5%; ++, 0.5~5%; +++, >5%; where polyfunctional T-cell response is defined as T cells producing double functions for IFN- γ , TNF- α , MIP-1 β , and CD107a; and the value $\geq 0.1\%$.

As used herein, a binding partner or an antibody is said to be “immunospecific,”
5 “specific for” or to “specifically bind” an immunogen of interest if the antibody reacts at a detectable level with the immunogen or immunogenic fragment thereof, preferably with an affinity constant, K_a , of greater than or equal to about 10^4 M⁻¹, or greater than or equal to about 10^5 M⁻¹, greater than or equal to about 10^6 M⁻¹, greater than or equal to about 10^7 M⁻¹, or greater than or equal to 10^8 M⁻¹. Affinity of an antibody for its cognate antigen is
10 also commonly expressed as a dissociation constant K_D , and an antibody specifically binds to the immunogen of interest if it binds with a K_D of less than or equal to 10^{-4} M, less than or equal to about 10^{-5} M, less than or equal to about 10^{-6} M, less than or equal to 10^{-7} M, or less than or equal to 10^{-8} M.

Affinities of binding partners or antibodies can be readily determined using
15 conventional techniques, for example, those described by Scatchard et al. (Ann. N.Y. Acad. Sci. USA 51:660 (1949)) and by surface plasmon resonance (SPR; BIAcore™, Biosensor, Piscataway, NJ). For surface plasmon resonance, target molecules are immobilized on a solid phase and exposed to a binding partner (or ligand) in a mobile phase running along a flow cell. If ligand binding to the immobilized target occurs, the local refractive index changes,
20 leading to a change in SPR angle, which can be monitored in real time by detecting changes in the intensity of the reflected light. The rates of change of the SPR signal can be analyzed to yield apparent rate constants for the association and dissociation phases of the binding reaction. The ratio of these values gives the apparent equilibrium constant (affinity) (see, e.g., Wolff et al., Cancer Res. 53:2560-2565 (1993)).

25 A biological sample may be obtained from the subject for determining the presence and level of an immune response to one or more tumor antigens in the subject who has received any one or more of the immunogenic compositions described herein, such as an immunogenic composition comprising an immunogen and an immunogenic composition comprising a recombinant expression vector comprising a nucleotide sequence encoding the
30 immunogen (e.g., tumor antigen) or who has received both immunogenic compositions, including one or more compositions comprising an adjuvant according to the methods described herein. A “biological sample” as used herein may be a blood sample (from which

serum or plasma may be prepared), biopsy specimen, body fluids (e.g., lung lavage, ascites, mucosal washings, synovial fluid), bone marrow, lymph nodes, tissue explant, organ culture, or any other tissue or cell preparation from the subject or a biological source. Biological samples may also be obtained from the subject prior to receiving any immunogenic composition, which biological sample is useful as a control for establishing baseline (i.e., pre-immunization) data. The biomarkers described herein and the methods for detecting such biomarkers may be carried out (such biomarkers may be detected in) on any one or more appropriate biological sample. Thus, in certain embodiments, multiple biological samples may be tested per patient and at multiple time points pre and post treatment to detect the multiple biomarkers (e.g., tumor antigens such as NY-ESO-1 may be detected by IHC in a tumor biopsy while the presence of a public TCR would be detected from a blood or PBMC sample). In one embodiment, the methods herein for identifying patients who are more likely to benefit from a particular treatment are carried out on a single pre-treatment biopsy, e.g. by using single cell analysis of tumor lysates. In certain embodiments, the biomarkers of interest, in particular, pTCR and the presence of tumor antigen (e.g., NY-ESO-1, one or more MAGEs, etc.) expression in tumor cells as described herein, are determined in a single pre-treatment biopsy.

With respect to all immunoassays and methods described herein for determining an immune response, a person skilled in the art will also readily appreciate and understand which controls are appropriately included when practicing these methods. Concentrations of reaction components, types of buffers, temperature, and time periods sufficient to permit interaction of the reaction components can be determined and/or adjusted according to methods described herein and with which persons skilled in the art are familiar.

Delivery of the Lentiviral Particles

The viral particles for delivery of a tumor antigen or an engineered TCR described herein (e.g. retroviral, lentiviral or other viral particles) may be delivered to a target cell in any way that allows the virus to contact the target cell, e.g., T cell, NK cell or dendritic cell, in which delivery of a polynucleotide of interest is desired. At times, a suitable amount of virus will be introduced into a human or other animal directly (*in vivo*), e.g., through injection into the body. Suitable animals include, without limitation, any mammal, non-human primates, horses, dogs, cats, cattle, pigs, sheep, rabbits, chickens or other birds. Viral particles may be injected by a number of routes, such as intravenous, intra-dermal,

subcutaneous, intratumoral, intranodal, intra-peritoneal cavity, or mucosal. In certain embodiments, intradermal delivery of lentiviral particles is preferred. The virus may be delivered using a subdermal injection device such the devices disclosed in U.S. Pat. Nos. 7,241,275, 7,115,108, 7,108,679, 7,083,599, 7,083,592, 7,047,070, 6,971,999, 6,808,506, 6,780,171, 6,776,776, 6,689,118, 6,670,349, 6,569,143, 6,494,865, 5,997,501, 5,848,991, 5,328,483, 5,279,552, 4,886,499. Other injection locations also are suitable, such as directly into organs comprising target cells. For example, intra-lymph node injection, intra-spleen injection, or intra-bone marrow injection may be used to deliver virus to the lymph node, the spleen and the bone marrow, respectively. Depending on the particular circumstances and nature of the target cells, introduction can be carried out through other means including for example, inhalation, or direct contact with epithelial tissues, for example those in the eye, mouth or skin.

Alternatively, target cells are provided and contacted with the virus *in vitro*, such as in culture plates. The target cells are typically populations of cells comprising dendritic cells or T cells obtained from a healthy subject or a subject in need of treatment or in whom it is desired to stimulate an immune response to an antigen. Methods to obtain cells from a subject are well known in the art and includes phlebotomy, surgical excision, and biopsy. Human DCs may also be generated by obtaining CD34 α + human hematopoietic progenitors and using an *in vitro* culture method as described elsewhere (e.g., Banchereau et al. *Cell* 106, 271-274 (2001)).

The virus may be suspended in media and added to the wells of a culture plate, tube or other container. Media containing the virus may be added prior to the plating of the cells or after the cells have been plated. Cells are typically incubated in an appropriate amount of media to provide viability and to allow for suitable concentrations of virus in the media such that transduction of the host cell occurs. The cells are preferably incubated with the virus for a sufficient amount of time to allow the virus to infect the cells. Preferably the cells are incubated with virus for at least 1 hour, at least 5 hours or at least 10 hours.

In both *in vivo* and *in vitro* delivery, an aliquot of viral particles containing sufficient number to infect the desired target cells may be used. When the target cell is to be cultured, the concentration of the viral particles is generally at least 1 IU/ μ L, more preferably at least 10 IU/ μ L, even more preferably at least 300 IU/ μ L, even more preferably at least 1×10^4 IU/ μ L, even more preferably at least 1×10^5 IU/ μ L, even more preferably at least

1X10⁶ IU/μL, or even more preferably at least 1X10⁷ IU/μL.

Following infection with the virus *in vitro*, target cells can be introduced (or re-introduced) into a human or other animal. The cells can be introduced into the dermis, under the dermis, or into the peripheral blood stream. The cells introduced into an animal are
5 preferably cells derived from that animal, to avoid an adverse immune response. Cells derived from a donor having a similar immune background may also be used. Other cells that also can be used include those designed to avoid an adverse immunologic response.

Target cells may be analyzed for integration, transcription and/or expression of the sequence or gene(s) of interest, the number of copies of the gene integrated, and the location
10 of the integration, for example. Such analysis may be carried out at any time and may be carried out by any method known in the art.

Subjects in which a virus, or virus-infected T cells, are administered can be analyzed for location of infected cells, expression of the virus-delivered polynucleotide or gene of interest, stimulation of an immune response, and monitored for symptoms associated with a
15 disease or disorder by any methods known in the art.

The methods of infecting cells disclosed herein do not depend upon individual-specific characteristics of the cells. As a result, they are readily extended to a variety of animal species. In some instances, viral particles are delivered to a human or to human T cells, and in other instances they are delivered to an animal such as a mouse, horse, dog, cat,
20 or mouse or to birds. As discussed herein, the viral vector genome is pseudotyped to confer upon it a broad host range as well as target cell specificity. One of skill in the art would also be aware of appropriate internal promoters and other elements to achieve the desired expression of a sequence of interest in a particular animal species. Thus, one of skill in the art will be able to modify the method of infecting dendritic cells from any species.

25 Combination Therapy

For those patients identified using the biomarkers and methods herein, as likely to benefit from (i.e., the subject is suitable for) tumor antigen cancer therapy, any tumor antigen specific therapy is contemplated herein, such as a cancer vaccine (e.g., a protein vaccine, an RNA vaccine, a viral vaccine, a retroviral vaccine, an adenoviral vaccine, a lentiviral vaccine,
30 or other such vaccine) that induces an immune response against the tumor antigen. It should

be understood that such immunotherapies may induce an immune response against one or more tumor antigens. Such tumor antigens are known in the art and are described elsewhere herein. In certain embodiments, the tumor antigen cancer therapy comprises adoptive immunotherapy with cells expressing a tumor antigen specific TCR, such as those described
5 herein. Combination therapies may also be used as described further herein.

Thus, provided herein are therapeutic compositions comprising tumor antigens, nucleic acids encoding tumor antigens (DNA, RNA), and viral vectors (e.g., lentiviral vectors) expressing the tumor antigens or TCR sequences as described herein.

The therapeutic compositions described herein may also be administered
10 simultaneously with, prior to, or after administration of one or more other therapeutic agents. Such combination therapy may include administration of a single pharmaceutical dosage formulation which contains a therapeutic composition described herein and one or more additional active agents, as well as administration of compositions (e.g., compositions comprising a tumor antigen or an engineered TCR as described herein, or compositions
15 comprising lentiviral vector particles comprising a sequence encoding a tumor antigen or an engineered TCR as described herein or compositions comprising isolated T cells modified to express an engineered TCR as described herein) and each active agent in its own separate pharmaceutical dosage formulation. For example, a therapeutic composition as described herein and the other active agent can be administered to the mammalian subject together in a
20 single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Similarly, the compositions described herein (e.g., comprising the lentiviral vector particles comprising a sequence encoding a tumor antigen or an engineered TCR as described herein, or a composition comprising T cells modified *ex vivo* with the such particles, or compositions comprising recombinant tumor antigen protein, or an
25 engineered TCR) and the other active agent can be administered to the mammalian subject together in a single parenteral dosage composition such as in a saline solution or other physiologically acceptable solution, or each agent administered in separate parenteral dosage formulations. Where separate dosage formulations are used, the compositions disclosed herein and one or more additional active agents can be administered at essentially the same
30 time, i.e., concurrently, or at separately staggered times, i.e., sequentially and in any order; combination therapy is understood to include all these regimens. Thus, in certain embodiments, also contemplated is the administration of one or more compositions disclosed

herein, in combination with one or more other therapeutic agents. Such therapeutic agents may be accepted in the art as a standard treatment for cancer. Exemplary therapeutic agents contemplated include cytokines, growth factors, immune checkpoint inhibitors, TLR agonists including TLR4 agonists such as glucopyranosyl lipid adjuvant (GLA) (as described for
5 example in US8273361, WO2008/153541 and WO2009143457), steroids, NSAIDs, DMARDs, anti-inflammatories, chemotherapeutics, radiotherapeutics, or other active and ancillary agents.

In certain embodiments, the therapeutic compositions disclosed herein may be administered in conjunction with any number of immune checkpoint inhibitors. Immune
10 checkpoint inhibitors include antibodies, or antigen binding fragments thereof, that bind to and block or inhibit the activity of one or more of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, and GAL9. Illustrative immune checkpoint inhibitors include Tremelimumab (CTLA-4 blocking antibody), anti-OX40, PD-L1 monoclonal Antibody (Anti-B7-H1; MEDI4736; MPLDL3280A (atezolizumab; TECENTRIQ), ipilimumab, MK-
15 3475 (PD-1 blocker). Nivolumab (anti-PD1 antibody).

In certain embodiments, the compositions disclosed herein may be administered in conjunction with any number of chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa,
20 carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil
25 mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic
30 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, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, 5 testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic 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; 10 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"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE®, Rhne- 15 Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoic acid 20 derivatives such as Targretin™ (bexarotene), Panretin™ (alitretinoin); ONTAK™ (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are 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, 25 trioxifene, keoxifene, LY117018, 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 compositions disclosed herein may be administered in conjunction with a histone deacetylase (HDAC) inhibitor. HDAC inhibitors include 30 hydroxamates, cyclic peptides, aliphatic acids and benzamides. Illustrative HDAC inhibitors contemplated for use herein include, but are not limited to, Suberoylanilide hydroxamic acid (SAHA/Vorinostat/Zolinza), Trichostatin A (TSA), PXD-101, Depsipeptide (FK228/romidepsin/ISTODAX®), panobinostat (LBH589), MS-275, Mocetinostat (MGCD0103),

ACY-738, TMP195, Tucidinostat, valproic acid, sodium phenylbutyrate, 5-aza-2' -
deoxycytidine (decitabine). See e.g., Kim and Bae, *Am J Transl Res* 2011;3(2):166-179;
Odunsi et al., *Cancer Immunol Res.* 2014 January 1; 2(1): 37–49. Other HDAC inhibitors
include Vorinostat (SAHA, MK0683), Entinostat (MS-275), Panobinostat (LBH589),
5 Trichostatin A (TSA), Mocetinostat (MGCD0103), ACY-738, Tucidinostat (Chidamide),
TMP195, Citarinostat (ACY-241), Belinostat (PXD101), Romidepsin (FK228,
Depsipeptide), MC1568, Tubastatin A HCl, Givinostat (ITF2357), Dacinostat (LAQ824),
CUDC-101, Quisinostat (JNJ-26481585) 2HCl, Pracinostat (SB939), PCI-34051,
Droxinostat, Abexinostat (PCI-24781), RGFP966, AR-42, Ricolinostat (ACY-1215),
10 Valproic acid sodium salt (Sodium valproate), Tacedinaline (CI994), CUDC-907, Sodium
butyrate, Curcumin, M344, Tubacin, RG2833 (RGFP109), Resminostat, Divalproex Sodium,
Scriptaid, and Tubastatin A (see e.g., selleckchem(dot)com).

In certain embodiments, the present disclosure provides a method of treating,
inhibiting the progression of or preventing a cancer associated with tumor antigen expression,
15 particularly in patients identified as likely to benefit from such a treatment using the
biomarkers and methods described herein, by administering to a mammalian subject afflicted
by a cancer associated with the tumor antigen expression a therapeutically effective amount
of a lentiviral vector comprising a nucleic acid encoding the tumor antigen, an engineered
TCR disclosed herein, lentiviral vectors comprising a nucleic acid encoding an engineered
20 TCR disclosed herein, or a composition comprising T cells modified *ex vivo* with such
particles. In certain embodiments, the method further comprises administering to the patient
a composition comprising a pseudotyped lentiviral vector particle comprising an envelope
that targets dendritic cells and can thus be used for dendritic cell vaccination (see e.g., US
Patent Nos. 8329162; 8372390; 8273345; 8187872; 8323662 and published PCT application
25 WO2013/149167). In this manner, antigen specific T-cells can be generated through *in vivo*
or *ex vivo* genetic modification using the lentiviral vectors described herein, and then are
boosted *in vivo* through active immunization of dendritic cells, using a DC-tropic lentiviral
vector.

In certain embodiments, the present disclosure provides a method of treating,
30 inhibiting the progression of or preventing a cancer associated with a tumor antigen, such as
NY-ESO-1 expression (particularly in those patients identified as likely to benefit from (i.e.,
the subject is suitable for) such a treatment using the biomarkers and methods herein) by

administering to a mammalian subject afflicted by a cancer associated with NY-ESO-1 expression a therapeutically effective amount of an engineered TCR disclosed herein, lentiviral vectors comprising a nucleic acid encoding an engineered TCR disclosed herein, or a composition comprising T cells modified *ex vivo* with such particles, and then further
5 administering to the patient a composition comprising a pseudotyped lentiviral vector particle comprising an envelope that targets dendritic cells and can thus be used for dendritic cell vaccination (see e.g., US Patent Nos. 8329162; 8372390; 8273345; 8187872; 8323662 and published PCT application WO2013/149167). In this manner, antigen specific T-cells can be generated through *in vivo* or *ex vivo* genetic modification using the lentiviral vectors
10 described herein, and then are boosted *in vivo* through active immunization of dendritic cells, using a DC-tropic lentiviral vector.

In another embodiment, the present disclosure provides a method of treating, inhibiting the progression of or preventing a cancer associated with tumor antigen expression, such as an NY-ESO-1 cancer, by administering to a mammalian subject afflicted with the
15 tumor antigen associated cancer a therapeutically effective amount of a composition comprising an engineered TCR specific for the tumor antigen, such as the NY-ESO-1 engineered TCRs disclosed herein, a composition comprising lentiviral vectors comprising a nucleic acid encoding an engineered TCR disclosed herein, or a composition comprising T cells modified *ex vivo* with such particles, and then further boosting the immune response by
20 administering to the patient a composition comprising a TLR4 agonist, such as Glucopyranosyl Lipid A (GLA) (see e.g., US Patent No. 8,273,361 and published applications WO2012/141984 and US20120328655), with or without an antigen. In this manner, antigen specific T-cells can be generated through *in vivo* or *ex vivo* genetic modification using the lentiviral vectors described herein, and then are boosted *in vivo*
25 through activation of dendritic cells.

For purposes of the inventive methods, wherein host cells or populations of cells are administered to the subject, the cells can be cells that are allogeneic or autologous to the host. Preferably, the cells are autologous to the subject.

The subject referred to herein can be any subject. Preferably, the subject is a mammal.
30 As used herein, the term “mammal” refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora,

including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). An especially preferred mammal is the human.

Pharmaceutical Compositions and Kits

Also contemplated herein are pharmaceutical compositions and kits containing one or more of (1) an engineered TCR as described herein; (2) viral particles comprising a nucleic acid encoding an engineered TCR; (3) immune cells, such as T cells or NK cells, modified to express an engineered TCR as described herein; (4) nucleic acids encoding an engineered TCR as described herein (5) viral particles comprising a nucleic acid encoding a tumor antigen; (6) other cancer therapeutic agent, in particular tumor antigen-specific therapeutic agents. In some embodiments, the present disclosure provides compositions comprising lentiviral vector particles comprising a nucleotide sequence encoding an engineered TCR described herein (or T cells that have been modified using the vector particles described herein to express an engineered TCR). Such compositions can be administered to subjects in the methods of the present disclosure as described further herein.

Compositions comprising the modified T cells as described herein can be utilized in methods and compositions for adoptive immunotherapy in accordance with known techniques, or variations thereof that will be apparent to those skilled in the art based on the instant disclosure. See, e.g., US Patent Application Publication No. 2003/0170238 to Gruenberg et al; see also US Patent No. 4,690,915 to Rosenberg.

In some embodiments, the cells are formulated by first harvesting them from their culture medium, and then washing and concentrating the cells in a medium and container system suitable for administration (a "pharmaceutically acceptable" carrier) in a treatment-effective amount. Suitable infusion medium can be any isotonic medium formulation, typically normal saline, Normosol R (Abbott) or Plasma-Lyte A (Baxter), but also 5% dextrose in water or Ringer's lactate can be utilized. The infusion medium can be supplemented with human serum albumin.

A treatment-effective amount of cells in the composition is typically greater than 10^2 cells, and up to 10^6 , up to and including 10^8 or 10^9 cells and can be more than 10^{10} cells. The

number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein. For example, if cells that are specific for a particular antigen are desired, then the population will contain greater than 70%, generally greater than 80%, 85% and 90-95% of such cells. For uses provided herein, the cells are generally in a volume of a liter or less, can be 500 ml or less, even 250 ml or 100 ml or less. Hence the density of the desired cells is typically greater than 10^6 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^9 , 10^{10} or 10^{11} cells.

Pharmaceutical compositions provided herein can be in various forms, e.g., in solid, liquid, powder, aqueous, or lyophilized form. Examples of suitable pharmaceutical carriers are known in the art. Such carriers and/or additives can be formulated by conventional methods and can be administered to the subject at a suitable dose. Stabilizing agents such as lipids, nuclease inhibitors, polymers, and chelating agents can preserve the compositions from degradation within the body. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

The engineered TCRs as described herein, or the viral vector particles comprising a nucleotide sequence encoding a tumor antigen or an engineered TCR provided herein, can be packaged as kits. Kits can optionally include one or more components such as instructions for use, devices, and additional reagents, and components, such as tubes, containers and syringes for practice of the methods. Exemplary kits can include the nucleic acids encoding the antigen, nucleic acids encoding the engineered TCRs, the engineered TCR polypeptides, or viruses provided herein, and can optionally include instructions for use, a device for detecting a virus in a subject, a device for administering the compositions to a subject, and a device for administering the compositions to a subject.

Kits comprising polynucleotides encoding a gene of interest (e.g., a tumor antigen or an engineered TCR) are also contemplated herein. Kits comprising a viral vector encoding a sequence of interest (e.g., an engineered TCR) and optionally, a polynucleotide sequence encoding an immune checkpoint inhibitor are also contemplated herein.

Kits contemplated herein also include kits for carrying out the methods for detecting

the presence of polynucleotides encoding any one or more public TCR V β CDR3 sequences, such as those disclosed herein. In particular, such diagnostic kits may include sets of appropriate amplification and detection primers and other associated reagents for performing deep sequencing to detect the polynucleotides encoding a public TCR V β CDR3 sequence, such as the public TCR V β CDR3 sequences disclosed herein. In further embodiments, the kits herein may comprise reagents for detecting the TCR polypeptide comprising the TCR V β CDR3, such as antibodies or other binding molecules. Diagnostic kits may also contain instructions for determining the presence of the polynucleotides encoding the public TCR V β CDR3 sequences or for determining the presence of the TCR polypeptides comprising the public TCR V β CDR3s disclosed herein.

In another embodiment, the kits contemplated herein include kits for carrying out the methods for identifying any one or more of the biomarkers described herein. Accordingly, kits herein include tumor antigen-specific antibodies, in particular, NY-ESO-1-specific antibodies or overlapping peptides spanning the tumor antigen (e.g., spanning NY-ESO-1 or one or more MAGE proteins), reagents for culturing and measuring CD8+ and/or CD4+ T cell responses (e.g., culture media, flow cytometric solutions and fluorescent dyes). In certain embodiments, such kits include instructions or protocols for determining the presence of a tumor antigen-specific antibodies, such as NY-ESO-1 specific antibodies or tumor antigen-specific T cells from a biological sample.

A kit may also contain instructions. Instructions typically include a tangible expression describing the virus and, optionally, other components included in the kit, and methods for administration, including methods for determining the proper state of the subject, the proper dosage amount, and the proper administration method, for administering the virus. Instructions can also include guidance for monitoring the subject over the duration of the treatment time.

Kits provided herein also can include a device for administering a composition described herein to a subject. Any of a variety of devices known in the art for administering medications or vaccines can be included in the kits provided herein. Exemplary devices include, but are not limited to, a hypodermic needle, an intravenous needle, a catheter, a needle-less injection device, an inhaler, and a liquid dispenser, such as an eyedropper. Typically, the device for administering a virus of the kit will be compatible with the virus of the kit; for example, a needle-less injection device such as a high pressure injection device

can be included in kits with viruses not damaged by high pressure injection, but is typically not included in kits with viruses damaged by high pressure injection.

Kits provided herein also can include a device for administering a compound, such as a T cell activator or stimulator, or a TLR agonist, such as a TLR4 agonist (see e.g., U.S. Patent No. 8,273,361), to a subject. Any of a variety of devices known in the art for administering medications to a subject can be included in the kits provided herein. Exemplary devices include a hypodermic needle, an intravenous needle, a catheter, a needle-less injection, but are not limited to, a hypodermic needle, an intravenous needle, a catheter, a needle-less injection device, an inhaler, and a liquid dispenser such as an eyedropper. Typically, the device for administering the compound of the kit will be compatible with the desired method of administration of the compound.

As used herein and in the appended claims, the singular forms “a,” “and,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “an antigen” includes a plurality of such antigens, and reference to “a cell” or “the cell” includes reference to one or more cells and equivalents thereof (e.g., plurality of cells) known to those skilled in the art, and so forth. Similarly, reference to “a compound” or “a composition” includes a plurality of such compounds or compositions, and refers to one or more compounds or compositions, respectively, unless the context clearly dictates otherwise. When steps of a method are described or claimed, and the steps are described as occurring in a particular order, the description of a first step occurring (or being performed) “prior to” (i.e., before) a second step has the same meaning if rewritten to state that the second step occurs (or is performed) “subsequent” to the first step. The term “about” when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary between 1% and 15% of the stated number or numerical range. The term “comprising” (and related terms such as “comprise” or “comprises” or “having” or “including”) is not intended to exclude that in other certain embodiments, for example, an embodiment of any composition of matter, composition, method, or process, or the like, described herein, may “consist of” or “consist essentially of” the described features.

The following examples are offered by way of illustration, and not by way of limitation.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

The following Examples describe the presence and levels of NY-ESO-1 specific T cells relative to cancer therapies, the presence and levels of NY-ESO-1 specific T cell CDR sequences relative to cancer therapies, as well as methods for determining whether subjects would benefit from NY-ESO-1 cancer therapy. Additional disclosure can be found in PCT Appl. No. PCT/US16/50826.

EXAMPLE 1: TREATMENT WITH LV305 INCREASED THE POLYCLONAL AFFINITY OF NY-ESO-1 SPECIFIC T CELLS POST-THERAPY

This Example demonstrates that treatment with LV305 increased the polyclonal affinity T cells that recognize NY-ESO-1 tumor antigen as measured by ELISPOT.

In this study on LV305, the patient received 3 injections with LV305, a dendritic cell tropic lentivector encoding the NY-ESO-1 tumor antigen. T cell response to NY-ESO-1 in pre-vaccination (pre-Tx) and post-vaccination (post-Tx) PBMC samples was measured by ELISPOT, in which the cells were stimulated with NY-ESO-1 peptide mix for 40 hr and the number of T cells that secreted IFN- γ was measured by counting the spots in plates that were pre-coated with anti-IFN- γ antibody.

As shown in Figure 1, post-Tx T cells had higher response to NY-ESO-1 peptide mix than pre-Tx T cells, at all the NY-ESO-1 concentrations tested (1670, 334, 60, and 12nM). At the two lower concentrations we tested (12 and 60 nM), there was no detectable T cell response in pre-Tx samples, yet there was significant T cell response in post-Tx sample. These data demonstrate that treatment with LV305 enhances T cell response to NY-ESO-1 and resulted in higher affinity NY-ESO-1 specific T cells, which recognize the antigen at low concentrations of NY-ESO-1 peptides.

EXAMPLE 2: TUMOR ANTIGEN-SPECIFIC TCR SEQUENCES ARE ENRICHED IN POST-TX PBMC AS COMPARED TO PRE-TX PBMC

This Example demonstrates that treatment with LV305 resulted enrichment of NY-ESO-1 specific TCR sequences in the peripheral blood.

In this study, PBMC were collected from the patient before LV305 treatment and after three vaccinations with LV305. A pre-Tx tumor sample was also collected from the patient. The PBMC and tumor sample were subjected to DNA extraction and subsequent sequencing analysis of the T cell receptor (TCR) beta chain. The sequence similarity between pre-Tx and post-Tx PBMC was analyzed using scatter plot. Then the TCR sequence from the tumor sample was also compared to the pre-Tx and post-Tx PBMC for similarity. The result showed that the TCR sequence from the T cells infiltrating the tumor samples are enriched in post-Tx PBMC as compared to pre-Tx PBMC.

EXAMPLE 3: AN OLIGOCLONAL CULTURE THAT IS HIGHLY ENRICHED FOR NY-ESO-1 SPECIFIC T CELLS HAS BEEN ESTABLISHED FROM POST-TX PBMC

This Example demonstrates that an oligoclonal culture that is highly enriched for NY-ESO-1 specific T cells has been established from post-Tx PBMC. The culture was started by using PBMC from a patient after vaccination with LV305. The PBMC was cultured in OpTmizer T cell expansion medium (Invitrogen, Carlsbad, CA) with NY-ESO-1 overlapping peptide (0.5 ug/mL, JPT Technologies, Berlin, Germany) in the presence of IL-2 and IL-7 (10 ng/mL). After repeated stimulation and long-term culture (>3 months), the PBMC culture was highly enriched for NY-ESO-1 specific T cells. As shown in Figure 3, the enriched T cells secreted high amount of IFN- γ upon stimulation with NY-ESO-1 peptides. TCR sequencing analysis showed that the culture is very oligoclonal as the top 6 clones accounts for more than 90% of all the T cells.

EXAMPLE 4: TCRB CDR3 SEQUENCES IN THE OLIGOCLONAL CULTURE ARE ENRICHED IN POST-TX PBMC AS COMPARED TO PRE-TX PBMC

This Example demonstrates that the TCR sequences from the NY-ESO-1 stimulated oligoclonal culture (PT151006 IVS3) are enriched in post-Tx PBMC as compared to pre-Tx PBMC. The sequence similarity between pre-Tx and post-Tx PBMC was analyzed using scatter plot (Figure 4). Then the top 6 TCR sequences from PT151006 IVS3 were also compared to the pre-Tx and post-Tx PBMC for similarity. The result showed that the TCR sequences from PT151006 IVS3, are enriched in post-Tx PBMC as compared to pre-Tx PBMC.

EXAMPLE 5: A TCRB CDR3 CLONE WITH A FREQUENCY OF 20.5% IN PT151006 IVS3 CAN BE DETECTED IN PT151016 POST-Tx PBMC

5 This Example demonstrates that the second dominant clone from PT151006 IVS3 is also detected in PT151016 post-Tx PBMC (Figure 5). Of note, the two patients have different Class I HLA background (see Table 4). The CDR3 sequence of the TCR β chain of the 2nd dominant clone (20.5% frequency) from IVS3 is CASSLNRDYGYTF (SEQ ID NO: 2). As shown in Figure 5, this amino sequence is detected at 0.0003% frequency in the post-Tx PBMC from PT151016, while it is not detected in the pre-Tx PBMC from PT151016.

EXAMPLE 6: A TCRB CDR3 CLONE WITH A FREQUENCY OF 8.5% IN PT151006 IVS3 CAN BE DETECTED IN PT151016 POST-Tx PBMC

15 This Example demonstrates that the fifth dominant clone from PT151006 IVS3 is also detected in PT151016 post-Tx PBMC. The CDR3 sequence of the TCRbeta chain of the 5th dominant clone (frequency 8.5%) from IVS3 is CASSLNRDQPQHF (SEQ ID NO: 3). As shown in Figure 6, this amino sequence is detected at 0.0006% frequency in the post-Tx PBMC from PT151016, while it is not detected in the pre-Tx PBMC from PT151016.

20 EXAMPLE 7: A TCRB CDR3 CLONE WITH A FREQUENCY OF 26.2% IN PT151006 IVS3 CAN BE DETECTED IN PT151014 POST-Tx PBMC

 This Example demonstrates that the dominant clone from PT151006 IVS3 (26.2% frequency) is also detected in PT151014 post-Tx PBMC. The CDR3 sequence of this clone is CASRLAGQETQYF (SEQ ID NO: 4). As shown in Figure 7, this amino sequence is detected at 0.000762% frequency in the post-Tx PBMC from PT151014, while it is not detected in the pre-Tx PBMC from PT151014.

EXAMPLE 8: THREE OUT OF THE TOP SIX TCRB CDR3 CLONES ARE PUBLIC CLONES THAT ARE SHARED BETWEEN MORE THAN ONE PATIENT

30 This Example demonstrates that the three public sequences we discovered are

detected in multiple patients with different HLA background and increase in frequency in PBMC sampled post LV305 therapy.

The first public CDR3 sequence of TCR β is CASSLNRDYGYTF (SEQ ID NO: 2). As shown in Table 1, this sequence is detected at 0.001% in pre-Tx PBMC from PT151006, and increased to 0.003% in post-Tx PBMC from the same patient. This sequence is non-detectable (0%) in pre-Tx PBMC from PT151016 and can be detected at 0.0003% in post-Tx PBMC from the same patient. This sequence is non-detectable (0%) in pre-Tx PBMC from PT151050 and can be detected at 0.0003% in post-Tx PBMC from the same patient.

Table 1: Frequency of the 1st Public TCRβ CDR3 Sequence, CASSLNRDYGYTF, in eight patients								
PT:	151006	151014	151016	151035	151039	151050	151119	151070
Pre-Tx PBMC	0.001%	0%	0%	0%	0%	0%	0%	0%
Post-Tx PBMC	0.003%	0%	0.0003%	0%	0%	0.0003%	0%	0%
IVS3 from PT151006	20.5%							

Table 1. The frequency of a 1st public TCR β CDR3 sequence in pre-Tx and post-Tx PBMC samples from eight patients. This table shows the frequency of the CDR3 sequence, CASSLNRDYGYTF (SEQ ID NO: 2), in different patient PBMC samples collected either before or after treatment with LV305. For example, this sequence is detected at 0.001% in pre-Tx PBMC from PT151006, and increased to 0.003% in post-Tx PBMC from the same patient. This sequence is non-detectable (0%) in pre-Tx PBMC from PT151016 and can be detected at 0.0003% in post-Tx PBMC from the same patient. This sequence is non-detectable (0%) in pre-Tx PBMC from PT151050 and can be detected at 0.0003% in post-Tx PBMC from the same patient.

The second public CDR3 sequence of TCR β is CASSLNRDQPQHF (SEQ ID NO: 3). As shown in Table 2, this sequence is detected at 0.0058% in pre-Tx PBMC from PT151006, and increased to 0.017% in post-Tx PBMC from the same patient. The sequence can also be detected in a pre-Tx tumor biopsy from this patient (0.06%) and a tumor infiltrating lymphocytes (TIL) culture from the same patient, 0.002% in TIL-PC12-04A1. This sequence is non-detectable (0%) in pre-Tx PBMC from PT151014 and can be detected

at 0.000109% in post-Tx PBMC from the same patient. This sequence is non-detectable (0%) in pre-Tx PBMC from PT151050 and can be detected at 0.0012% in post-Tx PBMC from the same patient. Overall, this TCR is detectable in 6 out of 8 patients, increased in frequency post-Tx sample in 5/8 patients and decreased in 1/8 patient.

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Table 2: Frequency of the 2nd Public TCRβ CDR3 Sequence, CASSLNRDQPQHF, in eight patients								
PT:	151006	151014	151016	151035	151039	151050	151119	151070
Pre-Tx PBMC	0.0058%	0%	0%	0.001%	0%	0%	0%	0%
Post-Tx PBMC	0.017%	0.000109%	0.0012%	0%	0%	0.0006%	0.000893%	0%
IVS3 from PT151006	8.5%							
TIL from PT151006	0.002%							
Fixed tumor from PT151006	0.06%							

Table 2. The frequency of a 2nd public TCR β CDR3 sequence in pre-Tx and post-Tx PBMC samples from eight patients. This table shows the frequency of the CDR3 sequence, CASSLNRDQPQHF (SEQ ID NO: 3), in different patient PBMC samples collected either before or after treatment with LV305. For example, this sequence is detected at 0.0058% in pre-Tx PBMC from PT151006, and increased to 0.017% in post-Tx PBMC from the same patient. The sequence can also be detected from fixed tumor from this patient (0.06%) and a TIL culture from this patient, 0.000647%. This sequence is non-detectable (0%) in pre-Tx PBMC from PT151016 and can be detected at 0.0006% in post-Tx PBMC from the same patient. This sequence is non-detectable (0%) in pre-Tx PBMC from PT151050 and can be detected at 0.0012% in post-Tx PBMC from the same patient.

The third public CDR3 sequence of TCR β is CASRLAGQETQYF (SEQ ID NO: 4). As shown in Table 3, this sequence is 0% in pre-Tx PBMC from PT151006, and increased to 0.000196% in post-Tx PBMC from the same patient. This sequence is non-detectable (0%)

in pre-Tx PBMC from PT151-014 and can be detected at 0.000761% in post-Tx PBMC from the same patient. This sequence is also detected at 0.000274% in pre-Tx PBMC from PT151050.

Table 3: Frequency of the 3rd Public TCRβ CDR3 Sequence, CASRLAGQETQYF, in eight patients								
PT:	151006	151014	151016	151035	151039	151050	151119	151070
Pre-Tx PBMC	0.0058%	0%	0%	0%	0%	0.000274%	0%	0%
Post-Tx PBMC	0.000196%	0.00076%	0%	0%	0%	0%	0%	0%
IVS3 PT151006	26%							

Table 3. The frequency of a 3rd public TCR β CDR3 sequence in pre-Tx and post-Tx PBMC samples from eight patients. This table shows the frequency of the CDR3 sequence, CASRLAGQETQYF (SEQ ID NO: 4), in different patient PBMC samples collected either before or after treatment with LV305. The sequence can be detected in PT151014 and PT151050 in addition to PT151006.

The frequency of the three identified public TCRs in pre-Tx and post-Tx PBMC from eight patients is summarized in Figure 8.

EXAMPLE 9: THE CDR3 OF THE THREE IDENTIFIED PUBLIC TCR β HAS THE TYPICAL FEATURES OF A PUBLIC TCR

This Example demonstrates that the identified TCR β CDR3 sequences have the typical features of public TCRs: (1) they are encoded by different nucleotide sequences in different patients; (2) they have relatively short CDR3 lengths; and (3) they have a relatively limited untemplated nucleotide addition. All of these features are characteristic of public TCR sequences (Venturi Nat Rev Immunol 2008).

Diversification of the TCR V α and V β gene segments depends on the use of different CDR1 and CDR2 regions (encoded in different V gene segments). CDR3 is created by the juxtaposition of different V(D)J germline segments after somatic recombination, with the diversity of the naive TCR repertoire increased further by a lack of precision during V(D)J gene rearrangement and by the addition of non-template encoded nucleotides (N) at the

V(D)J junctions (Turner, et al, Nature Review Immunology 2006).

One possible way that public TCRs are generated is through convergent recombination. That is, many different V(D)J recombination events “converge” to produce the same nucleotide sequence and many different nucleotide sequences “converge” to encode the same amino-acid sequence (Venturi, et al, Nature Review Immunology 2008). As shown in Figure 9 and Figure 12, the public TCR sequences we identified have different nucleotide sequences in different individuals.

It has been reported that compared to private TCRs, shared TCR CDR3 aa sequences tended to be shorter on average by about one aa residue and, in addition, showed a significantly lower number of nucleotide (nt) insertions in the VD and DJ junction (Madi, et al, Genome Research, 2014). A comparison of TCR β CDR3 length distribution in IVS3 showed that the T cells in IVS3 (Figure 10A, black columns) have relatively shorter CDR3 region than the unstimulated T cells in post-Tx PBMC (Figure 10A, hatched column). As shown in Figure 10B, all three public TCR have the same CDR3 length (n=39 nucleotides). These TCRs also have relatively few nontemplated (NT) nucleotide (nc) addition at the junction region (0 to 2 of nucleotide additions). One of the public TCRs we identified, CASRLAGQETQYF (SEQ ID NO: 4), had no NT nc addition at the N1 (VD) insertion site and no nt addition at the N2 (DJ) insertion site. The phenotypic features of shorter length and limited number of NT nc additions support the conclusion that these TCR β CDR3 sequences are public TCR sequences.

EXAMPLE 10: HLA TISSUE TYPING RESULTS FOR PATIENTS TREATED WITH LV305

Patient samples were forwarded to a commercial HLA tissue typing service. The results are summarized in Table 4 below and confirm no overlap in HLA-A or HLA-B alleles between patients sharing the public TCR CDR3. The HLA-DR, DP or DQ alleles are also different between the patients.

Table 4: HLA Tissue Typing Results for 4 Patients Treated with LV305

PT151006		
HLA-A	*02:01	*24:02
HLA-B	*13:02P	*35:01
HLA-C	*04:01	*06:02

HLA-DRB1	*11:01	*13:01
HLA-DRB3	*02:02	-
HLA-DRB4	-	-
HLA-DRB5	-	-
HLA-DQB1	*03:01	*06:03P
HLA-DPB1	*04:01	-
HLA-DQA1	*01:03/10	*05:01:01G
HLA-DPA1	*01:03P	-
PT151016		
HLA-A	*01:01	*29:02
HLA-B	*40:01	*57:01
HLA-C	*03:04	*06:02
HLA-DRB1	*04:01	*07:01P
HLA-DRB3	-	-
HLA-DRB4	*01:01	*01:03
HLA-DRB5	-	-
HLA-DQB1	*03:01	*03:03
HLA-DPB1	*04:01	*04:02
HLA-DQA1	*02:01	*03:01:01G
HLA-DPA1	*01:03P	-
PT151014		
HLA-A	*02:01	*03:01
HLA-B	*15:01	*40:01
HLA-C	*03:04	-
HLA-DRB1	*04:01	*08:01
HLA-DRB3	-	-
HLA-DRB4	*01:01	-
HLA-DRB5	-	-
HLA-DQB1	*03:02	*04:02
HLA-DPB1	*02:01P	*03:01:01G
HLA-DQA1	*03:01:01G	*04:01:01G
HLA-DPA1	*01:03P	-
PT151050		
HLA-A	*33:03	*74:01
HLA-B	*39:10	*58:01
HLA-C	*07:01	*12:03
HLA-DRB1	*01:02P	*15:03P
HLA-DRB3	-	-
HLA-DRB4	-	-
HLA-DRB5	*01:01P	-
HLA-DQB1	*05:01	*06:02
HLA-DPB1	No HLA-DPB type could be obtained for this sample. The probe reactivity from rPCR-SSOP does not correlate with any described HLA-DPB allele(s)	No HLA-DPB type could be obtained for this sample. The probe reactivity from rPCR-SSOP does not correlate with any described HLA-DPB allele(s)
HLA-DQA1	*01:01	*01:02
HLA-DPA1	*01:03P	*02:01P

EXAMPLE 11: SEQUENCING OF THE TCRA AND TCRB VARIABLE REGIONS OF PUBLIC TCRS
FROM PATIENTS TREATED WITH LV305

5'-RLM-RACE (Ambion, Austin, TX) was used for the TCR cloning. This method makes it feasible to identify a TCR without prior knowledge of the variable domain sequence.

5 In brief, RNA was isolated from the NY-ESO-1 specific T cells using a Trizol[®] extraction. Purified RNA was then dephosphorylated, de-capped using Tobacco Acid Pyrophosphatase, and ligated to a 5' adapter. Then, cDNA was prepared from the RNA using M-MLV reverse transcriptase (Ambion, Austin, TX) with random decamers following the manufacture's instruction. The cDNA was then used in the PCR. The PCR was performed with primers
10 annealing to 5' adapter and constant region of either TCR α or TCR β (illustrative primers and sequences such as pCa1 and pCb1 are listed in the publication by Walchli, et al, (2011) A Practical Approach to T-Cell Receptor Cloning and Expression. PLoS ONE 6(11): e27930; but have been modified to add a restriction site for cloning purposes). The amplicons were then digested, gel purified, and cloned into a prepared vector using restriction sites
15 incorporated into the PCR primers. Agar plates containing colonies were then sent out for sequencing of cloned TCR mRNA.

151 clones were sequenced from 2 separate rounds of PCR for TCR β . Two different TCR β variable region sequences were found to occur at high frequency, one of which contained the public V β CDR3 sequence previously identified and provided in SEQ ID NO:
20 4. The full-length TCR β variable region sequence containing the public V β CDR3 of SEQ ID NO: 4 is provided in SEQ ID NO: 9 and is shown in Figure 11. The other TCR β variable region sequence is provided in SEQ ID NO: 16 and shown in Figure 13 (the sequence may be annotated and the different regions of the TCR identified using standard methodologies such as those described at the IMGT database website). Almost 100 clones from 2 separate rounds
25 of PCR were sequenced for TCR alpha. A single TCR α chain variable region was identified and is provided in SEQ ID NO: 8 and shown in Figure 11. A BLAST search of the TCR α sequence indicates homology with a known NY-ESO-1 specific TCR α sequence (see PDB:2BNQ_D; Boulter et al., Protein Eng. (2003) 16 (9): 707-711; Chen, J.L. et al. (2000) J. Immunol., 165, 948-955). No known matches were identified in a similar search using the
30 TCR β sequence of SEQ ID NO: 9.

EXAMPLE 12: NY-ESO-1 SPECIFIC T CELLS WITH PUBLIC TCRS INFILTRATE INTO TUMOR
AFTER TREATMENT WITH G100

This Example demonstrates that the public TCR β CDR3 sequences that were originally identified from a sarcoma patient in the LV305 immunotherapy trial can be detected in patients from two different clinical trials using G100, intratumoral injection of glucopyranosyl lipid Adjuvant in stable emulsion (GLA-SE). The first G100 trial (NCT02035657) is a proof-of-concept trial of GLA-SE in patients with Merkel Cell Carcinoma (MCC). The second G100 trial (NCT02180698) is TLR4 agonist GLA-SE and radiation therapy in treating patients with soft tissue sarcoma that is metastatic or cannot be removed by surgery. Biopsies at the tumor site or a draining lymph node were taken before and after the administration of G100. DNA was extracted from the biopsy tissue and peripheral blood and deep sequencing of the TCR β CDR3 region was carried out to evaluate the diversity of the T cell repertoire. Unexpectedly, several public TCRs identified from LV305 patient PT151006 were also detected in patients with Merkel Cell Carcinoma (MCC) and sarcoma, who received local immune modulation by intratumoral G100 treatment combined with irradiation. Table 5 to Table 7 lists the frequency of the 3 public TCRs in pre-G100 and post-G100 PBMC and biopsy samples in one MCC patient and two sarcoma patients. The first public TCR β CDR3, CASSLNRDYGYTF (SEQ ID NO:2), was detectable in the MCC patient G2 and the sarcoma patient P13. The second public TCR β CDR3, CASSLNRDQPQHF (SEQ ID NO:3), was detectable in the MCC patient G2 and the sarcoma patient P12. The third public TCR β CDR3, CASRLAQETQYF (SEQ ID NO:4), was detectable in MCC patient G2.

Table 5: Frequency of the 1 st Public TCR β CDR3 Sequence, CASSLNRDYGYTF, in G100 patients						
Patient	G100-MCC-G2		G100-Sarcoma-P12		G100-Sarcoma-P13	
	G2-PBMC	G2-biopsy	P12-PBMC	P12-biopsy	P13-PBMC	P13-biopsy
Pre-G100	0%	0%	0%	0%	0.000069%	0%
Post-G100	0%	0.000442%	0%	0%	0%	0

Table 5: The frequency of the 1st public TCR β CDR3 sequence, CASSLNRDYGYTF (SEQ ID NO:2), in G100 patients. Shown are the presence (frequency) of the public TCR in pre-G100 or post-G100 PBMC and tumor biopsy from a G100-MCC patient (G100-MCC-G2) and two G100-Sarcoma patients (G100-Sarcoma-P12 and G100-Sarcoma-P13). The sequence was detected at 0.000442% in post-Tx biopsy from patient G2 but not detectable in pre-Tx biopsy or PBMC samples.

Table 6: Frequency of the 2 nd Public TCR β CDR3 Sequence, CASSLNRDQPQHF, in G100 patients						
Patient	G100-MCC-G2		G100-Sarcoma-P12		G100-Sarcoma-P13	
	G2-PBMC	G2-biopsy	P12-PBMC	P12-biopsy	P13-PBMC	P13-biopsy
Pre-G100	0.000421%	0%	0.000154%	0%	0%	0%
Post-G100	0%	0%	0%	0%	0%	0%

Table 6: The frequency of the 2nd public TCR TCR β CDR3 sequence, CASSLNRDQPQHF, in G100 patients. Shown are the presence (frequency) of the public TCR in pre-G100 or post-G100 PBMC and tumor biopsy from a G100-MCC patient (G100-MCC-G2) and two G100-Sarcoma patients (G100-Sarcoma-P12 and G100-Sarcoma-P13). This sequence was detected in pre-Tx PBMC from G2 and P12.

Table 7: Frequency of the 3 rd Public TCR β CDR3 Sequence, CASRLAGQETQYF, in G100 patients						
Patient	G100-MCC-G2		G100-Sarcoma-P12		G100-Sarcoma-P13	
	G2-PBMC	G2-biopsy	P12-PBMC	P12-biopsy	P13-PBMC	P13-biopsy
Pre-G100	0%	0%	0%	0%	0%	0%
Post-G100	0%	0.000442%	0%	0%	0%	0%

Table 7: The frequency of the 3rd public TCR TCR β CDR3 sequence, CASRLAGQETQYF, in G100 patients. Shown are the presence (frequency) of the public TCR in pre-G100 or post-G100 PBMC and tumor biopsy from a G100-MCC patient (G100-MCC-G2) and two G100-Sarcoma patients (G100-Sarcoma-P12 and G100-Sarcoma-P13). The sequence was detected at 0.000442% in post-Tx biopsy from patient G2 but not detectable in pre-Tx biopsy or PBMC samples.

G2 is a MCC patient with a NY-ESO-1 expressing tumor who had a complete response following intratumoral G100 treatment. As shown in Figure 14, the pre-G100 biopsy from this patient shows NY-ESO-1 expression, which was decreased by approximately 3 fold in the post-G100 biopsy, consistent with the disappearance of cytokeratin (CK20) positive tumor cells post-G100 treatment (data not shown). As shown in Figure 15, two public TCR β CDR3, which were non-detectable in pre-G100 biopsy, became detectable in post-G100 biopsy. These data suggest modulating the tumor microenvironment with G100 may have directed antigen-specific T cells with public TCRs into the tumor from

the peripheral blood. These findings support further exploration of public TCR β sequences as biomarkers for NY-ESO-1 specific immunotherapy.

EXAMPLE 13: THE PUBLIC TCR β CDR3 SEQUENCES IDENTIFIED IN PATIENTS FROM LV305 CLINICAL TRIAL CAN ALSO BE DETECTED IN PATIENTS FROM THE C131 CLINICAL TRIAL

This example demonstrates that the three public TCR β CDR3 sequences identified from PT151006, a patient from the LV305 trial (NCT02122861), can also be detected in patients from C131 (NCT02387125), a different clinical trial.

C131 is a Phase 1b safety study of CMB305 (sequentially administered LV305 and G305 (G305 consists of recombinant NY-ESO-1 protein formulated with a synthetic small molecule called glucopyranosyl lipid A (GLA), a TLR4 agonist, and is designed to boost the CTL response via the induction of antigen-specific CD4 "helper" T cells) in patients with locally advanced, relapsed, or metastatic cancer expressing NY-ESO-1. We examined the TCR β CDR3 repertoire from 13 C131 patients using the public sequences identified from PT151006. As shown in Table 8, the first public TCR β CDR3 sequence, CASSLNRDYG YTF (SEQ ID NO:2), was detected in 3 out of the 13 C131 patients; as shown in Table 9, the 2nd public TCR β CDR3 sequence, CASSLNRDQPQHF (SEQ ID NO:3), was detected in 6 out of 13 C131 patients; As shown in Table 10, the 3rd public TCR β CDR3 sequence, CASRLAGQETQYF (SEQ ID NO:4), was also detected in 6 out of 13 C131 patients. These data showed that the CDR3 sequences from PT151006 were shared in patients from different trials. In four patients these public TCR were detected after administration of the CMB305 regimen and are thus likely induced by the therapy.

Table 8: The 1st Public TCRβ CDR3 Sequence, CASSLNRDYGYTF, can be detected in 3 out of 13 C131 patients													
	C131-001	C131-002	C131-003	C131-007	C131-009	C131-010	C131-011	C131-012	C131-013	C131-017	C131-018	C131-020	C131-026
Pre-Tx PBMC	6.71E-04%	0%	0%	0%	0%	0%	0%	5.20E-04%	0%	0%	0%	0%	0%
Post-Tx PBMC	6.94E-04%	0%	0%	0%	0.001162%	0%	0%	0%	0%	0%	0%	0%	0%
IVS3 from PT151006	20%												

Table 8. The frequency of the 1st public TCRβ CDR3 sequence in pre-Tx and post-Tx PBMC samples from 13 patients in the C131 trial. This table shows the frequency of the CDR3 sequence, CASSLNRDYGYTF (SEQ ID NO:2), in 13 patients PBMC samples collected either before or after treatment with CMB305. The sequence can be detected in 3 out of 13 patients.

5

Table 9: The 2nd Public TCRβ CDR3 Sequence, CASSLNRDQPQHF, can be detected in 6 out of 13 C131 patients													
	C131-001	C131-002	C131-003	C131-007	C131-009	C131-010	C131-011	C131-012	C131-013	C131-017	C131-018	C131-020	C131-026
Pre-Tx PBMC	0%	0%	0%	0%	4.85E-04%	0%	0.001869%	2.85E-04%	0.00133%	0%	5.00E-04%	0%	7.92E-04%
Post-Tx PBMC	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.001153%

Table 9: The 2nd Public TCRβ CDR3 Sequence, CASSLNDRDQPQHF, can be detected in 6 out of 13 C131 patients													
	C131-001	C131-002	C131-003	C131-007	C131-009	C131-010	C131-011	C131-012	C131-013	C131-017	C131-018	C131-020	C131-026
IVS3 from PT151006	8.5%												

Table 9. The frequency of the 2nd public TCRβ CDR3 sequence in pre-Tx and post-Tx PBMC samples from 13 patients in the C131 trial. This table shows the frequency of the CDR3 sequence, CASSLNDRDQPQHF (SEQ ID NO:3), in 13 patients PBMC samples collected either before or after treatment with CMB305. The sequence can be detected in 6 out of 13 patients.

Table 10: The 3 rd Public TCRβ CDR3 Sequence, CASRLAGQETQYF, can be detected in 6 out of 13 C131 patients													
	C131-001	C131-002	C131-003	C131-007	C131-009	C131-010	C131-011	C131-012	C131-013	C131-017	C131-018	C131-020	C131-026
Pre-Tx PBMC	0.000336%	0.000407%	0%	0%	0.000485%	0%	0%	0%	0.000771%	0%	0%	0%	0%
Post-Tx PBMC	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.000344%	0.000429%	0%	0%
IVS3 from PT151006	26%												

Table 10. The frequency of the 3rd public TCRβ CDR3 sequence in pre-Tx and post-Tx PBMC samples from 13 patients in the C131 trial. This table shows the frequency of the CDR3 sequence, CASRLAGQETQYF (SEQ ID NO:4), in 13 patients PBMC samples collected either before or after treatment with CMB305. The sequence can be detected in 6 out of 13 patients.

EXAMPLE 14: THE CDR3 OF THE THREE PUBLIC TCR β IDENTIFIED FROM LV305 CLINICAL TRIAL CAN BE DETECTED IN PATIENTS FROM AN ANTI-CTLA-4 TRIAL

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This example demonstrates that the public TCR β CDR3 sequences identified from LV305 trial can be detected in patients receiving anti-CTLA4 mAb therapy with tremelimumab (Robert, et al, Clin Can Res, 2014).

10 In this trial with tremelimumab, PBMC were collected at baseline and 30 to 60 days after receiving tremelimumab. Next-generation sequencing was used to study the CDR3 region of TCR β . The sequencing data from 21 patients were deposited in the on-line database accessible through the Adaptive ImmunoSEQ Analyzer software. Database query comparing the CDR3 sequences from PT151006-IVS3 and the sequences of the 21 patients receiving anti-CTLA4 therapy showed that the 3 public sequences identified from PT151006
15 can also be detected in these patients on CTLA4 therapy (Figure 16).

Increased TCR V-beta CDR3 richness and Shannon index diversity was observed in patients with CTLA4 blockade therapy (Robert, et al, Clin Can Res, 2014). Regarding the frequency of public TCR, there was no clear trend of whether the frequency increases or decreases after anti-CTLA4 therapy, and it varies from patient to patient. Of note, in one of
20 the three patients that had completed response (CR) from the CTLA4 trial, GA18, all three public sequences were detected in post-Tx PBMC while none of them was detected in pre-Tx PBMC. The potential use of these public CDR3 sequences as biomarkers for treatment response needs to be further investigated.

25 EXAMPLE 15: DIFFERENT TCR β V USAGE FOR THE SAME CDR3 IN THE SAME OR DIFFERENT PATIENTS

This example demonstrates that the same CDR3 region can pair with different TCR β V genes in different patients, or even in the same patient.

Figure 17 lists the different TCR β V gene usage that were detected to be associated
30 with the same CDR3 by deep sequencing analysis. CASSLNRDQPQHF (SEQ ID NO:3) is

the second public CDR3, which was detected in both PT151006 and PT151119. This CDR3 mainly uses TCR β V07-07 in PT151006. It also uses TCR β V07-08, TCR β V07-06, TCR β V07-09, TCR β V07-02, TCR β V07-03, and TCR β V07-04 in PT151006. In PT151119, only TCR β V07-08 is used for this CDR3. This shows that different TCR β V-gene families can be used by different patients for the same CDR3, and different TCR β V-genes can also be used within the same patient for the same CDR3. Of note, the J gene usage (TCR β J01-05) is the same for both patients.

Figure 19 lists the nucleotide sequences, CDR3 amino acid sequences, and V gene and J gens of TCR β in patients from different trials. PT151006 is from the LV305 clinical trial; C131-001 and C131-013 are from the CMB305 trial; G2-C1W4B is from the G100 trial. The data in this figure demonstrated that patients from different trials can have the same CDR3 amino acid sequences but with different nucleotide sequences and different TCR β V-gene usage. In the case of patient C131-013, three different nucleotide sequences were found to encode the same CDR3, CASSLNRDQPQHF (SEQ ID NO:3), with different TCR β V-gene usage. This is similar to the observation for PT151006, as shown in Figure 17 and Figure 18.

EXAMPLE 16: THE PUBLIC CDR3 ARE IDENTIFIED FROM NY-ESO-1 SPECIFIC CD4 T CELLS

This example shows that the in vitro generated cell culture which was used to identify the public TCR β CDR3 was composed of CD4 T cells.

To characterize the phenotype of the PT151006-IVS3 T cell culture, we stained the cultured cells side-by-side with uncultured PBMC from a normal donor. The cells were stained with monoclonal antibodies against T cell markers (CD3, CD4, and CD8) and NK cell marker (CD56) using fluorochrome-conjugated monoclonal antibodies and then analyzed on a BD LSRII flow cytometer. Data analysis was done using the FlowJo software. As shown in Figure 20, the lymphocytes population was first gated on the FSC/SSC plot, then CD4 T cells were gated as CD3⁺CD4⁺ lymphocytes and CD8 T cells were gated as CD3⁺CD8⁺ lymphocytes. The NK cells were gated as CD3⁻CD56⁺ lymphocytes. The control donor PBMC has the expected percentages of CD4, CD8 T cells and NK cells, as normally observed in healthy donor PBMC. In contrast, the cultured cells from PT151006-IVS3 show a lack of NK cells and CD8 T cells and only contains CD4 T cells. This data showed that the

NY-ESO-1 specific T cell line that were cultured from PT151006 are CD4 T cells, and the public TCRs are CD4 TCRs.

EXAMPLE 17: IMMUNOLOGICAL BIOMARKERS ARE ASSOCIATED WITH SURVIVAL OF NY-ESO-1 POSITIVE CANCER PATIENTS FOLLOWING TREATMENT WITH LV305 OR CMB305, *IN VIVO* DENDRITIC CELL (DC) TARGETING ACTIVE IMMUNOTHERAPIES

Background: The correlation of immune response and clinical benefit in patients (pts) who receive active immunotherapy remains controversial. CMB305 is an active immunotherapy designed to generate and expand anti-NY-ESO-1 T cells, consisting of LV305 (a DC targeting lentiviral vector encoding NY-ESO-1) and a boost (NY-ESO-1 recombinant protein plus GLA-SE, a TLR-4 agonist). Phase 1 studies of LV305 and CMB305 evaluated the immune response and survival of pts with NY-ESO-1 positive tumors.

Methods: 64 patients with recurrent solid tumors expressing NY-ESO-1 were enrolled in two Phase 1 trials (see Table 12). NY-ESO-1 tumor expression was assessed by IHC. Peripheral blood was obtained pre and post therapy for anti-NY-ESO-1 T cell responses (IFN γ ELISPOT) and antibodies (Ab) (ELISA) (n=64), TCR- β CDR3 repertoire and conserved (public) TCR (n=55/64), and antigen spreading (ELISA, ELISPOT) (n=23/64). Relationship between progression-free survival (PFS), overall survival (OS) and up to 15 biomarkers were analyzed using the Kaplan-Meier method, log-rank test and Cox regression.

Table 12: LV305 and CMB305 Patient Demographics and Disease Characteristics		
	Soft Tissue Sarcoma (N=45)	All Patients (N=64)
Soft Tissue Sarcoma	45	45
Other Sarcoma	0	3
Ovarian	0	12
Melanoma	0	4
NSCLC	0	1
Median age, (range)	46 years (27-76, years)	52 years (27-76, years)
Female	44 %	53 %
Metastatic disease	93 %	89 %

Table 12: LV305 and CMB305 Patient Demographics and Disease Characteristics		
Median time from diagnosis, (range)	34 months (9-364, months)	38 months (9-364, months)
Prior chemotherapy	89 %	91 %
1 line of prior chemotherapy	57%	53%
≥2 prior lines of chemotherapy	43%	47%
Disease progression at study start	62 %	58 %
NY-ESO-1 expression 76-100 %, pt (%)	62 %	48 %
NY-ESO-1 expression 50-75%, pt (%)	16 %	13 %

Results: LV305 and CMB305 induced anti-NY-ESO-1 T-cells in 51% and 65% pts, and anti-NY-ESO-1 Ab responses in 13% and 68% pts, respectively. LV305 and CMB305 induced antigen spreading in 17% and 36% pts, as measured by ELISA using 24 recombinantly expressed tumor associated antigens. Overall, CMB305 induced stronger and broader IR (Figure 24). CMB305 also induced higher clonality in the T-cell receptor repertoire, as measured by deep sequencing of PBMC (Figure 21).

Both LV305 and CMB305 induced de novo IR and boosted pre-existing (baseline) IR, with highest frequency of responses observed in patients with pre-existing IR (Figure 25).

Surprisingly, the TCR-Vβ CDR3 amino acid sequences of three NY-ESO-1 specific TCR clones (SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4) obtained from a LV305 patient with a >2-year response were fully conserved in approximately half of all patients and 289 of 539 healthy blood donors (see Figure 8 and Table 13).

Table 13: Public TCRs in Patients and Blood Donors		
Three public TCR (pTCR) sequences in PBMC	pTCR pre Tx	pTCR post Tx
LV305	7/19	9/19

Table 13: Public TCRs in Patients and Blood Donors		
CMB305	11/24	13/24
Blood donors	289/539	na

These NY-ESO-1 specific “public TCR” were induced by treatment with LV305 and CMB305 and showed a possible association with improved survival (Figure 8 and Figure 29).

Analyzing the association of IR and survival, it was found that both pre-existing and induced IR (NY-ESO-1 antibodies or T-cells or pTCR) are associated with longer OS and PFS (Figure 27) (see also Figure 22, Figure 23 and Table 11). In addition, pre-existing (baseline) anti-NY-ESO-1 antibodies were found to be statistically significantly associated with NY-ESO-1 expression (Cox regression, $p=0.04$).

Conclusion: While LV305 and CMB305 are both immunogenic, CMB305 resulted in a stronger and broader and integrated IR, including antigen spreading. Patients with preexisting and treatment-induced NY-ESO-1 immune responses (any constellation of NY-ESO-1 specific antibodies, NY-ESO-specific T-cells and NY-ESO-1 specific public TCRs) had statistically significantly longer OS and PFS compared to patients without IR. The identified biomarkers will aid the design of pivotal trials of CMB305 and may be useful for an accelerated path to licensure. The presence of NY-ESO-1 specific public TCR sequences in over half of healthy blood donors is indicative of low-level pre-existing T-cell responses, a finding which may be applicable to other cancer testis antigens as well and can possibly be exploited for cancer vaccines in general.

Table 11: Summary Kaplan-Meier Analysis of Survival (OS/PFS): LogRank Test

Biomarkers	LV305 all pts (n=31)	LV305 sarcoma (n=23)	C131 all pts w/o s.c. (n=24)	C131 sarcoma pts w/o s.c. (n=15)	LV305 & C131 all pts (n=62)	LV305&C131 all pts w/o s.c. (n=55)	LV305&C131 all sarcoma pts (n=45)	LV305&C131 all sarcoma pts w/o s.c. (n=38)	LV305&C131 all non-sarc. Pts (n=17)
Sex	ns	OS 0.052	ns	ns	ns	ns	OS 0.032 PFS 0.047	OS 0.036	ns
Age	ns PFS 0.038	ns	ns	ns	ns	ns	ns	ns	ns
Pre-existing NY-ESO-1 Ab	OS 0.050 PFS 0.044	OS 0.008 PFS 0.021	ns	ns	ns	ns	OS 0.019 PFS 0.055	ns PFS 0.042	ns
Pre-existing T-cell response	ns	ns PFS 0.059	ns PFS 0.012	ns PFS 0.040	OS 0.053 PFS 0.007	OS 0.055 PFS 0.005	OS 0.042 PFS 0.009	OS 0.041 PFS 0.005	ns
Integrated IR (NY-ESO-1 Ab/CD/CD8)	ns PFS 0.012	ns PFS 0.025	ns	ns	OS 0.048 ns	OS 0.057 PFS 0.043	ns	ns PFS 0.041	ns
NY-ESO-1 express. Level >50%	ns	ns	ns	ns	OS 0.053 PFS 0.03	ns PFS 0.033	ns PFS 0.022	OS 0.018 PFS 0.027	0.054
CD4 T-cells induced	ns	ns	ns	ns	ns	ns	ns	ns	OS 0.032
CD8 T-cells induced	ns	ns	ns	ns	OS 0.059	ns	ns	ns	ns
pTCR present	Not done	Not done	Not done	Not done	ns PFS 0.035	Not done	OS 0.050 PFS 0.029	Not done	ns
pTCR induced	Not done	Not done	Not done	Not done	ns	Not done	ns	Not done	ns
pTCR x NY-ESO-1 expression >50%	Not done	Not done	Not done	Not done	ns PFS 0.002	Not done	ns PFS 0.008	Not done	ns
Clonality increase post Tx	Not done	Not done	Not done	Not done	ns PFS 0.058	Not done	ns PFS 0.046	Not done	ns

EXAMPLE 18: SURVIVAL IMPACT FROM CMB305 IN NY-ESO-1+ RECURRENT SOFT TISSUE SARCOMAS (C131 STUDY)

The overall survival in soft tissue sarcoma patient (STS; including synovial sarcoma (N=14), MRCL (N=9) and spindle cell sarcoma (N=2) patients) on CMB305 (C131 Study; NCT02387125; Arm B SQ dose excluded) compares favorably to currently approved agents for recurrent soft tissue sarcoma where the survival ranges from 12.4 to 13.5 months (see Figure 28). The majority of sarcoma patients on the study had synovial histology. The published METASARC study reports a median survival of 11.7 months in synovial sarcoma patients entering their second line of therapy.

In conclusion, CMB305 therapy is safe, well tolerated, and has an overall survival rate that compares favorably with currently approved agents for recurrent soft tissue sarcoma patients. Disease control was observed in more than half of sarcoma patients, including durable tumor growth arrest in patients who had progressive disease at study entry. CMB305 generates broad anti-NY-ESO-1 immune responses superior to immune response to LV305 vector alone. The induction of anti-NY-ESO-1 immune responses appear to correlate with improved overall survival. Further, anti-NY-ESO-1 immune biomarkers identify cancer patients who have prolonged survival following CMB305 therapy (see also Example 17). A combination study of CMB305 and Atezolizumab in synovial sarcoma and myxoid round cell liposarcoma patients is currently ongoing (see also Example 19). Baseline Immune biomarkers and evidence of induction of immune response with CMB305 will inform development of a pivotal randomized study design of CMB305 in STS patients.

EXAMPLE 19: A PHASE 2 STUDY OF CMB305 AND ATEZOLIZUMAB IN NY-ESO-1+ SOFT TISSUE SARCOMA: INTERIM ANALYSIS OF IMMUNOGENICITY, TUMOR CONTROL AND SURVIVAL

BACKGROUND: CMB305 is an active immunotherapy designed to generate and expand anti-NY-ESO-1 immune response (IR). CMB305 consists of a dendritic cell-targeting lentiviral vector encoding NY-ESO-1 (LV305), and a boost with an NY-ESO-1 recombinant protein plus GLA-SE (G305), a TLR-4 agonist. Phase 1 studies of LV305 and CMB305 showed this approach is safe, generates IR and appears to impact survival with 81% 1-yr survival in NY-ESO-1+ sarcoma patients (pts) following LV305 treatment. The phase 2 study described in this example was designed to evaluate whether CMB305 has activity in a randomized setting and whether addition of CMB305 improves activity of atezolizumab in a

tumor type where checkpoint inhibitors have limited single agent activity.

STUDY DESIGN: This is a randomized, open-label, Phase 2 trial of CMB305 in combination with atezolizumab (C+A) or with atezolizumab alone (A) in patients with in NY-ESO-1⁺ synovial sarcoma (SS) and myxoid round cell liposarcoma (MRCL) who have had an inadequate response, relapse, and/or unacceptable toxicity with one or more prior systemic, surgical, or radiation cancer therapies. Safety run-in was followed by a stratified by histology randomization between arms. Eighty-nine (89) patients were enrolled (88 dosed) at 15 sites in the United States (US). Tumor biopsies were optional. No formal comparison analysis was planned between treatment arms.

METHODS: Treatment: Arm C+A: CMB305 (LV305 Intradermal Days 0, 14, 42, 70 + G305 Intramuscular Days 28, 56, 84 then q6wk up to one year) + atezolizumab (1200 mg IV q3wk); Arm A: atezolizumab alone.

INTERIM ANALYSIS: Exploratory analysis of progression-free survival rate (PFR) at 6 months for the first 36 patients enrolled after the safety run-in was performed; No formal interim analysis was performed for efficacy; Final analysis will be conducted when 72 deaths have occurred or earlier as determined by Sponsor.

STUDY OBJECTIVES: Primary objectives are progression free survival (PFS) and overall survival (OS) with secondary objectives of safety, PFS rate at 3 and 6 months, immune response in the tumor tissue and peripheral blood, time to next treatment (TTNT) and distant metastasis free survival (DMFS).

RESULTS: Patients in arm C+A were a more advanced, refractory STS population compared to A alone (see Table 14). In particular, this group had a higher % of ECOG PS1, metastatic disease, synovial sarcomas, Grade 3 at diagnosis, ≥ 2 prior lines of chemotherapy. Safety run-in 13 patients were excluded from PFS IA due to lack of stratification by histology.

Combination C+A demonstrated partial responses (PR) (Table 15), better disease control (Table 15, Figure 30) and a trend to a longer PFS (Table 15) than A alone; Overall Survival (OS) data is immature at the time of IA.

Table 14: Demographics and Disease Characteristics				
Demographics/Characteristics	Interim Analysis* (n=36)		Safety Population (n=88)	
	Atezo (n=18)	CMB305 + Atezo (n=18)	Atezo (n=43)	CMB305 + Atezo (n=45)
Median age, yrs	43.0	46.5	47.0	48.0
Female, %	50	38.9	44.2	42.2
ECOG PS1, %	44.4	55.6	46.5	57.8
Synovial sarcoma, %	55.6	61.1	67.4	73.3
Metastatic disease, %	72.2	100	79.1	97.8
Time from diagnosis, median (range), mos	26.1 (7.7, 186)	27.4 (3.7, 78.1)	27.8 (6.2, 116.8)	46.8 (14.2, 146.8)
>2 lesions at baseline, %	83.3	100	83.7	95.6
≥2 prior lines of chemo, %	53.3	76.5	48.6	61.1
Progression at study entry, %	50	50	51.2	48.9
NY-ESO-1 expression 50- 100%, %	94.5	88.9	90.7	86.7
Grade 3 at Diagnosis	38.9	61.1	32.6	46.7
*The IA of PFS included the first 36 patients with a median 7.0 months follow up (first 13 patients in safety run-in are excluded from IA as they were not stratified by histology)				

Table 15: Preliminary Clinical Efficacy in Patients on Arm A and Arm C+A				
Outcomes	Interim Analysis (n=36)		Safety Population (n=88)	
	Atezo (n=18)	CMB305 + Atezo (n=18)	Atezo (n=43*)	CMB305 + Atezo (n=45*)
Median Duration of Observation, mos	8.9	7.7	5.1	5.3
Median Progression Free Survival, mos (95% CI)	1.4 (1.3- 1.9)	2.6 (1.4-2.8)	Not reported	Not reported
PFS Rate 6 months (%)	16.7	16.7	Not reported	Not reported
Partial Response (%)	0	1 (5.6)	0	3 ^s (6.8)
Disease Control Rate (%)	5 (27.8)	11 (61.1)	16 (38.1)	25 (56.8)
Time to Next Treatment (mos)**	6.3	9.0	Not reported	Not reported
*1 pt in each Arm did not have post baseline tumor assessment				
**For TTNT 19 patients in A and 13 patients in C+A received subsequent therapy after progression				
^s 1 patient with unconfirmed PR				

For the immune response analysis (IR), pre- and post-treatment PBMC and plasma samples were tested. T cell assays included NY-ESO-1 IFN γ ELISPOT (ex vivo ELISPOT

without in vitro stimulation (IVS) and ELISPOT after IVS), and intracellular cytokine staining (ICS). Antibody assays included NY-ESO-1 ELISA (recombinant protein & peptides), antigen epitope spreading: 24 recombinant Tumor Associated Antigens ELISA (p53, MAGE, SSX, PRAME, etc.). Patients with collected and analyzable peripheral blood samples (N=60) were included in the immune response (IR) analysis. Combination C+A resulted in stronger induction of anti-NY-ESO-1 specific antibodies (Ab) and T cell responses than A alone; Antigen epitope spreading was observed only on Arm C+A (Figure 31).

Preliminary biomarker analysis showed a trend to a better OS in patients with induced anti-NY-ESO-1 T Cells on C+A arm than A alone (Table 16 and Figure 32).

Table 16: Preliminary biomarker analysis				
	Induced T cell Negative	Induced T cell Positive	Hazard Ratio *	P value **
All patients	11.0 (7.6, NA) (n=27)	NA (10.3, NA) (n=16)	0.47	0.26
CMB305 + Atezolizumab	11.0 (3.9, 18.6) (n=12, 48%)	NA (5.3, NA) (n=13, 52%)	0.22	0.04
Atezolizumab	NA (6.6, NA) (n=15, 83%)	NA (10.3, NA) (n=3, 17%)	1.62	0.70
* Kaplan-Meier method; ** not adjusted for multiplicity of analyses				

Preliminary biomarker analysis shows a trend to a better OS in patients with induced anti-NY-ESO-1 Antibodies on C+A arm than A alone (Table 17, Figure 33).

Table 17: Preliminary biomarker analysis, Induced anti-NY-ESO-1 Antibodies				
	Induced Antibody Negative (n=36)	Induced Antibody Positive (n=14)	Hazard Ratio	P value

Table 17: Preliminary biomarker analysis, Induced anti-NY-ESO-1 Antibodies

	Induced Antibody Negative (n=36)	Induced Antibody Positive (n=14)	Hazard Ratio	P value
All Patients	18.6 (7.6, NA) (n=36)	NA (13.4, NA) (n=14)	0.23	0.12
CMB305 + Atezolizumab	7.6 (4.8, 18.6) (n=13, 48%)	NA (13.4, NA) (n=14, 52%)	0.132	0.025
Atezolizumab	NA (10.3, NA) (n=23, 100%)	0 (n=0, 0%)	Not applicable	Not applicable

* Kaplan-Meier method; ** not adjusted for multiplicity of analyses

Patients with partial response (PR) treated on combination C+A had advanced metastatic disease after multiple prior lines of therapy. ELISPOT analysis demonstrated that these patients had evidence of preexisting anti-NY-ESO-1 immunity and induction of anti-NY-ESO-1 IR while on combination C+A therapy (Figure 34). T cell immune response included both CD4 and CD8 cells based on intracellular cytokine staining (data not shown).

Tumor biopsy IHC staining showed that STS baseline and post treatment PD-L1 expression was low and restricted to immune cells and not on tumor cells. Paired biopsies showed evidence of a possible increase of CD8 tumor infiltrating lymphocytes (TILs) on C+A Arm.

CONCLUSIONS: Interim analysis indicates that addition of CMB305 to atezolizumab may result in clinical efficacy improved over atezolizumab alone in NY-ESO-1+ STS patients with low/absent PD-L1.

Despite more advanced patients with worse prognostic factors enrolled to the combination arm, there were partial responses, higher disease control rate and a trend to a better PFS. Overall survival data is immature for this interim analysis. Addition of CMB305 to atezolizumab is well tolerated and no new safety signal was detected.

C+A combination induced a more robust anti-NY-ESO-1 immune response including stronger T cell induction than A alone, antibody induction (not seen on arm A), faster immune response than CMB305 monotherapy (based on historic data comparison). Exploratory analysis found that induction of anti-NY-ESO-1 T cell and antibody IR may be associated with a better OS on C+A arm when compared to patients without induction of IR. Baseline and on treatment PD-L1 expression was low in NY-ESO-1 STS and restricted to immune cells and not on tumor cells. Tumor biopsies showed evidence of CD8 tumor infiltrating lymphocytes (TILs) with possibly increased infiltration on C+A combination.

The various embodiments described above can be combined to provide further embodiments. All U.S. patents, U.S. patent application publications, U.S. patent application, foreign patents, foreign patent application and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified if necessary to employ concepts of the various patents, applications, and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

What is claimed is:

1. A method of identifying a mammalian subject that is likely to benefit from an NY-ESO-1 cancer therapy comprising detecting in one or more biological samples from the mammalian subject the presence of at least two of the following:

(i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both, or a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both;

(ii) an NY-ESO-1 specific antibody;

(iii) an NY-ESO-1 specific CD4+ or CD8+ T cell; and

(iv) the presence of NY-ESO-1 in > about 50% of tumor cells in a tumor sample;

wherein the presence of at least two of (i) – (iv) is indicative that the subject is likely to benefit from the NY-ESO-1 cancer therapy.

2. The method of claim 1, wherein the V β CDR3 comprises an amino acid sequence selected from the group consisting of CASSLNRDYGTYF (SEQ ID NO: 2), CASSLNRDQPQHF (SEQ ID NO: 3) and CASRLAGQETQYF (SEQ ID NO: 4), or any combination of one or more of the foregoing.

3. A method of treating cancer in a mammalian subject comprising first identifying the mammalian subject according to claim 1 or claim 2 and then administering a NY-ESO-1 cancer therapy.

4. The method of claim 3 wherein the cancer therapy comprises administering to the subject a therapeutic composition, said composition comprising one or more therapeutic agents selected from (a) an isolated cell, wherein said isolated cell is a T cell comprising a nucleic acid, or expression vector, wherein said nucleic acid comprises a polynucleotide sequence that encodes a chimeric heterodimeric TCR; wherein the expression vector is a retroviral vector comprising a polynucleotide sequence that encodes a chimeric heterodimeric TCR; (b) an isolated cell, wherein said isolated cell is a T cell comprising a nucleic acid or

expression vector, wherein said nucleic acid comprises a polynucleotide sequence that encodes a single chain TCR; wherein the expression vector is a retroviral vector comprising a polynucleotide sequence that encodes a single chain TCR; wherein the therapeutic composition is administered in an amount effective to treat the cancer in the subject.

5 5. The method of claim 4 wherein the chimeric heterodimeric TCR comprises: a) a first polypeptide comprising a TCR beta chain variable region, a TCR beta chain constant region, and optionally a transmembrane domain and a cytoplasmic signaling domain; b) a second polypeptide comprising a TCR alpha chain variable region, a TCR alpha chain constant region, and optionally a transmembrane domain and a cytoplasmic signaling domain; wherein the heterodimeric TCR specifically binds to an NY-ESO-1/MHC complex, wherein the TCR beta chain variable region comprises the TCR beta chain variable region amino acid sequence set forth in SEQ ID NO:9; wherein the TCR alpha chain variable region comprises the cognate TCR alpha chain variable region amino acid sequence set forth in SEQ ID NO:8; and wherein there is at least one disulfide bond between the first polypeptide and
10 the second polypeptide.
15

6. The method of claim 5 wherein the TCR beta chain variable region CDR3 comprises the amino acid sequence CASRLAGQETQYF (SEQ ID NO: 4).

7. The method of claim 5 wherein the chimeric heterodimeric TCR is soluble, and wherein the first polypeptide and the second polypeptide do not comprise the
20 transmembrane domain and the cytoplasmic signaling domain.

8. The method of claim 4 wherein the single chain TCR comprises a beta chain variable region comprising a TCR beta chain variable region amino acid sequence set forth in SEQ ID NO:9; and a TCR alpha chain variable region comprising the cognate TCR alpha chain variable region amino acid sequence as set forth in SEQ ID NO:8.

25 9. The method of claim 4 wherein the single chain TCR is a soluble single chain TCR and wherein the single chain TCR does not comprise the transmembrane domain and the cytoplasmic signaling domain.

10. The method of any one of claims 3-9, wherein the NY-ESO-1 cancer therapy comprises administering a vector encoding an NY-ESO-1 polypeptide.

11. The method of claim 10, wherein the vector comprises a dendritic cell targeting retroviral vector.

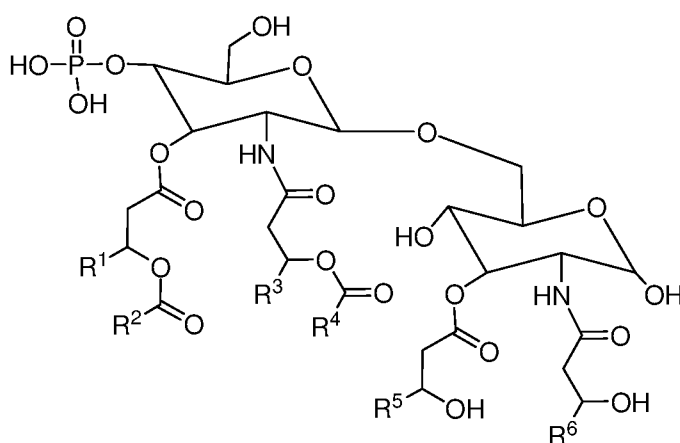
12. The method of any one of claims 3-11, further comprising administering an adjuvant to the subject.

5 13. The method of claim 12, wherein the adjuvant is glucopyranosyl lipid A (GLA), optionally wherein the GLA is formulated in a stable oil-in-water emulsion.

14. The method of claim 11, wherein the vector is a lentiviral vector.

15. The method of any one of claims 3-14, wherein the NY-ESO-1 cancer therapy comprises administering to the subject a composition comprising GLA, said composition
10 comprising:

(a) GLA of the formula:



wherein:

R1, R3, R5 and R6 are C11-C20 alkyl; and

15 R2 and R4 are C12-C20 alkyl; and

(b) a pharmaceutically acceptable carrier or excipient;

wherein the composition does not comprise antigen.

16. The method of claim 15, wherein R1, R3, R5 and R6 are undecyl and R2 and R4 are tridecyl.

17. The method of claim 16, wherein the mammal is human.

18. The method of claim 16, wherein the composition is an aqueous formulation.

19. The method of claim 16, wherein the composition is in the form of an oil-in-water emulsion, a water-in-oil emulsion, liposome, micellar formulation, or a microparticle.

5 20. The method of any of claims 3-19, wherein the cancer comprises a solid tumor.

21. The method of any one of claims 3-20, wherein the cancer is selected from the group consisting of a sarcoma, prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, oesophageal cancer, non-small-cell
10 lung cancer, non-Hodgkin's lymphoma, multiple myeloma, melanoma, hepatocellular carcinoma, head and neck cancer, gastric cancer, endometrial cancer, colorectal cancer, cholangiocarcinoma, breast cancer, bladder cancer, myeloid leukemia and acute lymphoblastic leukemia.

22. The method of claim 15, wherein the composition is administered by
15 subcutaneous, intradermal, intramuscular, intratumoral, or intravenous injection.

23. The method of any of claims 3-22, wherein the composition is administered in conjunction with one or more additional therapeutic agents or treatments.

24. The method of claim 23, wherein the therapeutic agent is an immune checkpoint inhibitor.

20 25. The method of claim 23, wherein the therapeutic agent is an antibody that activates a costimulatory pathway.

26. The method of claim 25, wherein the antibody is an anti-CD40 antibody.

27. The method of claim 23, wherein the therapeutic agent is a cancer therapeutic agent.

25 28. The method of claim 27, wherein the cancer therapeutic agent is selected from the group consisting of taxotere, carboplatin, trastuzumab, epirubicin, cyclophosphamide, cisplatin, docetaxel, doxorubicin, etoposide, 5-FU, gemcitabine, methotrexate, and paclitaxel,

mitoxantrone, epothilone B, epidermal-growth factor receptor (EGFR)-targeting monoclonal antibody 7A7.27, vorinostat, romidepsin, docosahexaenoic acid, bortezomib, shikonin and an oncolytic virus.

29. The method of claim 23, wherein the one or more additional therapeutic
5 treatments is radiation therapy.

30 A method of identifying a mammalian subject undergoing an NY-ESO-1 cancer therapy that is likely to benefit from an extended NY-ESO-1 cancer therapy comprising detecting in a sample from the mammalian subject the presence of at least two of the following as compared to before the cancer therapy:

10 (i) induction of an NY-ESO-1 specific CD8 T cell response;

(ii) induction of an integrated immune response comprising each of an NY-ESO-1 specific CD4 T cell response, an NY-ESO-1 specific CD8 T cell response, and NY-ESO-1 antibody production;

(iii) antigen spreading;

15 (iv) increased T cell clonality; and

(v) increase in the presence of (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both, or a TCR polypeptide comprising a V β CDR3 that is specific for NY-
20 ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both;

wherein the presence of at least two of the foregoing indicates that the subject is likely to benefit from an extended NY-ESO-1 cancer therapy.

31. The method of claim 30, wherein the V β CDR3 comprises an amino acid
25 sequence selected from the group consisting of CASSLNRDYGTYF (SEQ ID NO: 2), CASSLNRDQPQHF (SEQ ID NO: 3) and CASRLAGQETQYF (SEQ ID NO: 4), or any combination of one or more of the foregoing.

32. A method of treating cancer in a mammalian subject comprising identifying

the mammalian subject according to claim 30 or claim 31 and then continuing administration of the NY-ESO-1 cancer therapy.

33. The method of any one of claims 30-32, wherein the NY-ESO-1 cancer therapy comprises administering a vector encoding an NY-ESO-1 polypeptide.

5 34. The method of claim 33, wherein the vector comprises a dendritic cell targeting retroviral vector.

35. The method of any one of claims 30-34, further comprising administering an adjuvant to the subject.

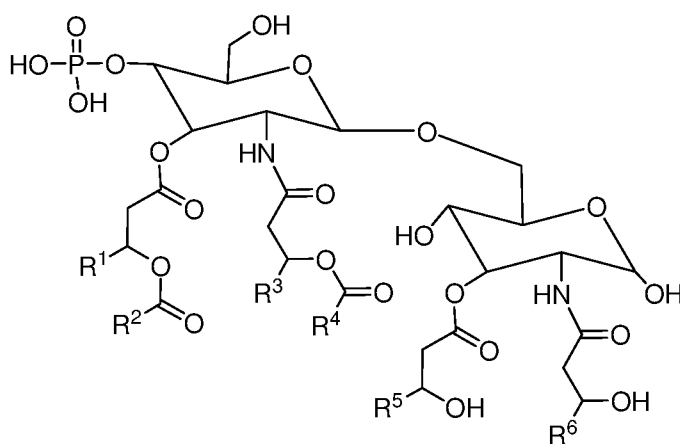
10 36. The method of claim 35, wherein the adjuvant is glucopyranosyl lipid A (GLA).

37. The method of claim 36, wherein GLA is formulated in a stable oil-in-water emulsion.

38. The method of claim 34, wherein the vector is a lentiviral vector.

15 39. The method of any one of claims 30-38, wherein the NY-ESO-1 cancer therapy comprises administering to the subject a composition comprising GLA, said composition comprising:

(a) GLA of the formula:



wherein:

20 R1, R3, R5 and R6 are C11-C20 alkyl; and

R2 and R4 are C12-C20 alkyl; and

(b) a pharmaceutically acceptable carrier or excipient;

wherein the composition does not comprise antigen.

40. The method of claim 39, wherein R1, R3, R5 and R6 are undecyl and R2 and
5 R4 are tridecyl.

41. The method of claim 40, wherein the mammal is human.

42. The method of claim 40, wherein the composition is an aqueous formulation.

43. The method of claim 40, wherein the composition is in the form of an oil-in-
water emulsion, a water-in-oil emulsion, liposome, micellar formulation, or a microparticle.

10 44. The method of any of claims 30-43, wherein the cancer comprises a solid
tumor.

45. The method of any one of claims 30-44, wherein the cancer is selected from
the group consisting of a sarcoma, prostate cancer, uterine cancer, thyroid cancer, testicular
cancer, renal cancer, pancreatic cancer, ovarian cancer, oesophageal cancer, non-small-cell
15 lung cancer, non-Hodgkin's lymphoma, multiple myeloma, melanoma, hepatocellular
carcinoma, head and neck cancer, gastric cancer, endometrial cancer, colorectal cancer,
cholangiocarcinoma, breast cancer, bladder cancer, myeloid leukemia and acute
lymphoblastic leukemia.

20 46. The method of claim 39, wherein the composition is administered by
subcutaneous, intradermal, intramuscular, intratumoral, or intravenous injection.

47. The method of any of claims 30-46, wherein the composition is administered
in conjunction with one or more additional therapeutic agents or treatments.

48. The method of claim 47, wherein the therapeutic agent is an immune
checkpoint inhibitor.

25 49. The method of claim 47, wherein the therapeutic agent is an antibody that
activates a costimulatory pathway.

50. The method of claim 49, wherein the antibody is an anti-CD40 antibody.

51. The method of claim 47, wherein the therapeutic agent is a cancer therapeutic agent.

52. The method of claim 51, wherein the cancer therapeutic agent is selected from the group consisting of taxotere, carboplatin, trastuzumab, epirubicin, cyclophosphamide, cisplatin, docetaxel, doxorubicin, etoposide, 5-FU, gemcitabine, methotrexate, and paclitaxel, mitoxantrone, epothilone B, epidermal-growth factor receptor (EGFR)-targeting monoclonal antibody 7A7.27, vorinostat, romidepsin, docosahexaenoic acid, bortezomib, shikonin and an oncolytic virus.

53. The method of claim 47, wherein the one or more additional therapeutic treatments is radiation therapy.

54. A method of identifying a mammalian subject that is likely to benefit from an in vivo DC targeted lentiviral vector NY-ESO-1 cancer therapy comprising detecting in one or more biological samples from the mammalian subject the presence of the following:

(i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises an amino acid sequence selected from the group consisting of CASSLNRDYGYTF (SEQ ID NO: 2), CASSLNRDQPQHF (SEQ ID NO: 3) and CASRLAGQETQYF (SEQ ID NO: 4), or any combination of one or more of the foregoing.; and

(ii) the presence of NY-ESO-1 in > about 50% of tumor cells in a tumor sample;

wherein the presence of (i) and (ii) is indicative that the subject is likely to benefit from the NY-ESO-1 cancer therapy.

55. The method of claim 48 wherein the immune checkpoint inhibitor is atezolizumab.

56. A method of identifying a mammalian subject that is likely to benefit from a tumor antigen-specific cancer therapy comprising detecting in one or more biological samples from the mammalian subject the presence of at least two of the following:

(i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is

specific for the tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence;

(ii) a tumor antigen-specific antibody;

(iii) a tumor antigen-specific CD4+ or CD8+ T cell; and

5 (iv) the presence of the tumor antigen in > about 50% of tumor cells in a tumor sample;

wherein the presence of at least two of (i) – (iv) is indicative that the subject is likely to benefit from the tumor antigen-specific cancer therapy.

57. A method of treating cancer in a mammalian subject comprising first
10 identifying the mammalian subject according to claim 56 and then administering a tumor antigen-specific cancer therapy.

58. The method of claim 57 wherein the cancer therapy comprises administering to the subject a therapeutic composition, said composition comprising one or more therapeutic agents selected from:

15 (a) an isolated cell, wherein said isolated cell is a T cell comprising a nucleic acid, or expression vector, wherein said nucleic acid comprises a polynucleotide sequence that encodes a chimeric heterodimeric TCR; wherein the expression vector is a retroviral vector comprising a polynucleotide sequence that encodes a chimeric heterodimeric TCR;

(b) an isolated cell, wherein said isolated cell is a T cell comprising a nucleic acid or
20 expression vector, wherein said nucleic acid comprises a polynucleotide sequence that encodes a single chain TCR; wherein the expression vector is a retroviral vector comprising a polynucleotide sequence that encodes a single chain TCR;

wherein the therapeutic composition is administered in an amount effective to treat the cancer in the subject.

25 59. The method of claim 58 wherein the chimeric heterodimeric TCR comprises:

a. a first polypeptide comprising a TCR beta chain variable region, a TCR beta chain constant region, and optionally a transmembrane domain and a cytoplasmic signaling

domain;

b. a second polypeptide comprising a TCR alpha chain variable region, a TCR alpha chain constant region, and optionally a transmembrane domain and a cytoplasmic signaling domain;

5 wherein the heterodimeric TCR specifically binds to a tumor antigen/MHC complex; and wherein there is at least one disulfide bond between the first polypeptide and the second polypeptide.

60. The method of claim 59 wherein the chimeric heterodimeric TCR is soluble, and wherein the first polypeptide and the second polypeptide do not comprise the
10 transmembrane domain and the cytoplasmic signaling domain.

61. The method of claim 59 wherein the single chain TCR is a soluble single chain TCR and wherein the single chain TCR does not comprise the transmembrane domain and the cytoplasmic signaling domain.

62. The method of any one of claims 56-61, wherein the tumor antigen-specific
15 cancer therapy comprises administering a vector encoding the tumor antigen polypeptide.

63. The method of claim 62, wherein the vector comprises a dendritic cell targeting retroviral vector.

64. The method of any one of claims 56-61, wherein the tumor antigen-specific cancer therapy comprises administering a nucleic acid encoding the tumor antigen
20 polypeptide.

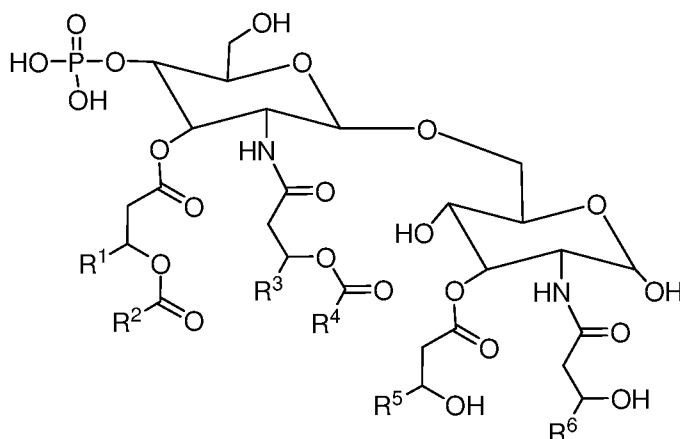
65. The method of claim 64, wherein the nucleic acid comprises an RNA encoding the tumor antigen polypeptide.

66. The method of claim 63, further comprising administering an adjuvant to the subject.

25 67. The method of claim 66, wherein the adjuvant is glucopyranosyl lipid A (GLA), optionally wherein the GLA is formulated in a stable oil-in-water emulsion.

68. The method of claim 63, wherein the vector is a lentiviral vector.

69. The method of claim 66, wherein the GLA comprises a a GLA of the formula:



wherein:

R1, R3, R5 and R6 are C11-C20 alkyl; and

5 R2 and R4 are C12-C20 alkyl..

70. The method of claim 69, wherein R1, R3, R5 and R6 are undecyl and R2 and R4 are tridecyl.

71. The method of any one of claims 56-61, wherein the tumor antigen is a cancer testis antigen..

10 72. The method of claim 62, wherein the tumor antigen is a cancer testis antigen.

73. The method of any one of claims 56-61, wherein the composition is administered in conjunction with one or more additional therapeutic agents or treatments.

74. The method of claim 62, wherein the composition is administered in conjunction with one or more additional therapeutic agents or treatments.

15 75. The method of claim 73 or 74, wherein the therapeutic agent is an immune checkpoint inhibitor.

76. The method of claim 73 or 74, wherein the therapeutic agent is an antibody that activates a costimulatory pathway.

77. The method of claim 76, wherein the antibody is an anti-CD40 antibody.

78. The method of claim 73 or 74, wherein the therapeutic agent is a cancer therapeutic agent.

79. The method of claim 78, wherein the cancer therapeutic agent is selected from the group consisting of taxotere, carboplatin, trastuzumab, epirubicin, cyclophosphamide, cisplatin, docetaxel, doxorubicin, etoposide, 5-FU, gemcitabine, methotrexate, and paclitaxel, mitoxantrone, epothilone B, epidermal-growth factor receptor (EGFR)-targeting monoclonal antibody 7A7.27, vorinostat, romidepsin, docosahexaenoic acid, bortezomib, shikonin and an oncolytic virus.

80. The method of claim 73 or 74, wherein the one or more additional therapeutic treatments is radiation therapy.

81 A method of identifying a mammalian subject undergoing a tumor antigen-specific cancer therapy that is likely to benefit from an extended tumor antigen-specific cancer therapy comprising detecting in a sample from the mammalian subject the presence of at least two of the following as compared to before the cancer therapy:

(i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for the tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence;

(ii) a tumor antigen-specific antibody;

(iii) a tumor antigen-specific CD4⁺ or CD8⁺ T cell; and

(iv) the presence of the tumor antigen in > about 50% of tumor cells in a tumor sample;

wherein the presence of at least two of (i) – (iv) is indicative that the subject is likely to benefit from the extended tumor antigen-specific cancer therapy.

82. A method of treating cancer in a mammalian subject comprising first identifying the mammalian subject according to claim 81 and then continuing administration of the tumor antigen-specific cancer therapy.

83. The method of any one of claims 81-82, wherein the tumor antigen-specific cancer therapy comprises administering a vector encoding the tumor antigen polypeptide.

84. The method of claim 83, wherein the vector comprises a dendritic cell targeting retroviral vector.

85. The method of claim 84, further comprising administering an adjuvant to the subject.

5 86. The method of claim 85, wherein the adjuvant is glucopyranosyl lipid A (GLA).

87. The method of claim 84, wherein the vector is a lentiviral vector.

88. The method of any one of claims 81-87, wherein the tumor antigen is a cancer testis antigen.

10 89. The method of any of claims 81-87, wherein the composition is administered in conjunction with one or more additional therapeutic agents or treatments.

90. The method of claim 89, wherein the therapeutic agent is an immune checkpoint inhibitor.

15 91. The method of claim 89, wherein the therapeutic agent is an antibody that activates a costimulatory pathway.

92. The method of claim 91, wherein the antibody is an anti-CD40 antibody.

93. The method of claim 89, wherein the therapeutic agent is a cancer therapeutic agent.

20 94. The method of claim 93, wherein the cancer therapeutic agent is selected from the group consisting of taxotere, carboplatin, trastuzumab, epirubicin, cyclophosphamide, cisplatin, docetaxel, doxorubicin, etoposide, 5-FU, gemcitabine, methotrexate, and paclitaxel, mitoxantrone, epothilone B, epidermal-growth factor receptor (EGFR)-targeting monoclonal antibody 7A7.27, vorinostat, romidepsin, docosahexaenoic acid, bortezomib, shikonin and an oncolytic virus.

25 95. The method of claim 89, wherein the one or more additional therapeutic treatments is radiation therapy.

96. A method of identifying a mammalian subject that is likely to benefit from an

in vivo DC targeted lentiviral vector tumor antigen-specific cancer therapy comprising detecting in one or more biological samples from the mammalian subject the presence of the following:

5 (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for the tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3; and

(ii) the presence of the tumor antigen in > about 50% of tumor cells in a tumor sample;

wherein the presence of (i) and (ii) is indicative that the subject is likely to benefit from the tumor antigen-specific cancer therapy.

10 97. The method of claim 96 wherein the wherein the mammalian subject is being treated with an immune checkpoint inhibitor.

98 A method of treating a soft tissue sarcoma in a patient comprising, detecting in one or more biological samples from the patient the presence of at least two of the following:

15 (i) a polynucleotide encoding a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both, or a TCR polypeptide comprising a V β CDR3 that is specific for NY-ESO-1, wherein the V β CDR3 comprises the amino acid sequence of CASSLNRDXXXXF (SEQ ID NO: 1) or CASRLAGQETQYF (SEQ ID NO: 4), or both;

20 (ii) an NY-ESO-1 specific antibody;

(iii) an NY-ESO-1 specific CD4+ or CD8+ T cell; and

administering a NY-ESO-1 cancer therapy.

99. The method of claim 98 wherein the soft tissue sarcoma is synovial sarcoma or myxoid round cell liposarcoma.

25 100. A kit for identifying a patient likely to benefit from a tumor antigen-specific cancer therapy comprising two or more of the following:

(i) a set of polynucleotides for detecting a polynucleotide encoding a TCR

polypeptide comprising a V β CDR3 that is specific for a tumor antigen, wherein the V β CDR3 comprises a public TCR V β CDR3 amino acid sequence;

(ii) ELISA reagents for detecting a tumor antigen-specific antibody;

5 (iii) a sufficient amount of recombinant tumor antigen, or a set of overlapping peptides spanning the tumor antigen, suitable for stimulating tumor antigen-specific CD4+ or CD8+ T cells or both; and

(iv) an antibody specific for the tumor antigen useful to detect the presence of the tumor antigen in a tumor sample.

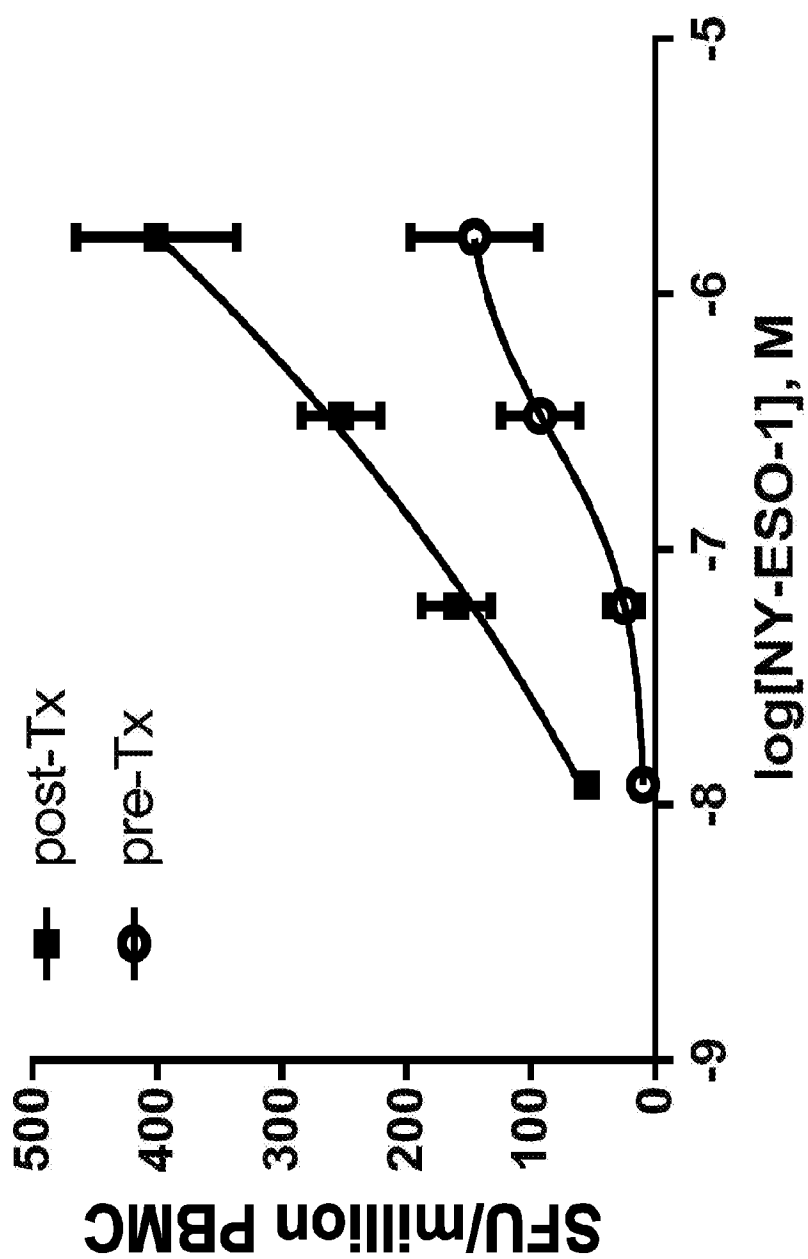


Figure 1

Figure 2

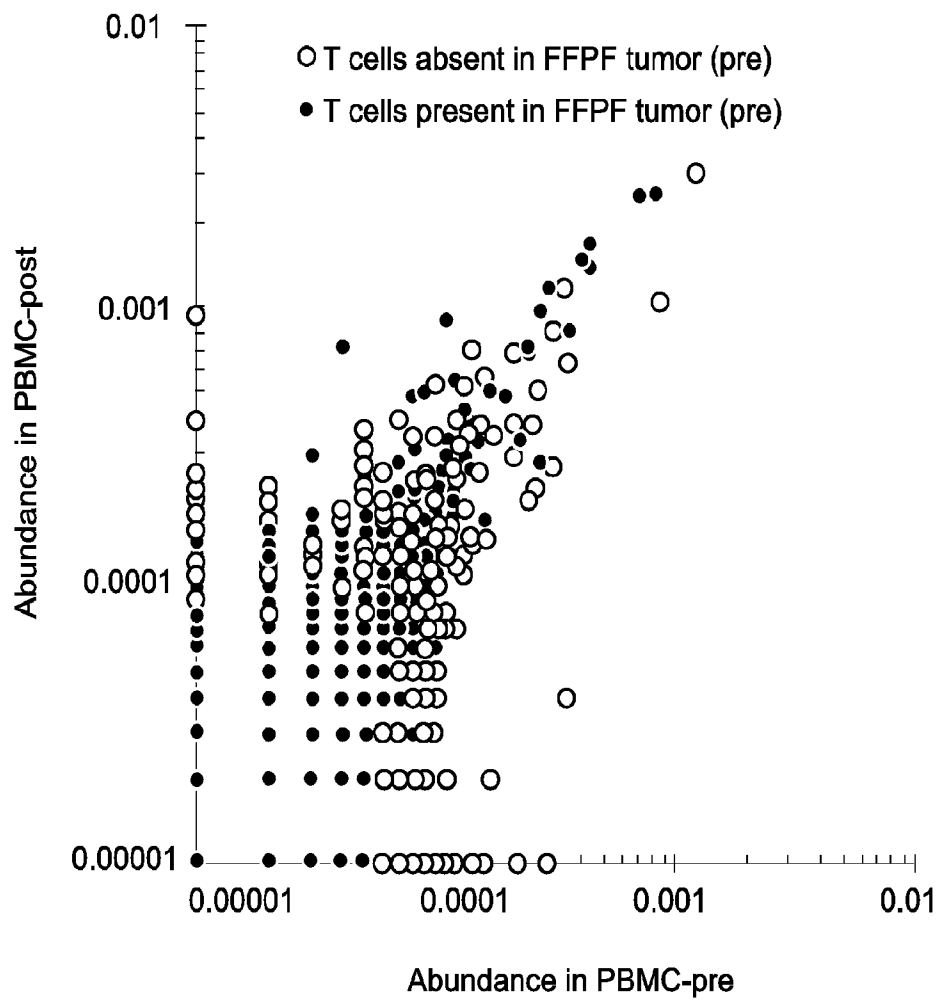


Figure 3

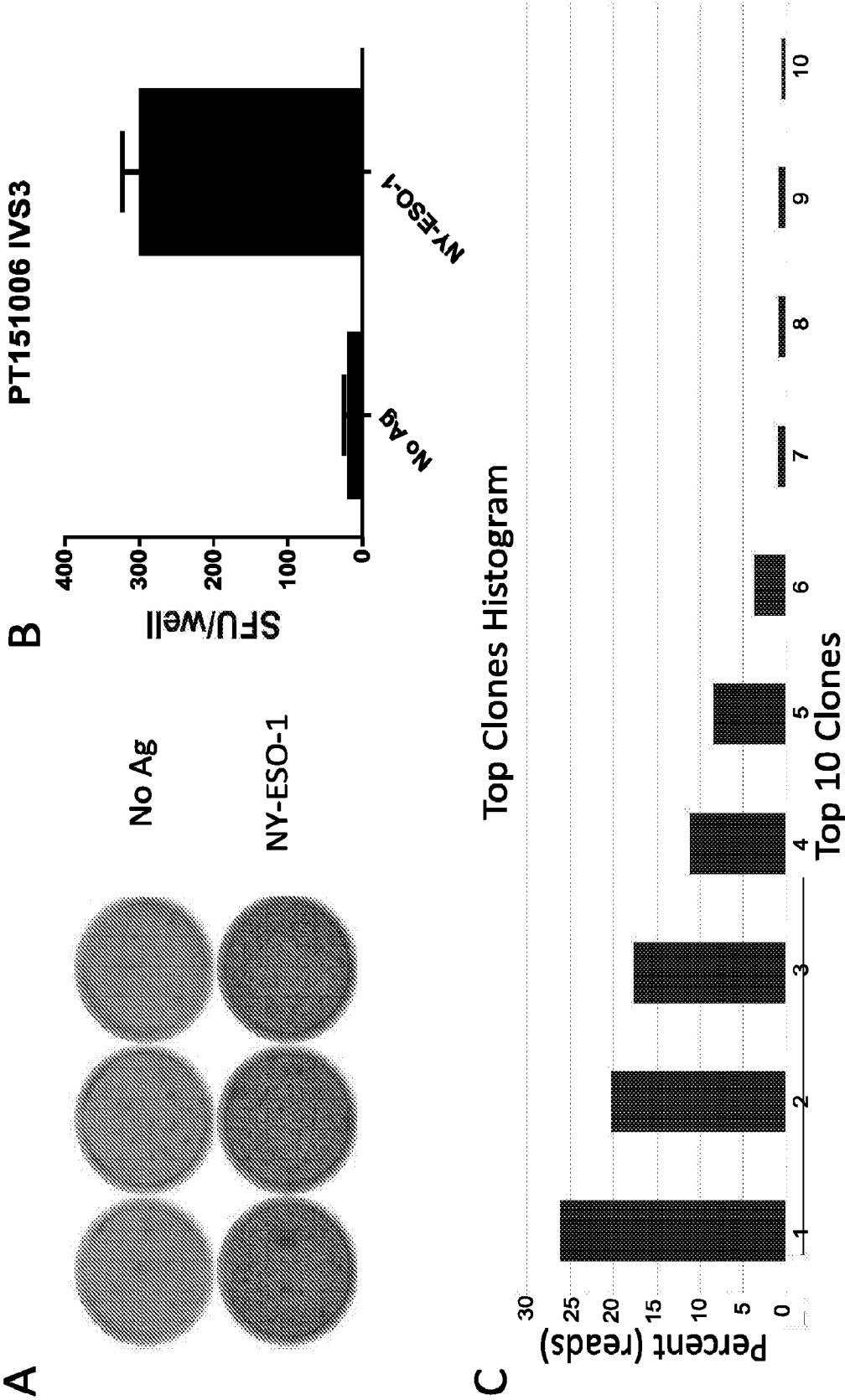


Figure 4

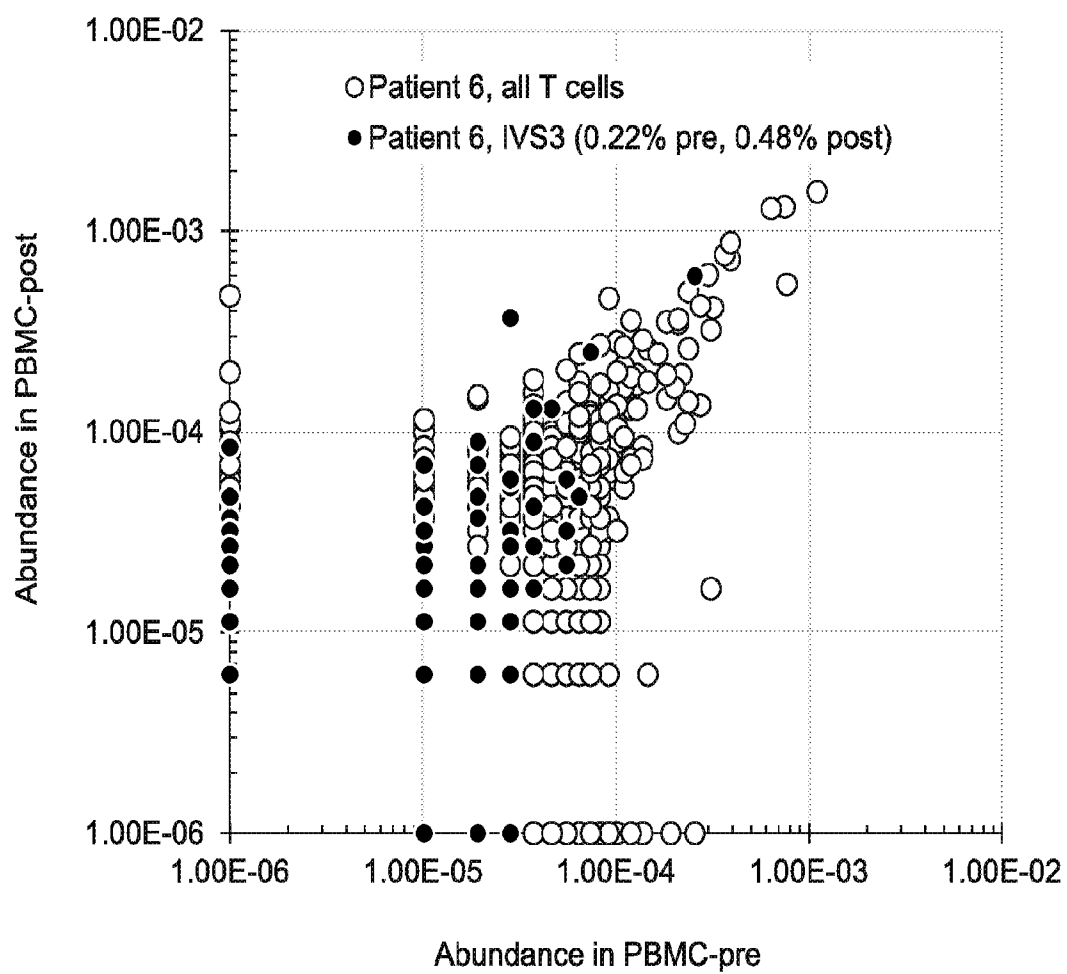
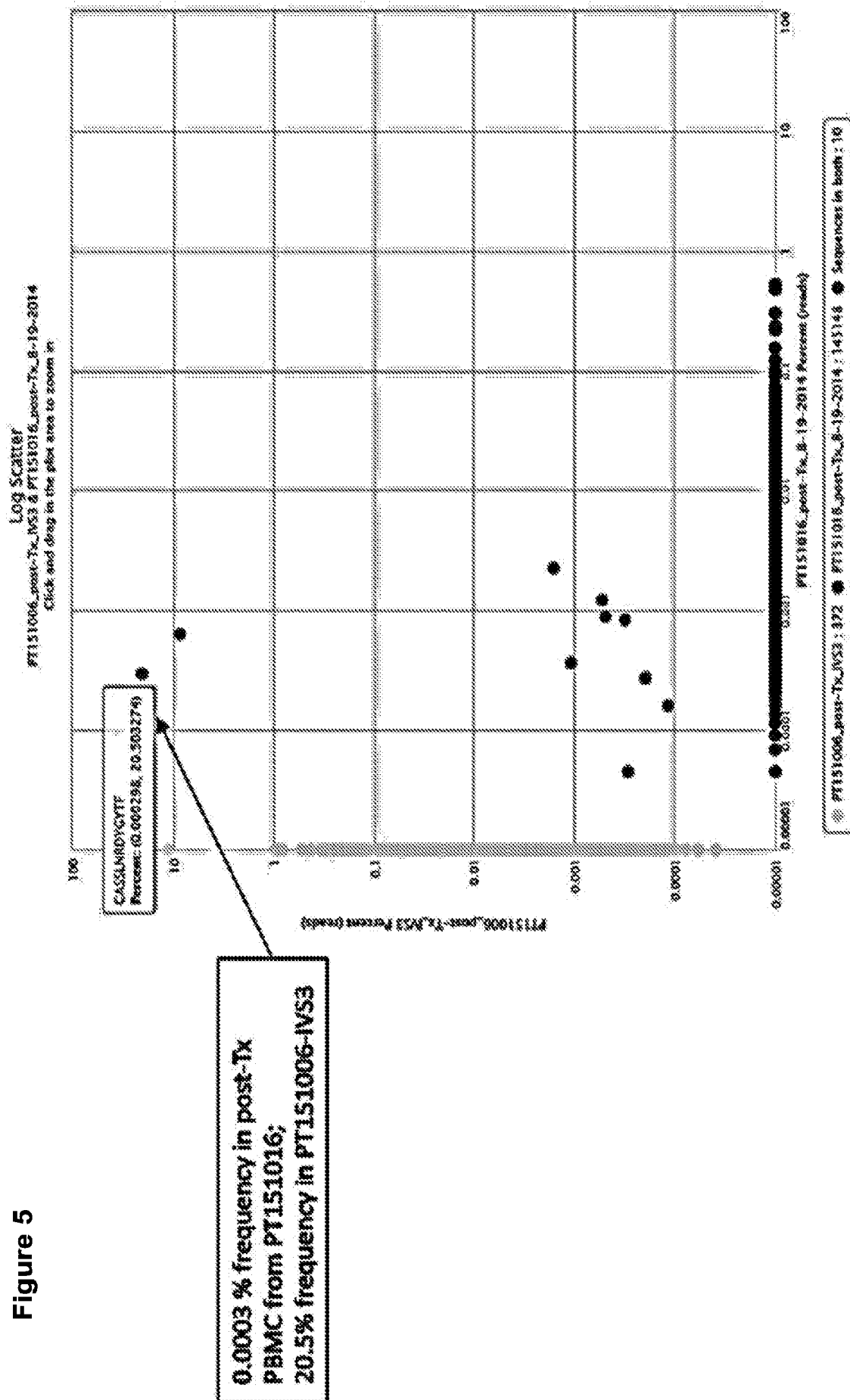


Figure 5



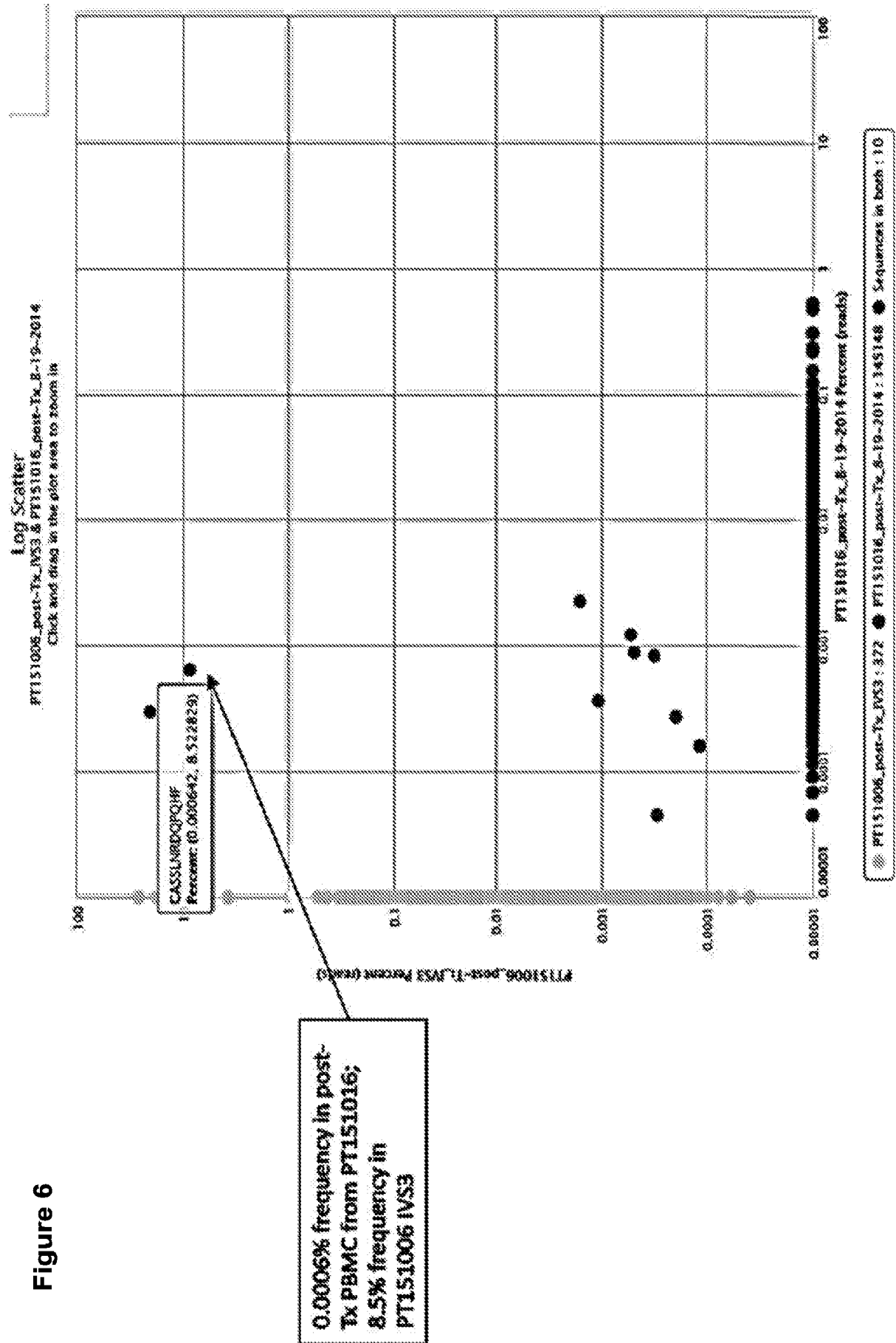
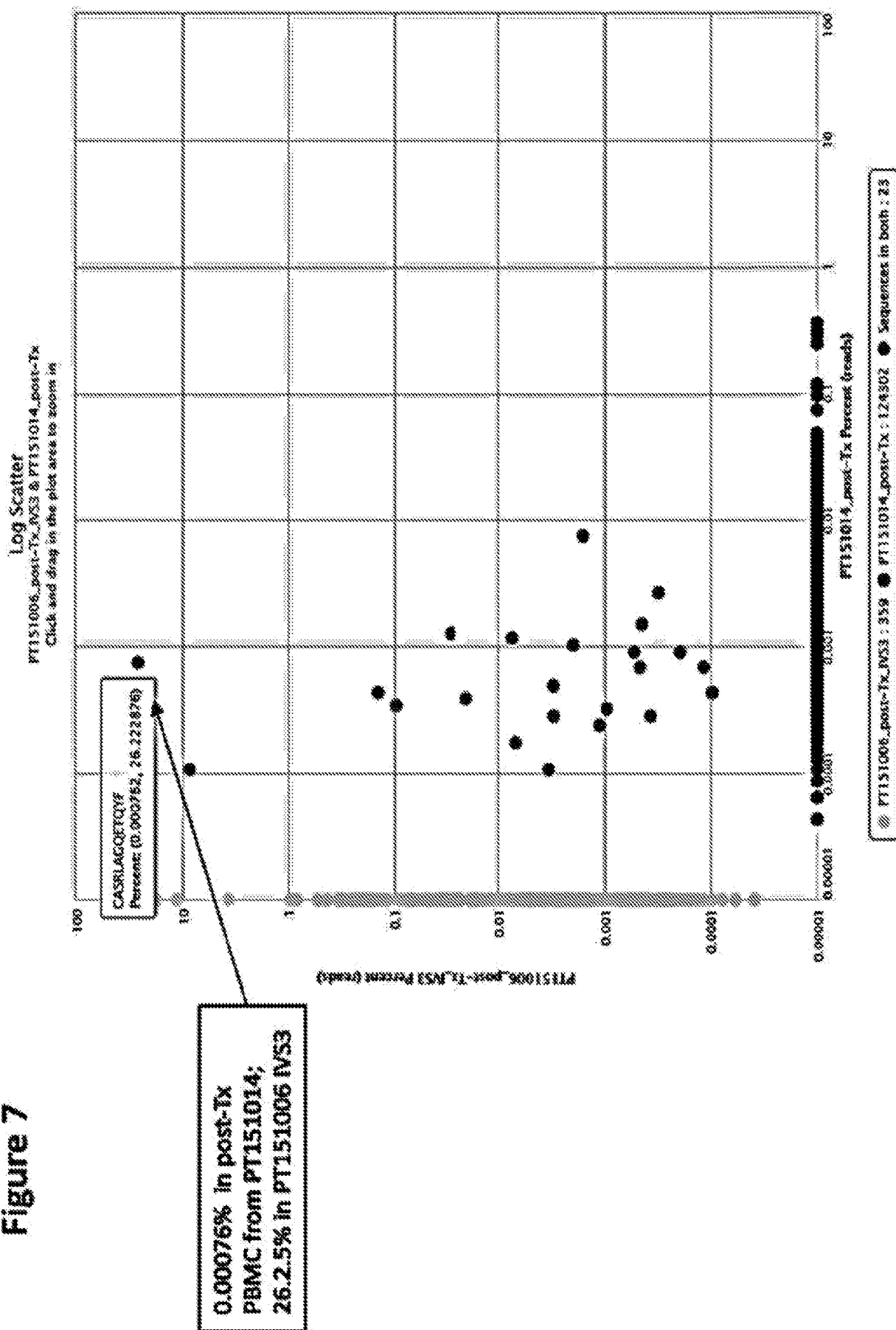


Figure 7



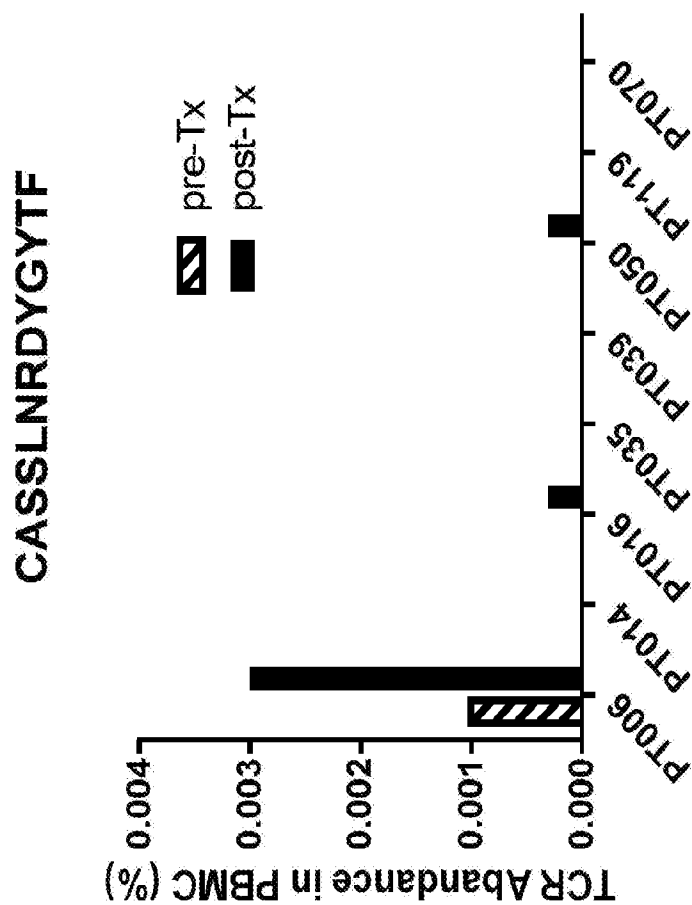


Figure 8A

Figure 8B

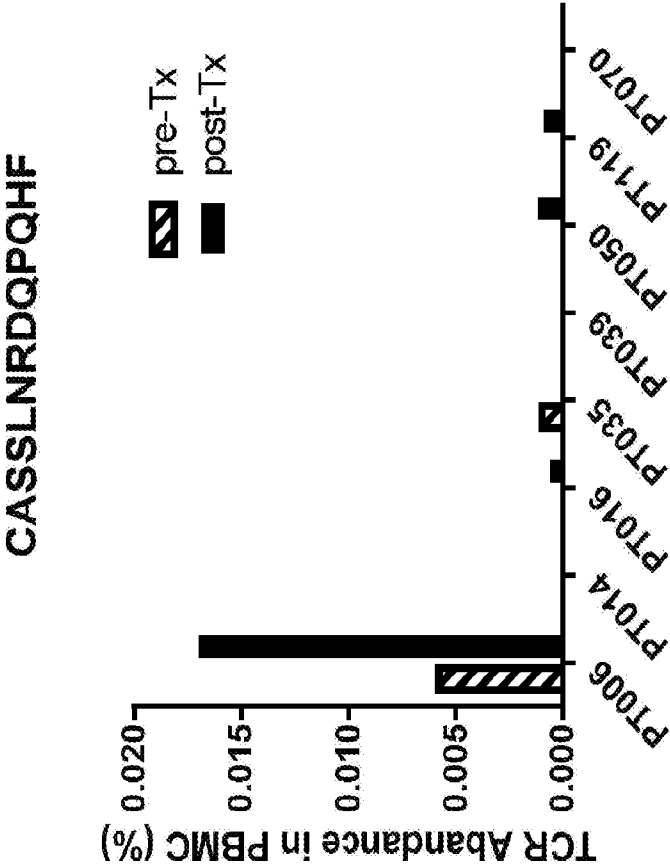


Figure 8C

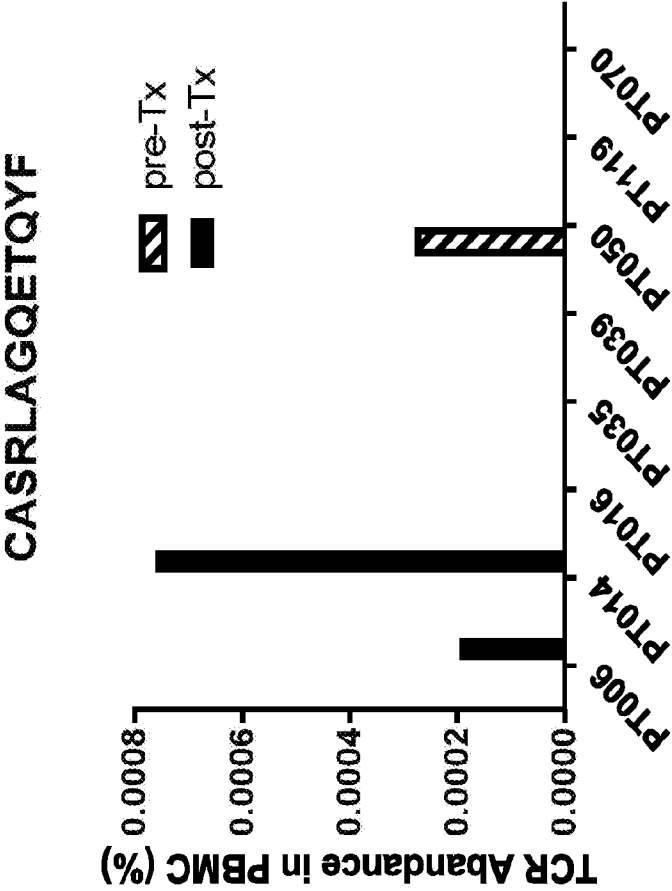


Figure 9

Patient ID	Nucleotide Sequence	Amino Acid Sequence
PT151006	<u>AAATCCAGCGCACACAGCAGGAGGACTCCGCGTGATCTCTGTGCCAGCAGC</u> <u>TTGAACAGGGGACTATGGCTACACCTTCGGTTCG (SEQ ID NO:5)</u>	CASSLNRDYGYTF (SEQ ID NO:2)
PT151016	<u>AAATCCAGCGCCCTCAGAACCCAGGGACTCAGCTGTGTACTTCTGTGCCAGCAGTT</u> <u>TGAACCGGGGACTATGGCTACACCTTCGGTTCG (SEQ ID NO:10)</u>	CASSLNRDYGYTF (SEQ ID NO:2)
PT151050	<u>AAATCCAGCGCACAGAGCGGGGGGACTCAGCCGTGTATCTCTGTGCCAGCAGC</u> <u>TTAAACAGGGGACTATGGCTACACCTTCGGTTCG (SEQ ID NO: 11)</u>	CASSLNRDYGYTF (SEQ ID NO:2)
Patient ID	Nucleotide Sequence	Amino Acid Sequence
PT151006	<u>ACGATTACAGCGCACAGAGCAGCGGGGACTCAGCCATGTATCGTCTGTAGCAGC</u> <u>TTGAACAGGGGACAGCCCCAGCATTTGGTGAT (SEQ ID NO: 6)</u>	CASSLNRDQPQHF (SEQ ID NO: 3)
PT151016	<u>GAGATCCAGCGCACAGAGCAGGGGGACTCGGCCATGTATCTCTGTGCCAGCAGC</u> <u>TTAAACCGGGGACAGCCCCAGCATTTGGTGAT (SEQ ID NO: 12)</u>	CASSLNRDQPQHF (SEQ ID NO: 3)
PT151050	<u>AATGTGAACGCCTTGGAGCTGGACGACTCGGCCCTGTATCTCTGTGCCAGCAGCT</u> <u>TGAATCGTGATCAGCCCCAGCATTTGGTGAT (SEQ ID NO: 13)</u>	CASSLNRDQPQHF (SEQ ID NO: 3)
Patient ID	Nucleotide Sequence	Amino Acid Sequence
PT151006	<u>ATTCTGGAGTCCGCCAGCACCAACAGACATCTATGTACCTCTGTGCCAGCAGAC</u> <u>TAGCGGGACAAGAGACCCAGTACTTCGGGCCA (SEQ ID NO: 7)</u>	CASRLAGQETQYF (SEQ ID NO: 4)
PT151014	<u>AGGCTGGAGTTGGCTGCTCCCTCCAGACATCTGTGTACTTCTGTGCCAGCAGAC</u> <u>TAGCGGGGCAAGAGACCCAGTACTTCGGGCCA (SEQ ID NO: 14)</u>	CASRLAGQETQYF (SEQ ID NO: 4)
PT151050	<u>AATGTGAGCACCTTGGAGCTGGGGGACTCGGCCCTTATCTTTCGCCAGCAGCA</u> <u>CTAGCGGGGCAAGAGACCCAGTACTTCGGGCCA (SEQ ID NO: 15)</u>	CASRLAGQETQYF (SEQ ID NO: 4)

Figure 10A

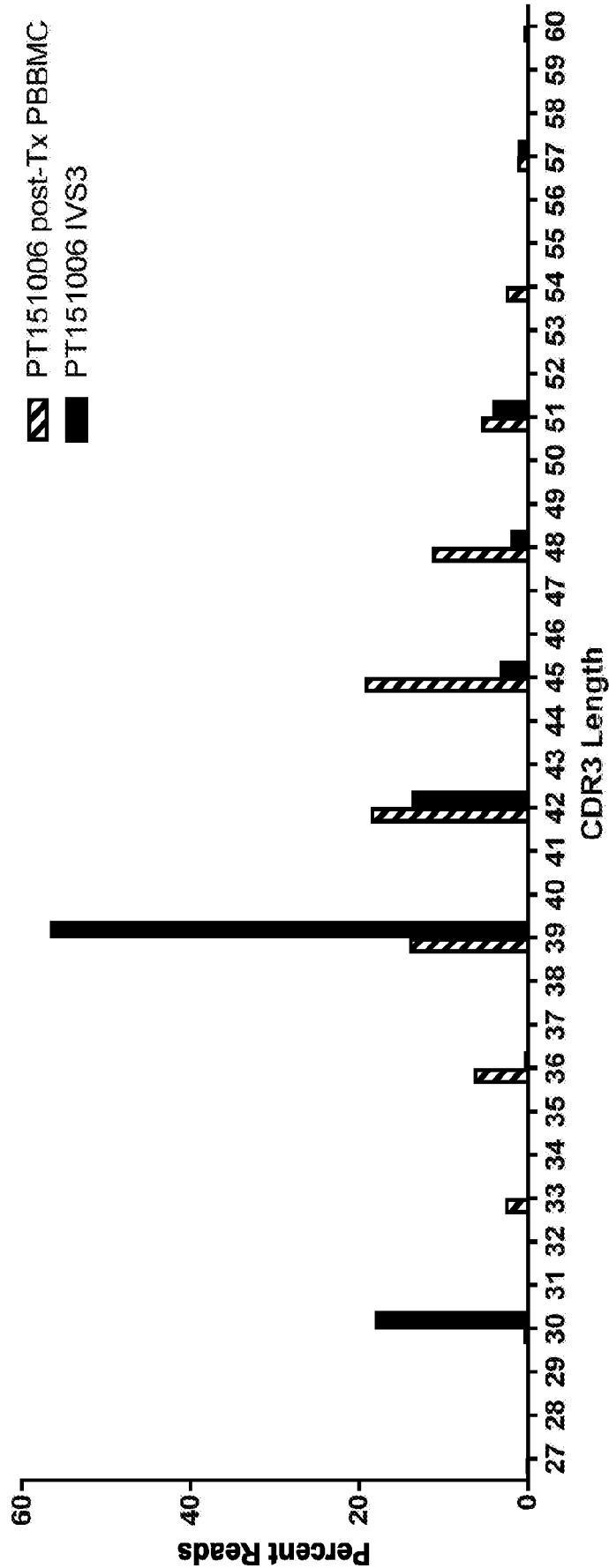


Figure 10B

PT151006 Nucleotide Sequence	Amino Acid	Cdr3 nt Length	V GeneName	D GeneName	J GeneName	V Deletion	N1 Insertion	D5 Deletion	D3 Deletion	N2 Insertion	J Deletion
AAGATCCAGCGCACACAGCAGG AGGACTCCGCCGTGTCTCTGT GCCAGCAGCTTGAACAGGGACT ATGGCTACACCTTCGGTTTCG (SEQ ID NO: 5)	CASSLNRDYGYTF (SEQ ID NO: 2)	39	TCRBV07- 08	TCRBD01- 01	TCRBJ01- 02	3	2	3	3	0	3
ACGATTTCAGCGCACAGCAGC GGGACTCAGCCATGTATCGCTGT GCTAGCAGCTTGAACAGGGACC AGCCCCAGCATTTTGGTGAT (SEQ ID NO: 6)	CASSLNRDQPQHIF (SEQ ID NO: 3)	39	TCRBV07- 07	TCRBD01- 01	TCRBJ01- 05	3	2	3	3	2	7
ATTCTGGAGTCCGCCAGCACCAACCA GACATCTATGTACCTCTGTGCCAGCA GACTAGCGGGACAAGAGACCCAGTA CTTCGGGCCA (SEQ ID NO: 7)	CASRLAGQETQYF (SEQ ID NO: 4)	39	TCRBV28- 01	TCRBD02- 01	TCRBJ02- 05	6	0	3	3	0	2

Figure 11

TCR alpha:

METLLGLLILWLQLQWVSSKQEV^{VαCDR1}QTQIPAA^{~J region = double underline}LSVPEGENLV^{CDRs bolded}LNC^{Variable Region = single underline + double underline}SFTD^{~J region = double underline}SAIYNLQWFRQDPGKGLT
 SLLLIQSSQREQTSGR^{Constant region = dotted underline}LNASLDKSSGRSTLYIAASQPGDSATYLCAVVGGSYIPTFGRGTS^{CDRs bolded}LIVHP
 YIQKPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKNSAVA
 WSNKSD (SEQ ID NO:8)

Vα CDR2
 Vα CDR3

TCR beta variable region:

DVKVTQSSRYLVKRTGEKV^{VβCDR1}FLECVQDMDHENMF^{~J region = double underline}WYRQDPGLGLRLIYFSYDV^{CDRs bolded}KMKEKGD^{Variable Region = single underline + double underline}PIEG
 YSVSREKKERFSLILESASTNQTSMYL^{~J region = double underline}CASRLAGQETQYFGPGTRLLVLEDLKN (SEQ ID NO:9)

VβCDR2
 VβCDR3

Figure 12

1 st public TCRbeta CDR3																									
C	A	S	S	L	N	R	D	Y	G	Y	T	F													
PT006	T	G	T	G	C	C	A	G	C	A	G	G	A	C	T	A	C	A	C	C	T	T	C		
PT016	T	G	T	G	C	C	A	G	C	A	G	I	T	T	G	A	A	C	C	G	G	A	C	T	C
PT050	T	G	T	G	C	C	A	G	C	A	G	C	T	T	A	A	A	C	A	G	G	A	C	T	C

2 nd public TCRbeta CDR3																									
C	A	S	S	L	N	R	D	Q	P	Q	H	F													
PT006	T	G	T	G	C	T	A	G	C	A	G	C	C	C	A	G	C	C	A	T	T	T	T	T	
PT016	T	G	T	G	C	C	A	G	C	T	T	A	A	A	C	C	G	G	A	C	C	A	T	T	T
PT050	T	G	T	G	C	C	A	G	C	T	T	G	A	A	I	C	A	G	C	C	A	T	T	T	T

3 rd public TCRbeta CDR3																											
C	A	S	R	L	A	G	Q	E	T	Q	Y	F															
PT006	T	G	T	G	C	C	A	G	C	A	G	A	C	A	A	G	A	C	C	A	G	T	A	C	T	C	
PT014	T	G	T	G	C	C	A	G	A	C	T	A	G	C	G	G	G	A	C	C	A	G	T	A	C	T	C
PT050	T	G	C	G	C	C	A	G	A	C	T	A	G	C	G	G	G	A	C	C	A	G	T	A	C	T	C

Figure 13

MEAVVTTLPREGGVRPSRKMLLLLLLLGPGSGLGAVVSQHPS
WVICKSGTSVKIECRSLDFEQATTMFWYRQFPKQSLMLMATSN
EGSKATYEQGVEKDKFLINHASLTSLTSTLTVTSAHPEDSSFY**ICSA**
RKGLAGRETQYFGPGTRLLVLEDLKNVFPPEVAVFEPSEAE

SEQ ID NO: 16

CDR3 – bolded
Variable region (VDJ) – underlined

Figure 14

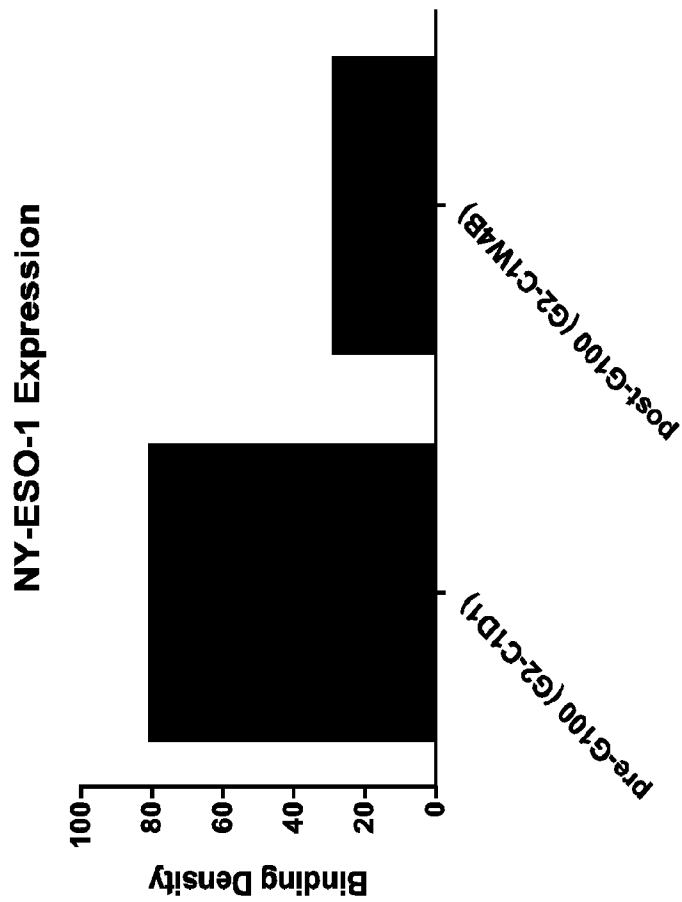


Figure 15A

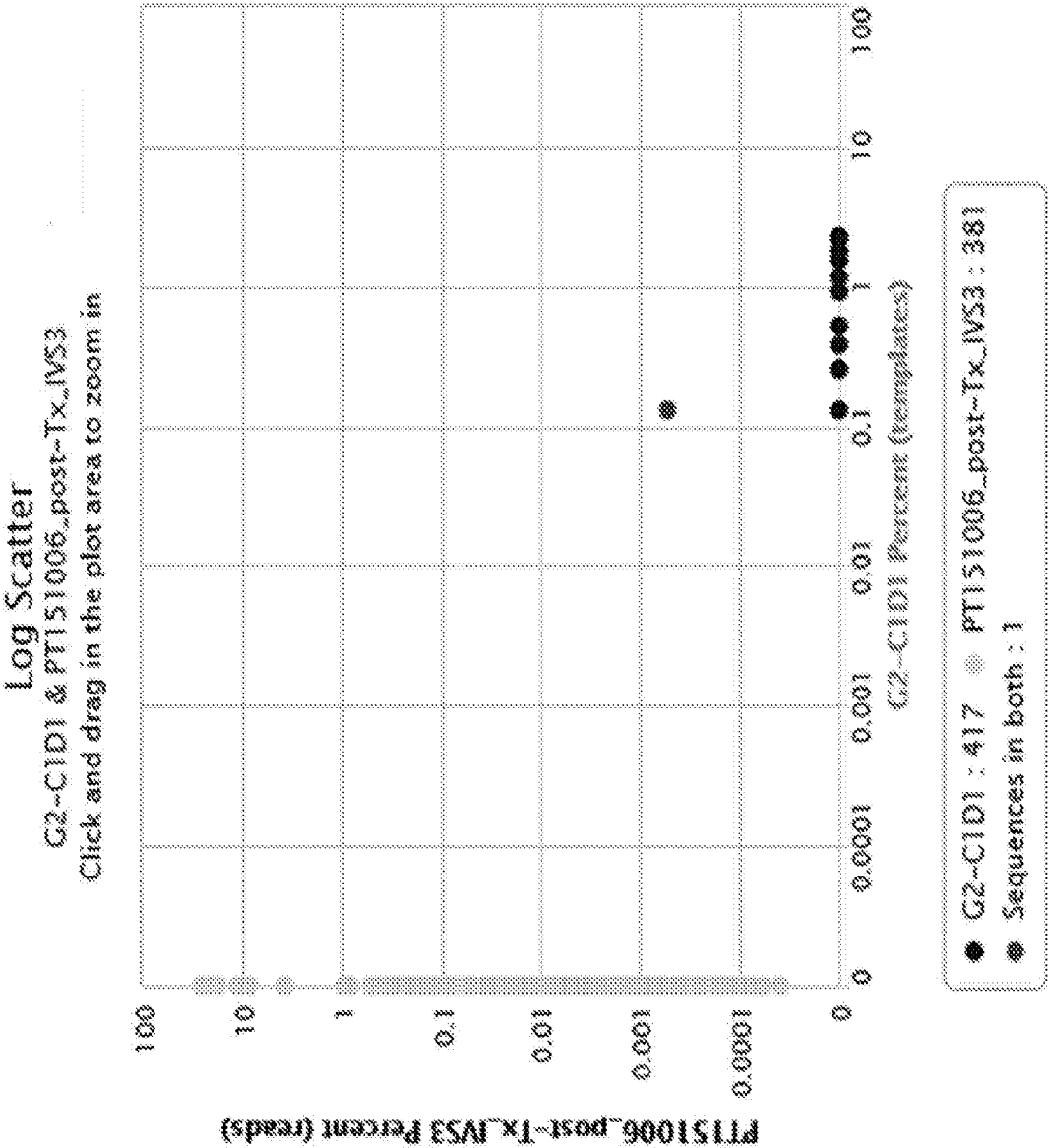


Figure 16A

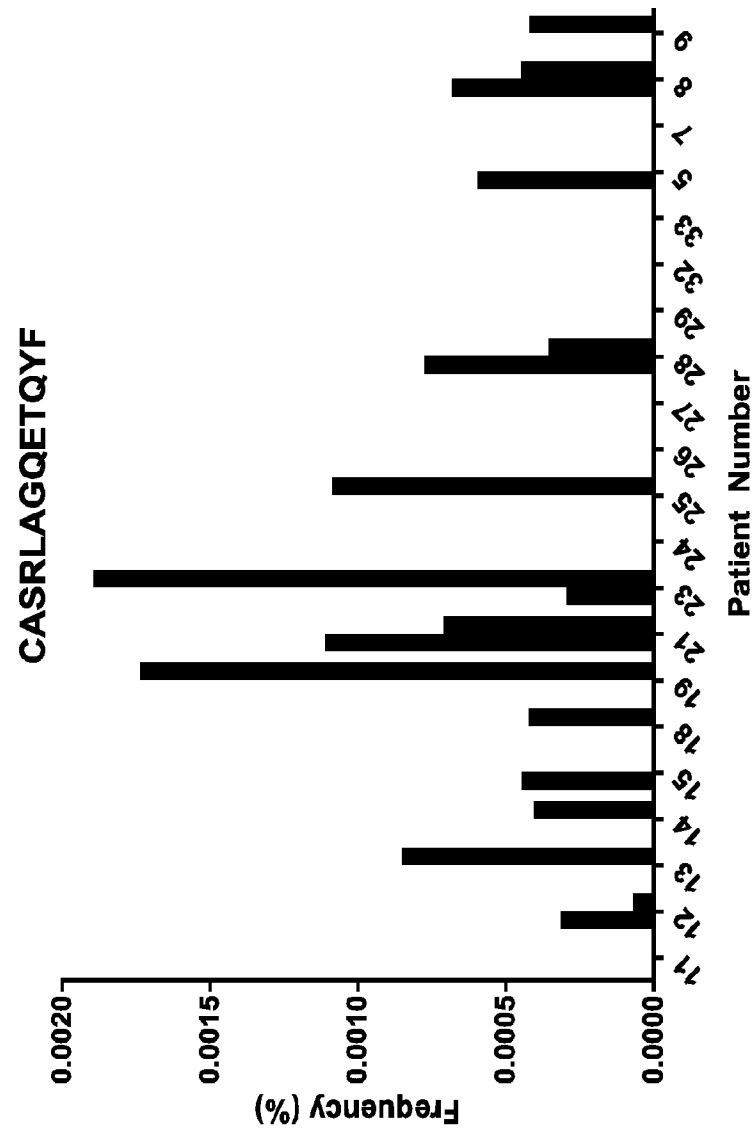


Figure 16B

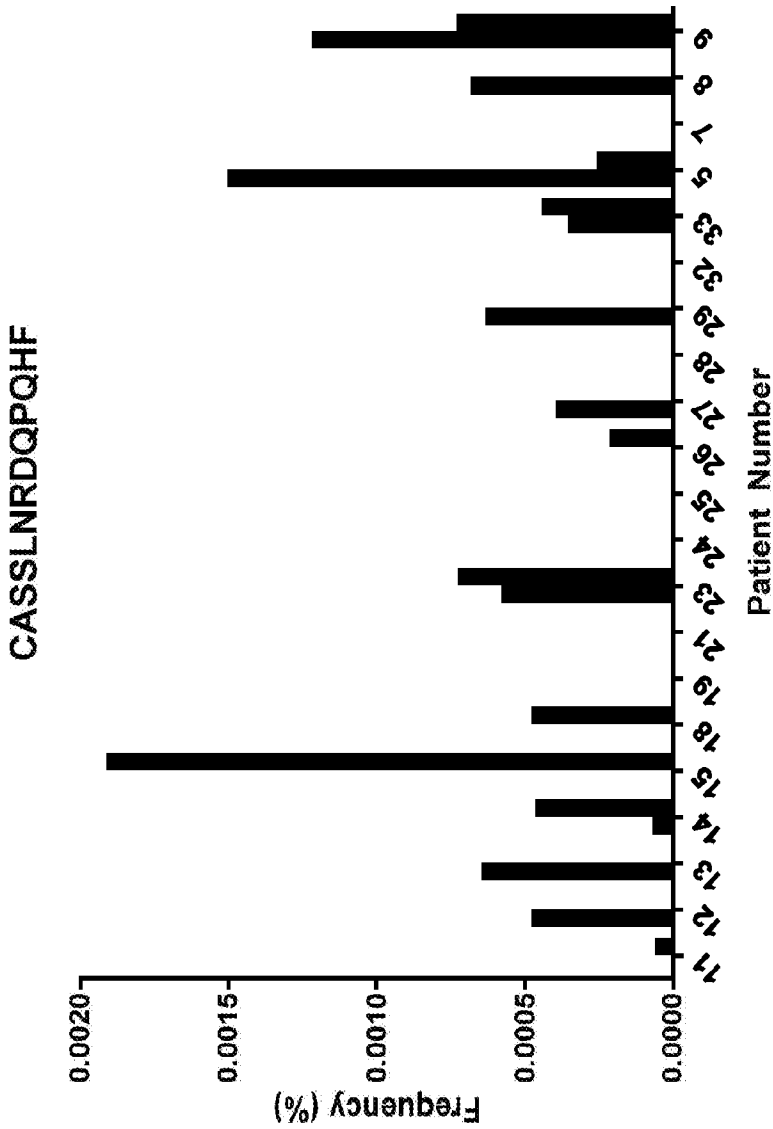


Figure 16C

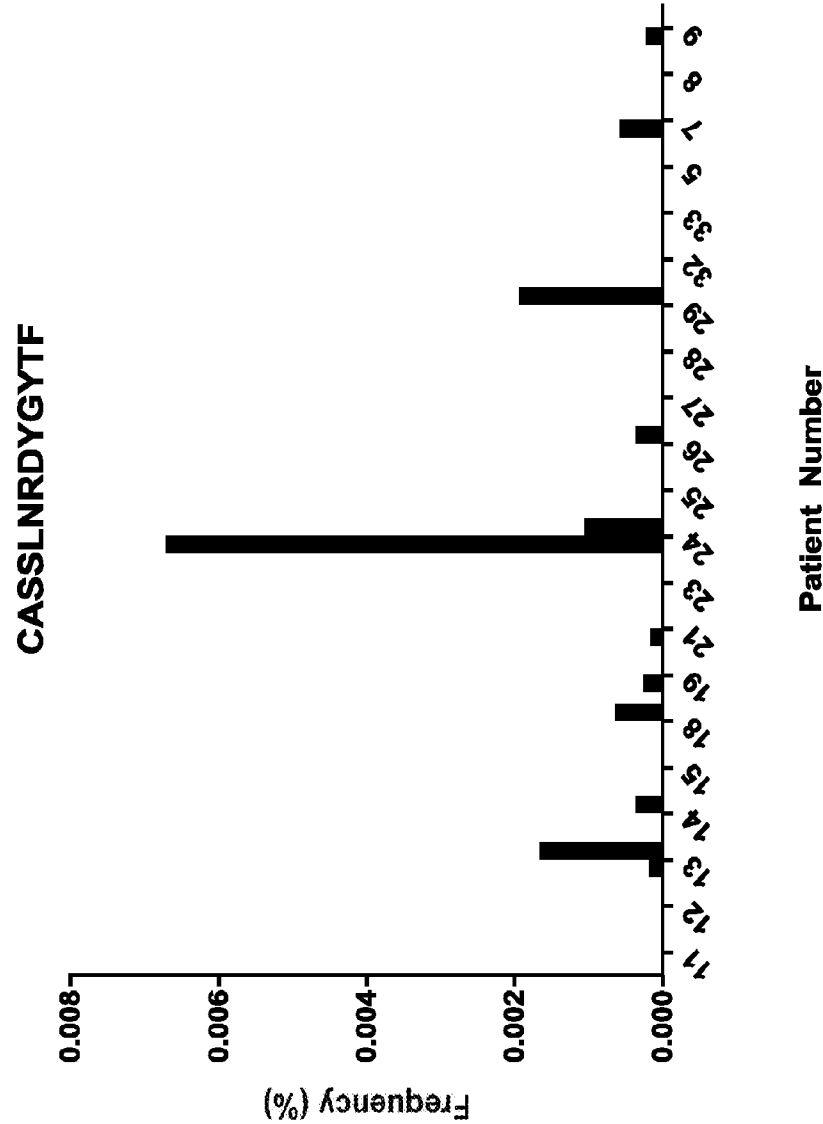


Figure 17

V Gene	J Gene	Total	PT151006_post-Tx_VSS frequency	PT151119_post-Tx frequency
TCRBV07-07	TCRBJ01-05	434351	0.461619302870072 %	0 %
TCRBV07-08	TCRBJ01-05	1929	0.036741265633300154 %	0.0008929722873319008 %
TCRBV07-09	TCRBJ01-05	140	0.002833197367979015 %	0 %
TCRBV07-09	TCRBJ01-05	97	0.0018896631529321922 %	0 %
TCRBV07-02	TCRBJ01-05	88	0.0017143336820415765 %	0 %
TCRBV07-03	TCRBJ01-05	78	0.00151952294771867 %	0 %
TCRBV07-04	TCRBJ01-05	18	0.0003506591417812316 %	0 %
TCRBV11-02	TCRBJ01-05	6	0.0001688632059374385 %	0 %

Figure 18

	C	A	S	S	L	N	R	D	Q	P	Q	H	F													
TCRBV07-07	T	G	C	T	A	G	C	A	G	G	A	C	C	C	C	A	G	C	A	T	T	T				
TCRBV11-02	T	G	T	G	C	C	A	G	C	T	T	A	A	A	C	A	G	G	A	C	C	A	T	T	T	
TCRBV07-03	T	G	T	G	C	C	A	G	C	T	T	A	A	A	C	A	G	G	A	C	C	C	A	T	T	T

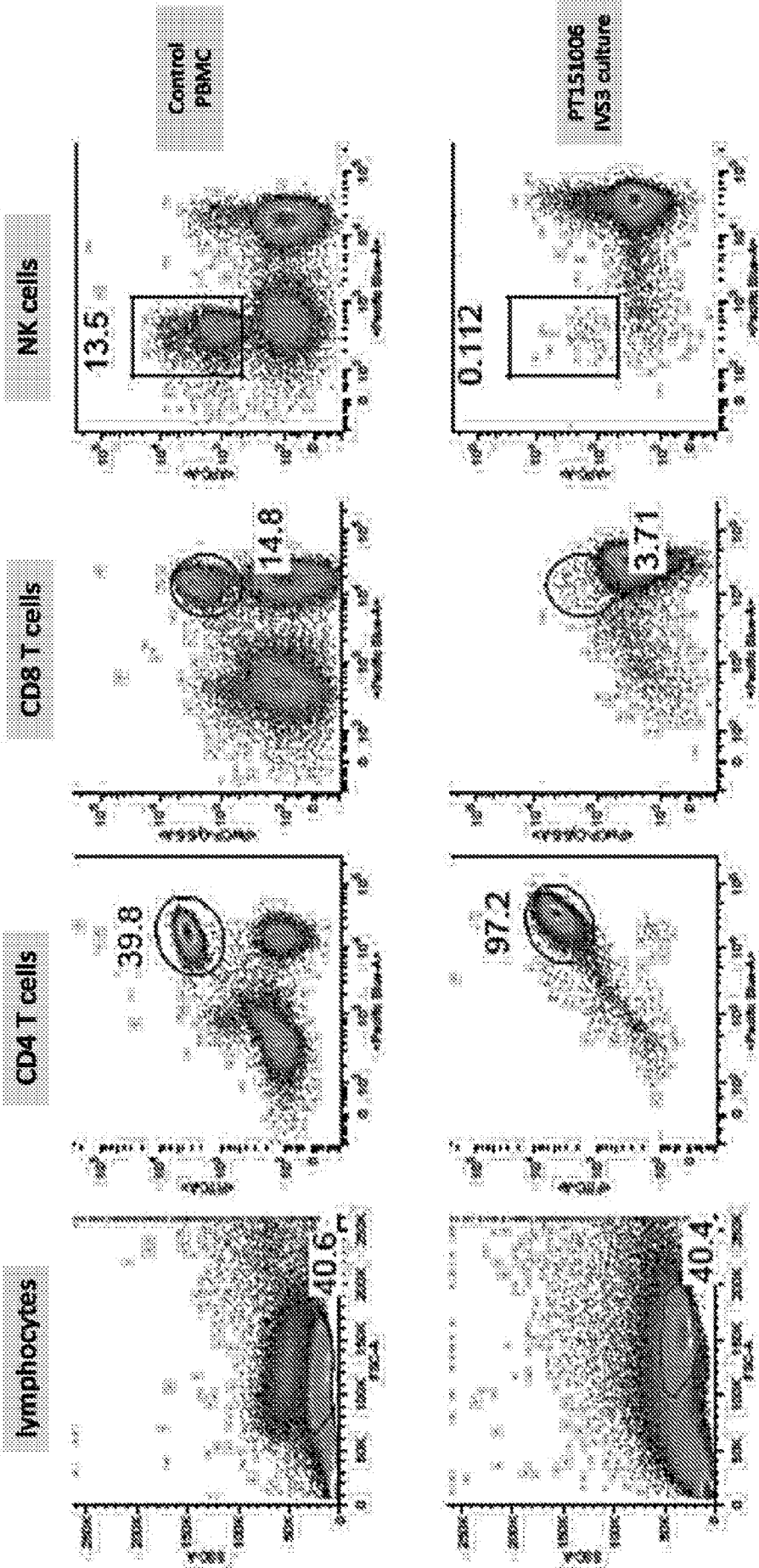
Figure 19

Patient ID	Nucleotide Sequence	Amino Acid SEQ ID NO:2	V Gene	J Gene
PT151006	AAGATCCAGCGCACACAGCAGCAGGAGGACTCCGCCGTGTATCTCTGTGCCAGCA GCTTGAACAGGGACTATGGCTACACCTTCGGTTCG (SEQ ID NO:5)	CASSLNRDYGYTF	TCRBV07-08	TCRBJ01-02
C131-001	AAGATCCGGTCCACAAAGCTGGAGGACTCAGCCATGTACTCTGTGCCAGCA GTCTAAACAGGGACTATGGCTACACCTTCGGTTCG (SEQ ID NO:17)	CASSLNRDYGYTF	TCRBV02-01	TCRBJ01-02
G2-C1W4B	ATTCTGGAGTCCGCCAGCACCAACAGACATCTATGTACCTCTGTGCCAGCAG TTTAAACCGGGATTATGGCTACACCTTCGGTTCG (SEQ ID NO:18)	CASSLNRDYGYTF	TCRBV28-01	TCRBJ01-02

Patient ID	Nucleotide Sequence	Amino Acid SEQ ID NO:3	V Gene	J Gene
PT151006	ACGATTACGCGCACAGAGCAGCGGAGCTCAGCATGTATCGCTGTGCTAGCA GCTTGAACAGGGAGCAGGCCCGCATTTTGGTGAT (SEQ ID NO:6)	CASSLNRDQPQHF	TCRBV07-07	TCRBJ01-05
C131-013	AATGTGAACGCCTTGGAGCTGGAGGACTCGGCCCTGTATCTCTGTGCCAGCAG CTTGAACAGGGATCAGCCCGCATTTTGGTGAT (SEQ ID NO:19)	CASSLNRDQPQHF	TCRBV05-08	TCRBJ01-05
C131-013	AACATGAGCTCCTTGGAGCTGGGGAGCTCAGCCCTGTACTCTGTGCCAGCAG CTTGAACAGGGATCAGCCCGCATTTTGGTGAT (SEQ ID NO:20)	CASSLNRDQPQHF	TCRBV13-01	TCRBJ01-05
C131-013	AATGTGAACGCCTTGTGCTGGGGAGCTCGGCCCTGTATCTCTGTGCCAGCAG CTTGAACAGGGATCAGCCCGCATTTTGGTGAT (SEQ ID NO:21)	CASSLNRDQPQHF	TCRBV05-05	TCRBJ01-05

Patient ID	Nucleotide Sequence	Amino Acid SEQ ID NO:4	V Gene	J Gene
PT151006	ATTCTGGAGTCCGCCAGCACCAACAGACATCTATGTACCTCTGTGCCAGCAG ACTAGCGGACAAAGAGACCCAGTACTTCGGGCCA (SEQ ID NO:7)	CASRLAGQETQYF	TCRBV28-01	TCRBJ02-05
C131-001	AGGCTGGAGTTGGCTGCTCCCTCCAGACATCTGTACTTCTGTGCCAGCAG ACTTGGGGCCAAAGAGACCCAGTACTTCGGGCCA (SEQ ID NO:22)	CASRLAGQETQYF	TCRBV06-06	TCRBJ02-05
G2-C1W4B	ACCCTGGAGTCTGCCAGGCCCTCACATACCTCTCAGTACCTCTGTGCCAGCAG CTAGCGGGACAGGAGACCCAGTACTTCGGGCCA (SEQ ID NO:23)	CASRLAGQETQYF	TCRBV25-01	TCRBJ02-05

Figure 20



Staining panel: CD3-PB, CD4-FITC, CD8-PerCP, CD56-APC

Figure 21

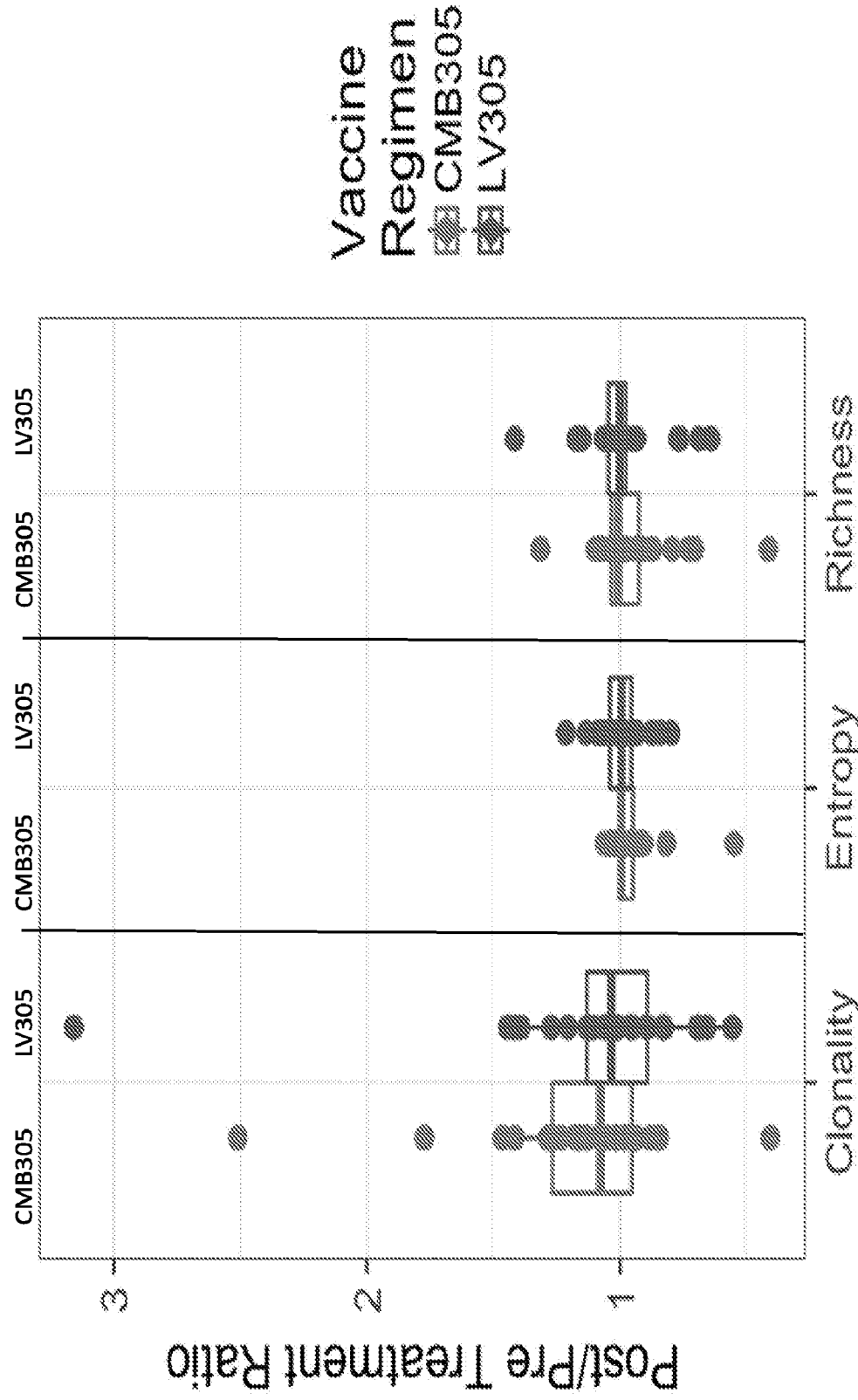


Figure 22

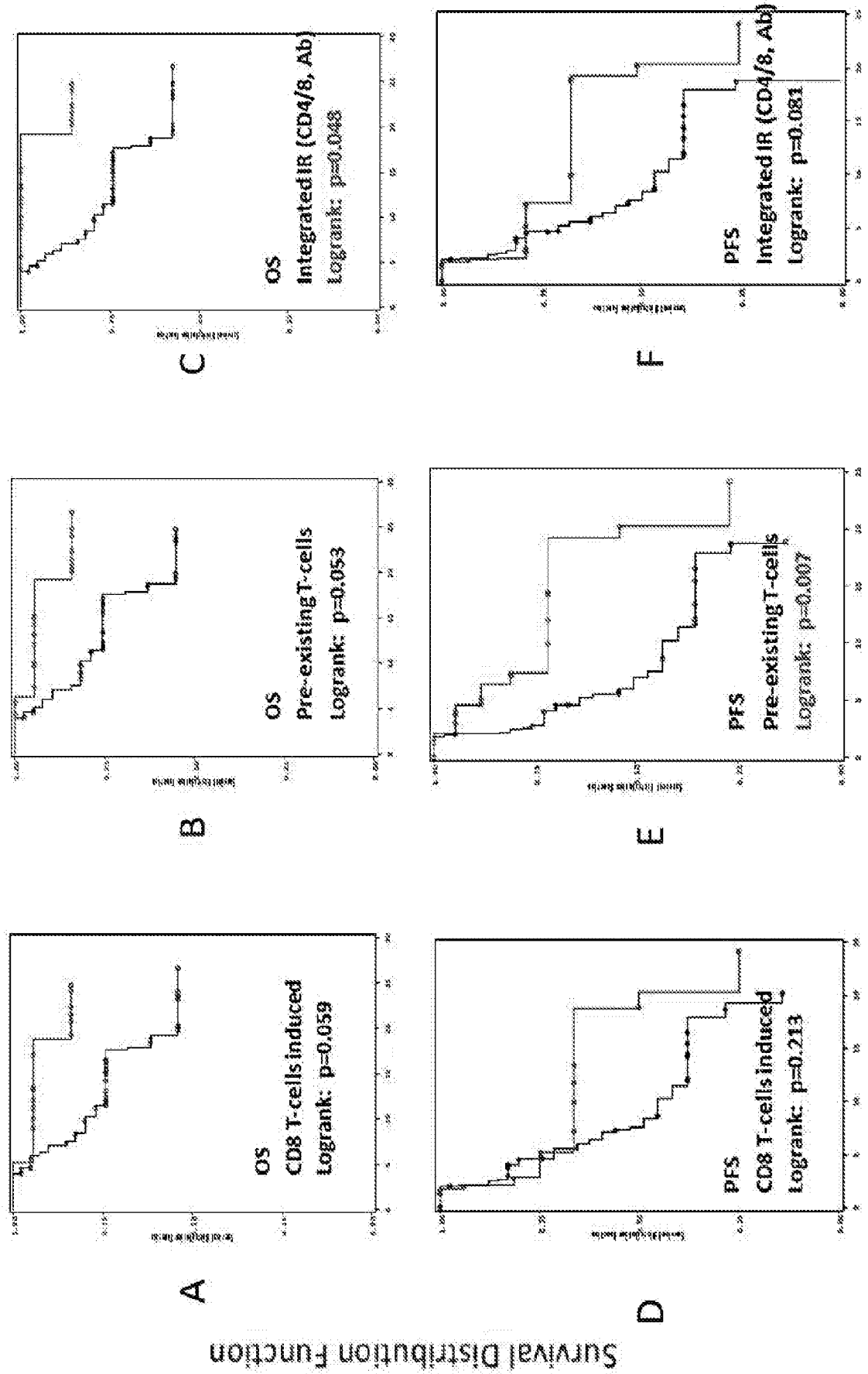
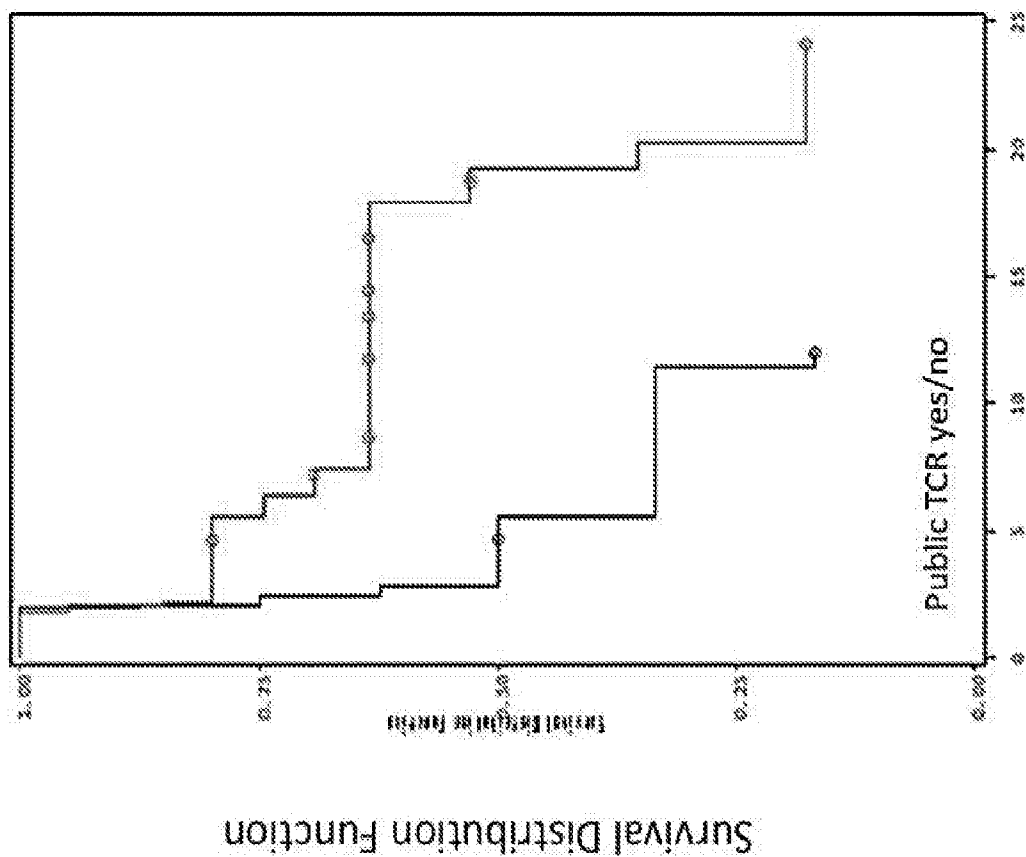


Figure 23

A



B

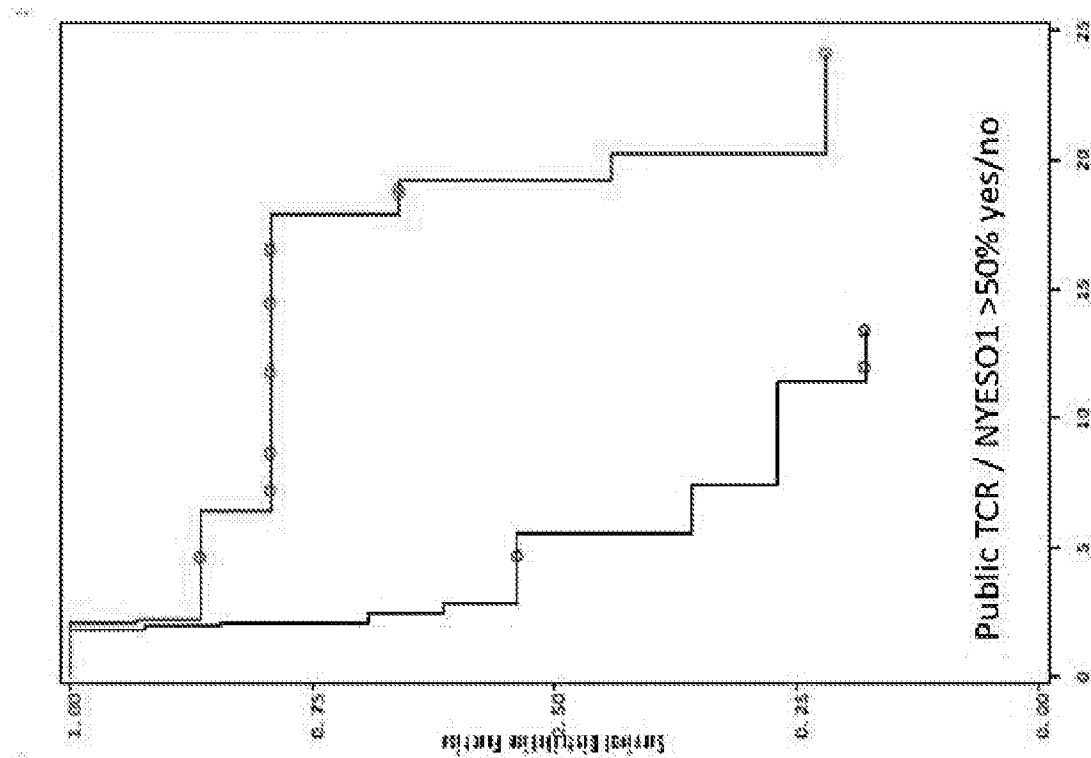


Figure 24

LV305 & CMB305 pts (n=62)
 *23+20 sarcomas, 3+9 ovarian, 4+0 melanomas, and 2+1 NSCLC

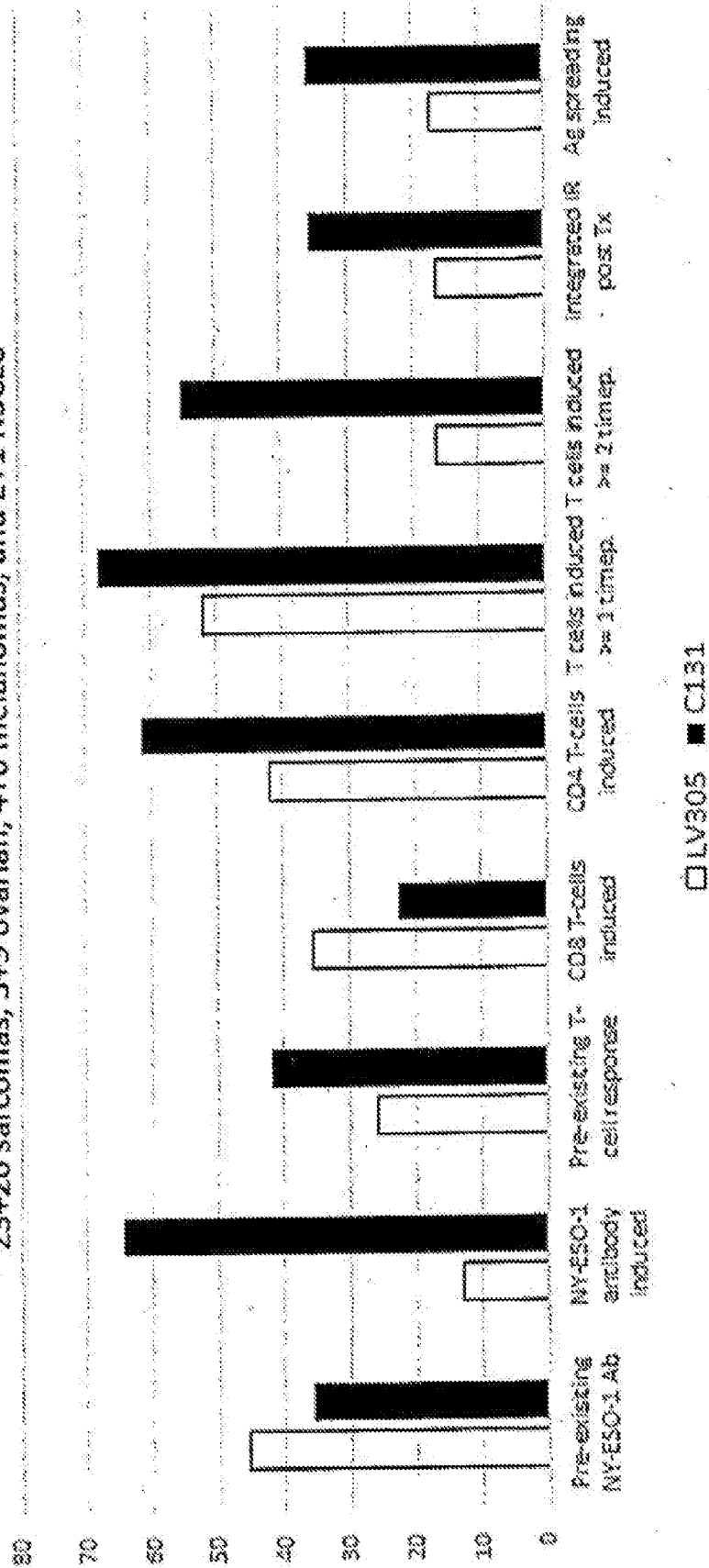
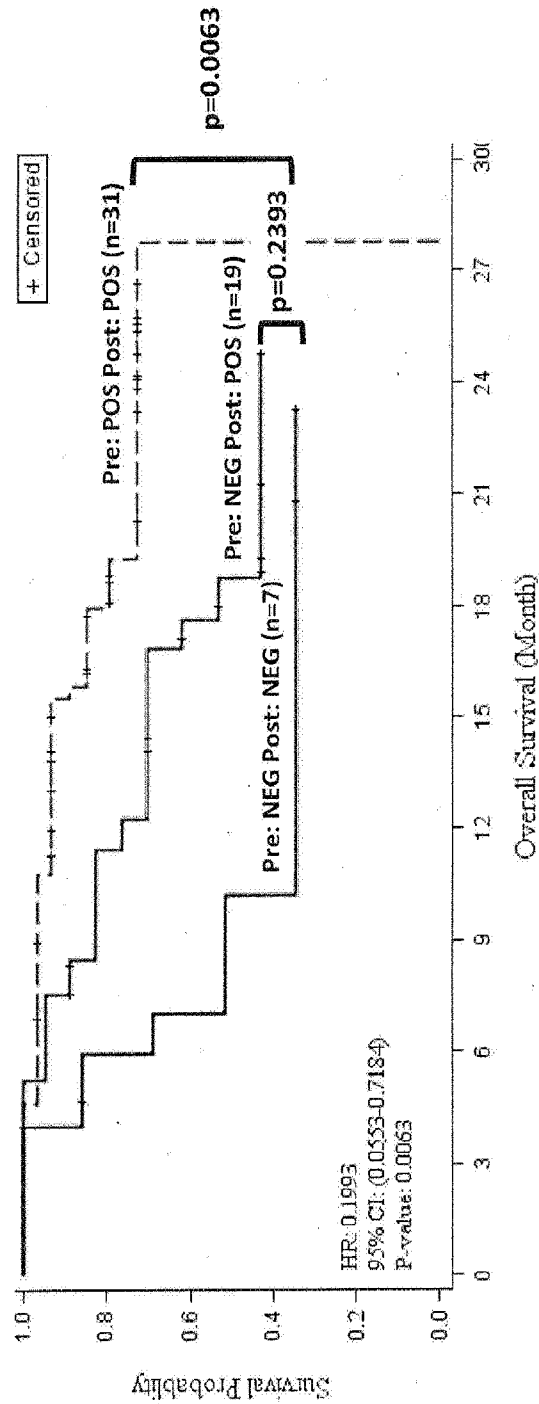


Figure 25

<u>Baseline Immunity</u>		<u>Induced Immunity</u>		N=64 Patients
Negative N=26 (26%)	Positive N=38 (59%)	Negative N=14 (22%)	Positive N=50 (78%)	
7 (11%)	7 (11%)	19 (30%)	31 (48%)	

Figure 26



All patients treated with LV305 or CMB305 (ID/IM and SQ dosing) and who had biomarker samples were analyzed (n=64) 50/64 pts (78%) of patients had an induced anti-NY-ESO-1 immune response on LV305 or CMB305 therapy (assessed by one of the antibody or T cell assays)

Figure 27

A
All Patients (N=64)
Overall Survival

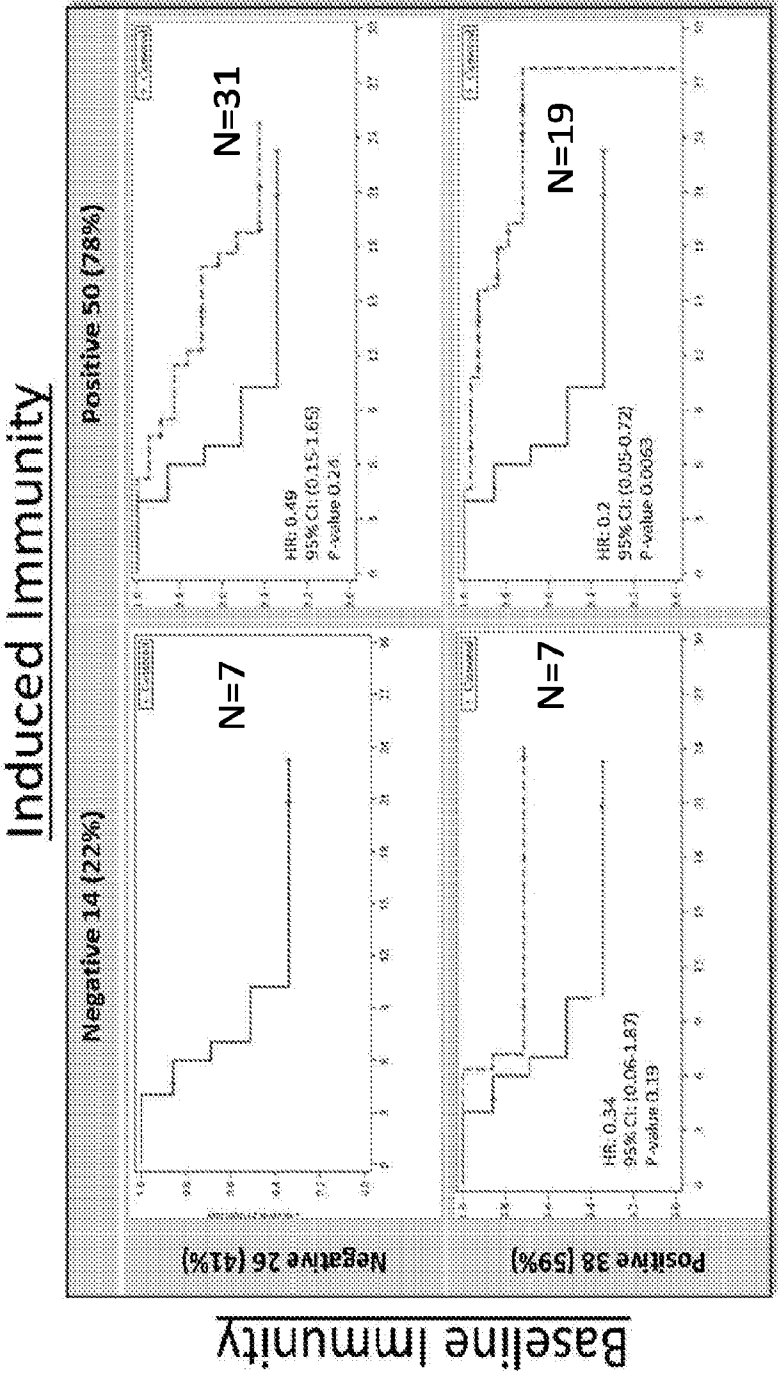


Figure 27

B
All Patients (N=64)
Progression Free Survival
Induced Immunity

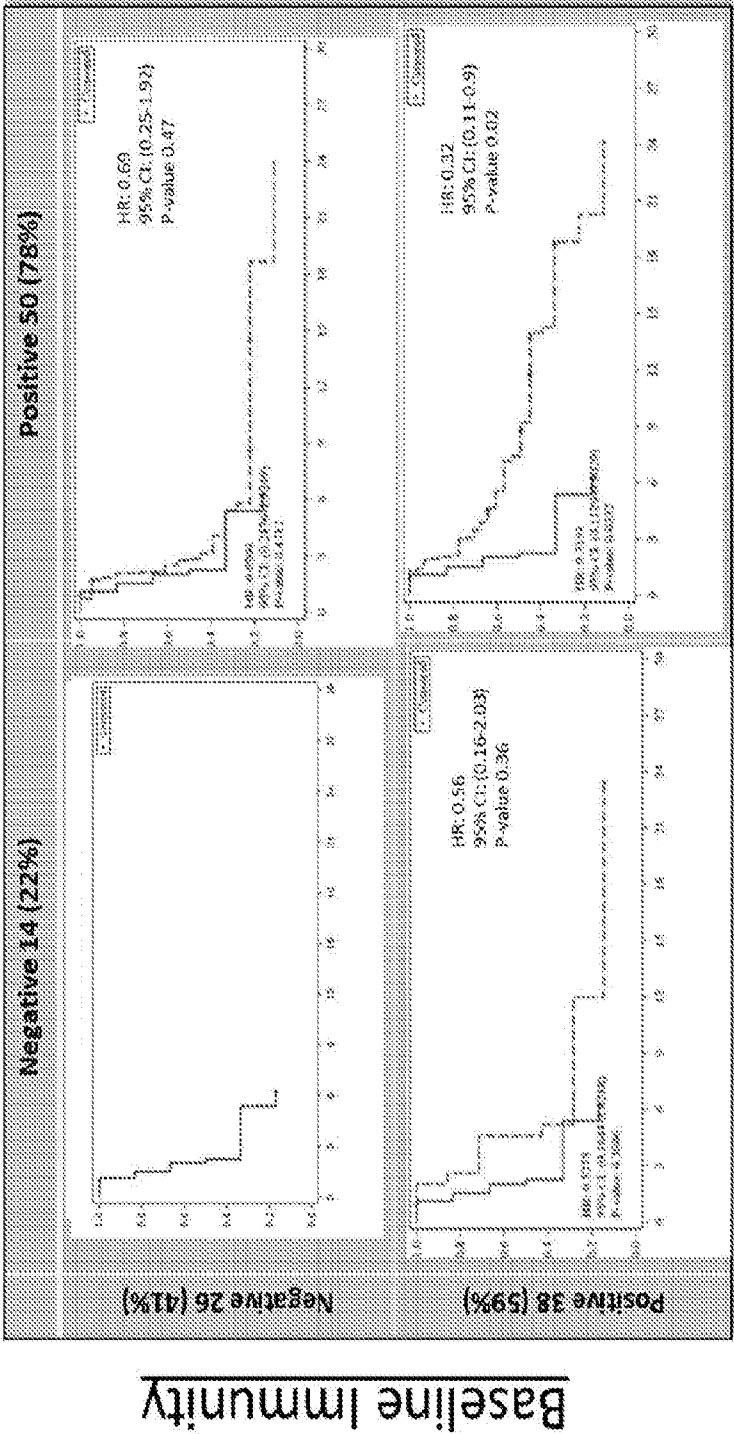


Figure 27

C
Soft Tissue Sarcoma Patients (N=45)
Overall Survival

Induced Immunity

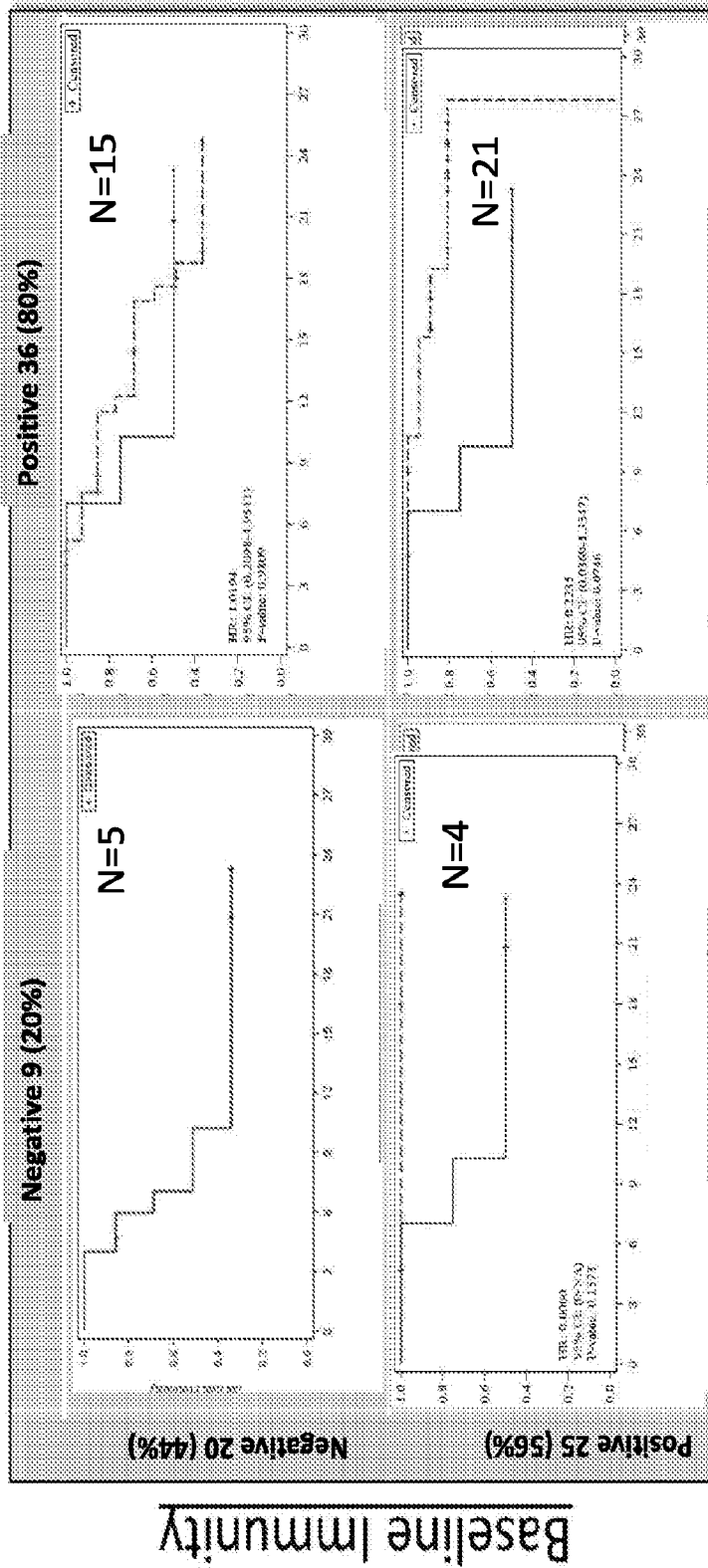


Figure 27

D
Soft Tissue Sarcoma Patients (N=45)
Progression Free Survival
Induced Immunity

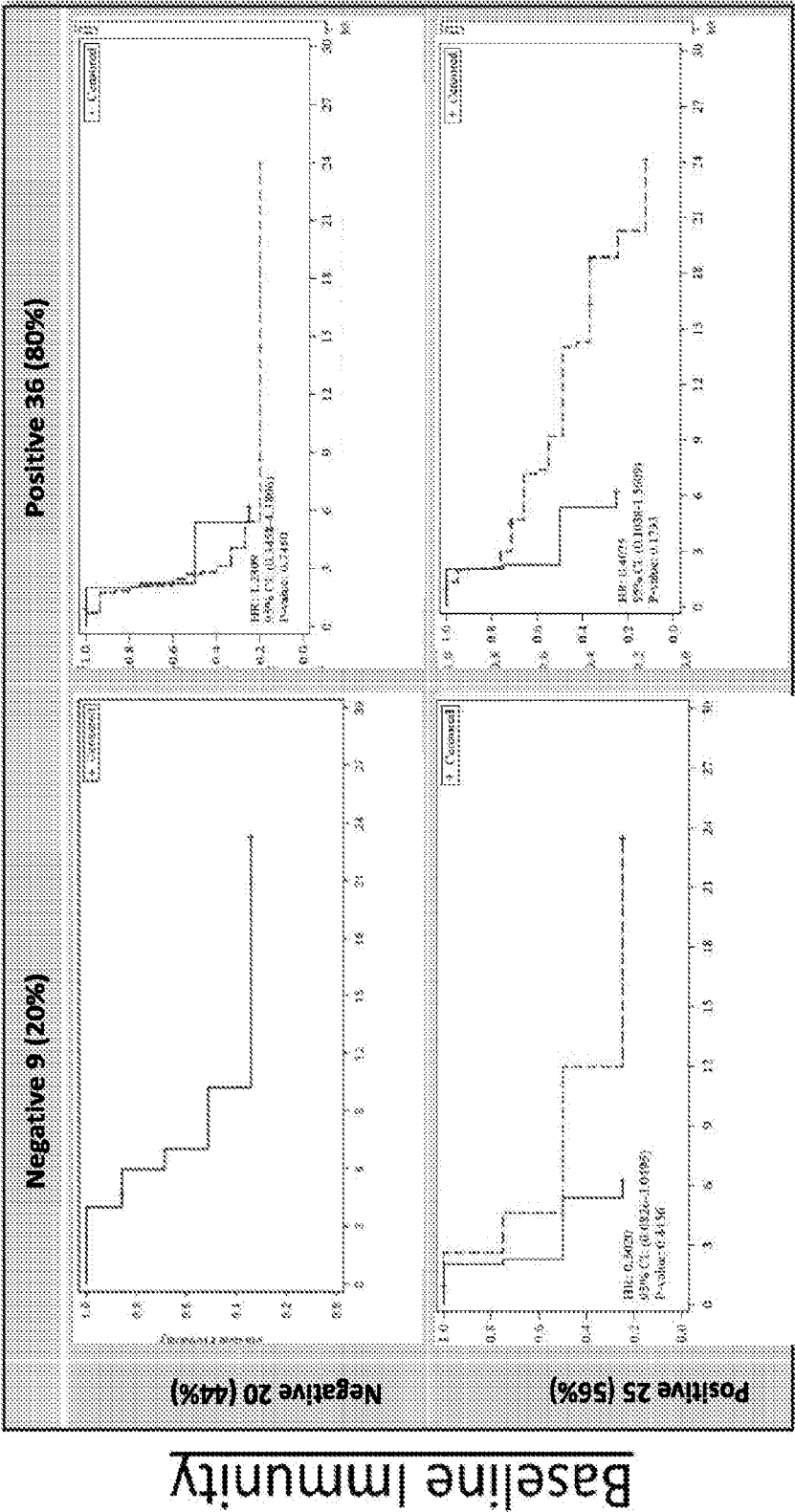


Figure 28

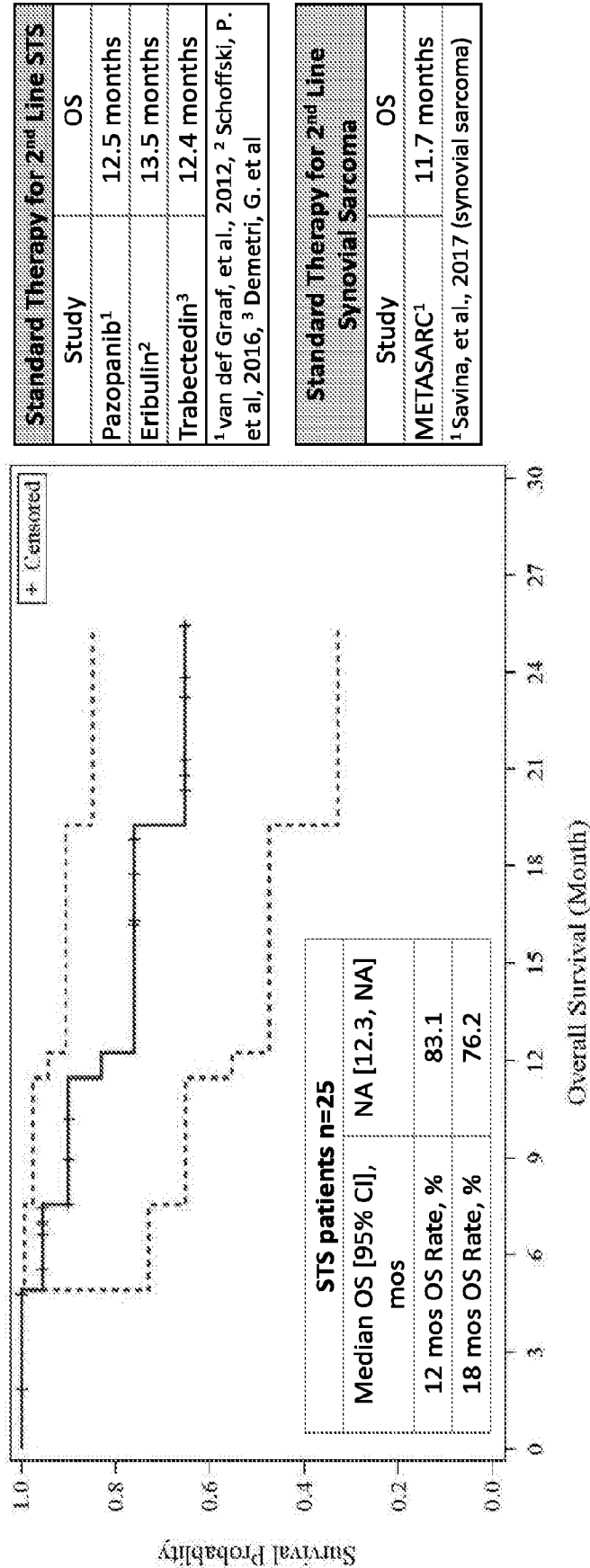
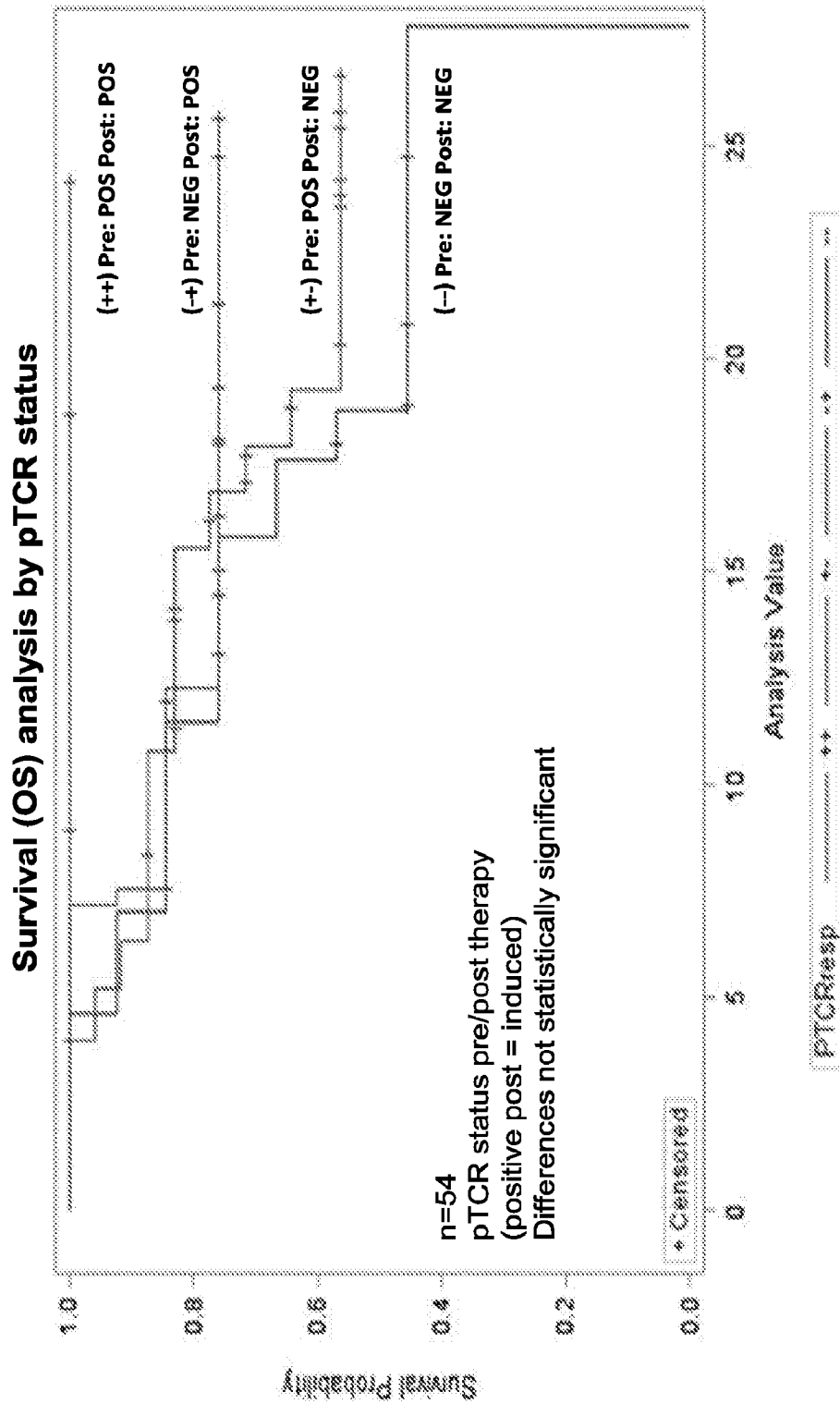
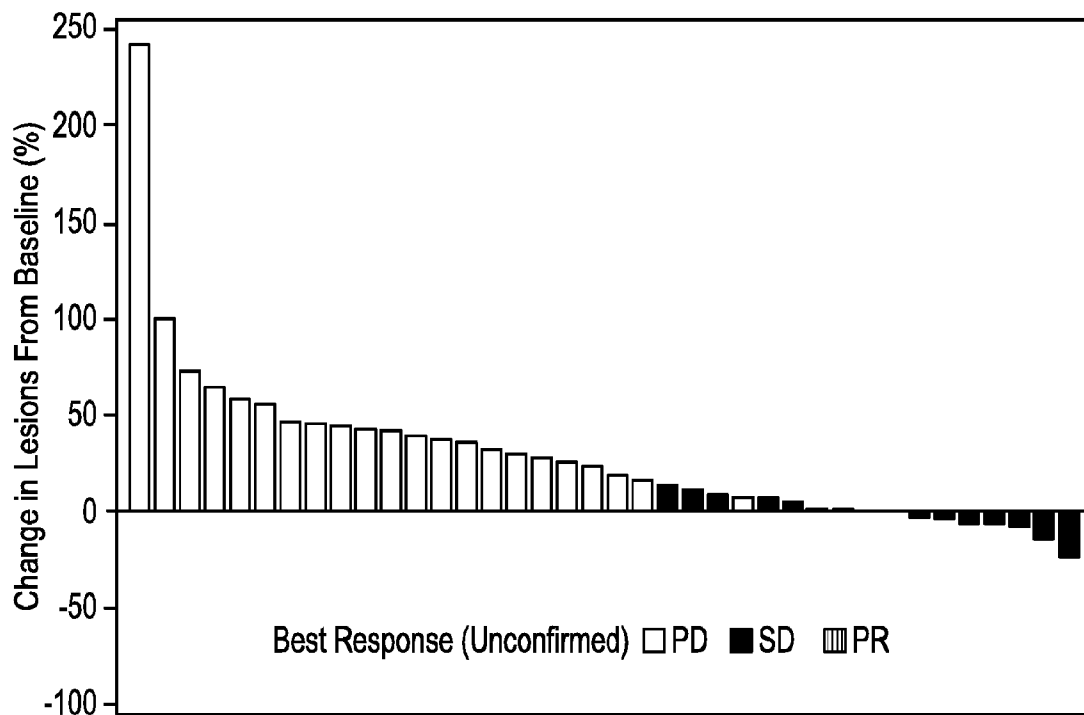


Figure 29



Atezolizumab



CMB305 + Atezolizumab

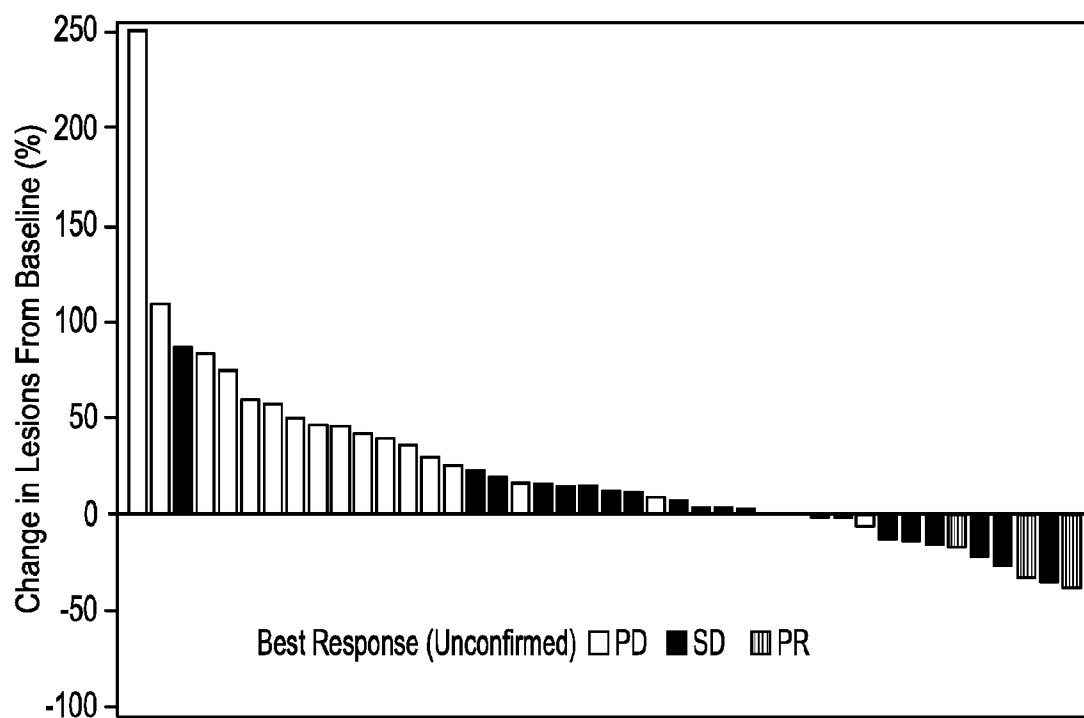


Figure 30

Figure 31

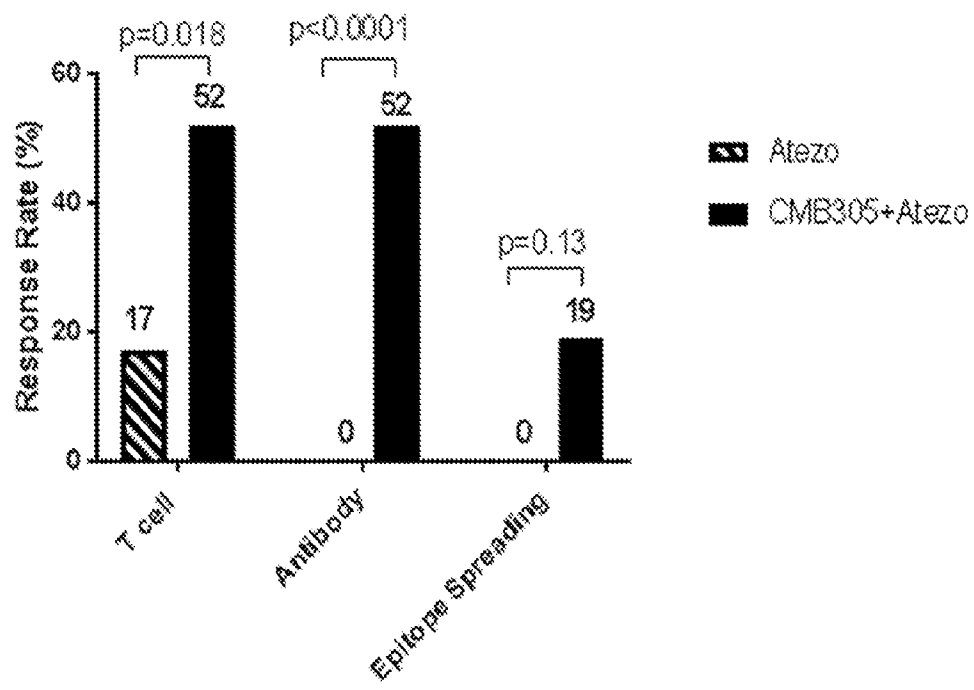


Figure 32A

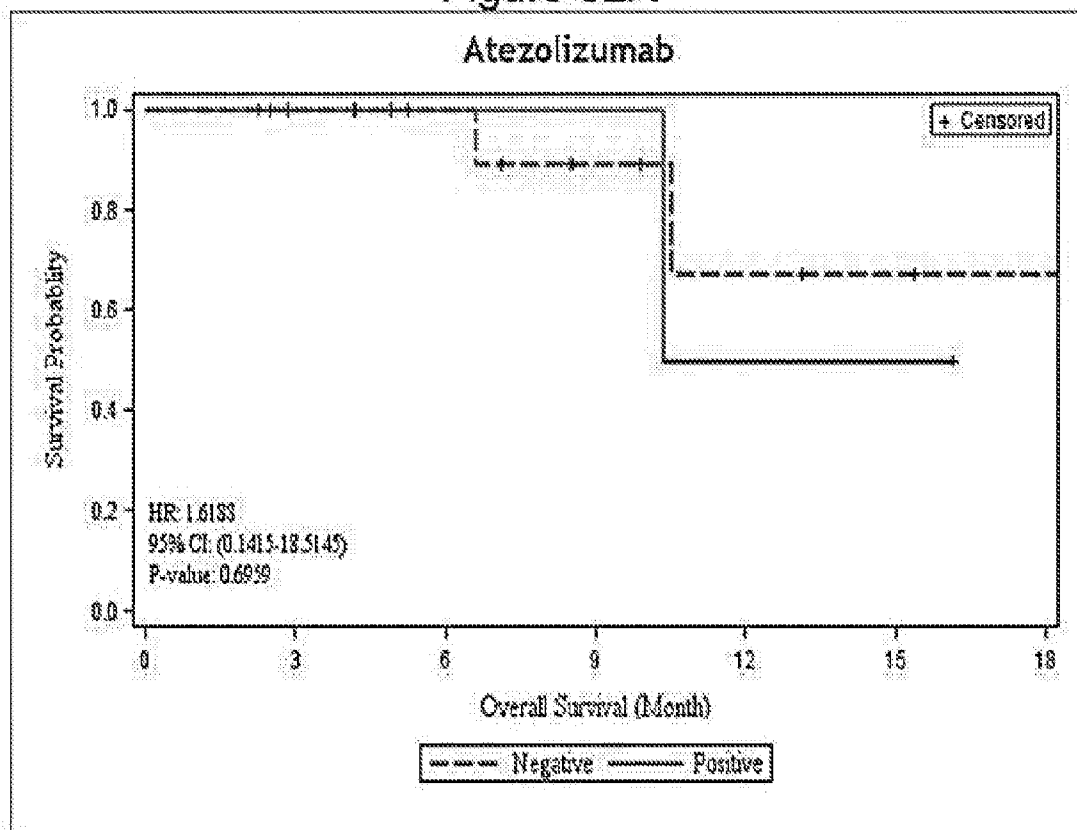


Figure 32B

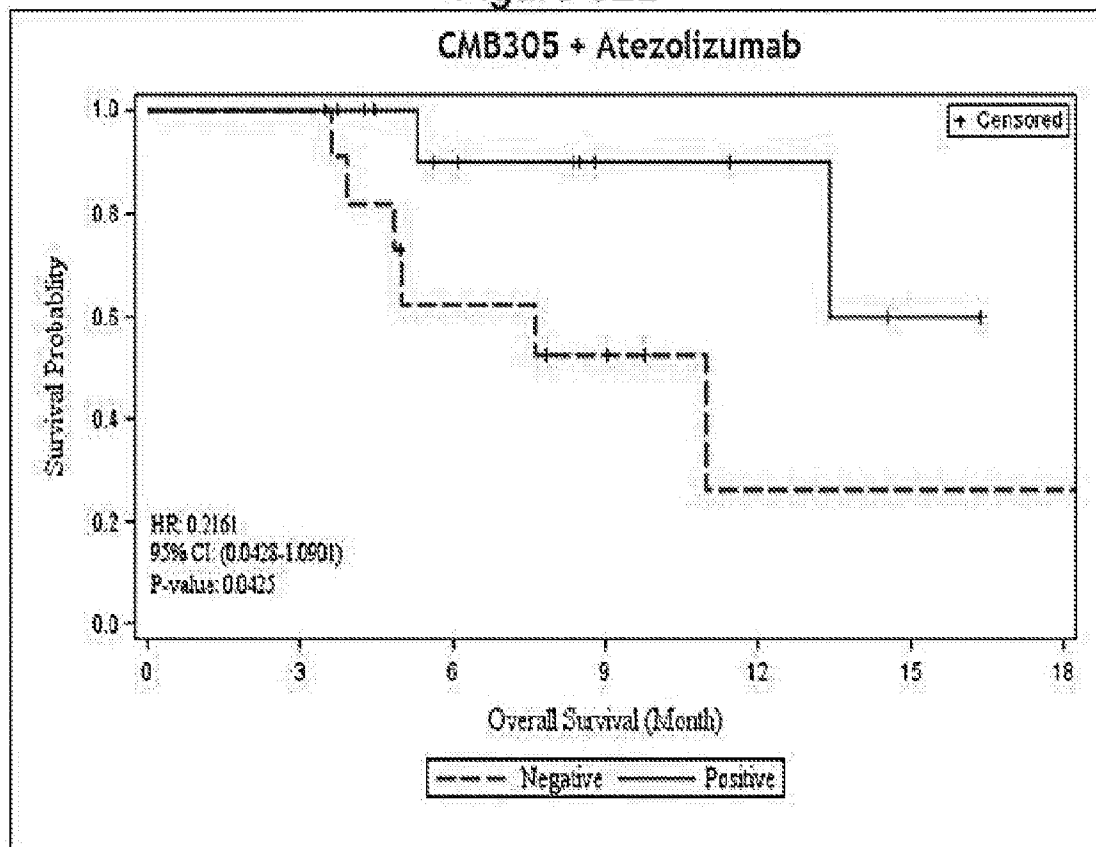


Figure 33A

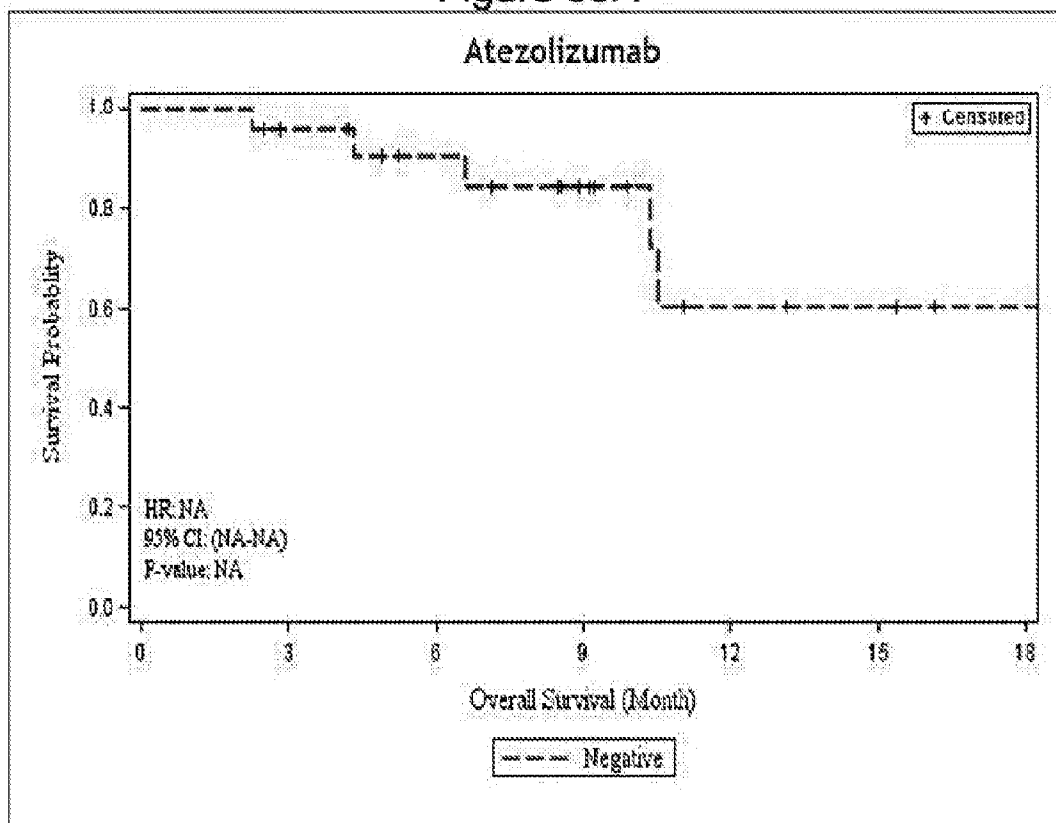


Figure 33B

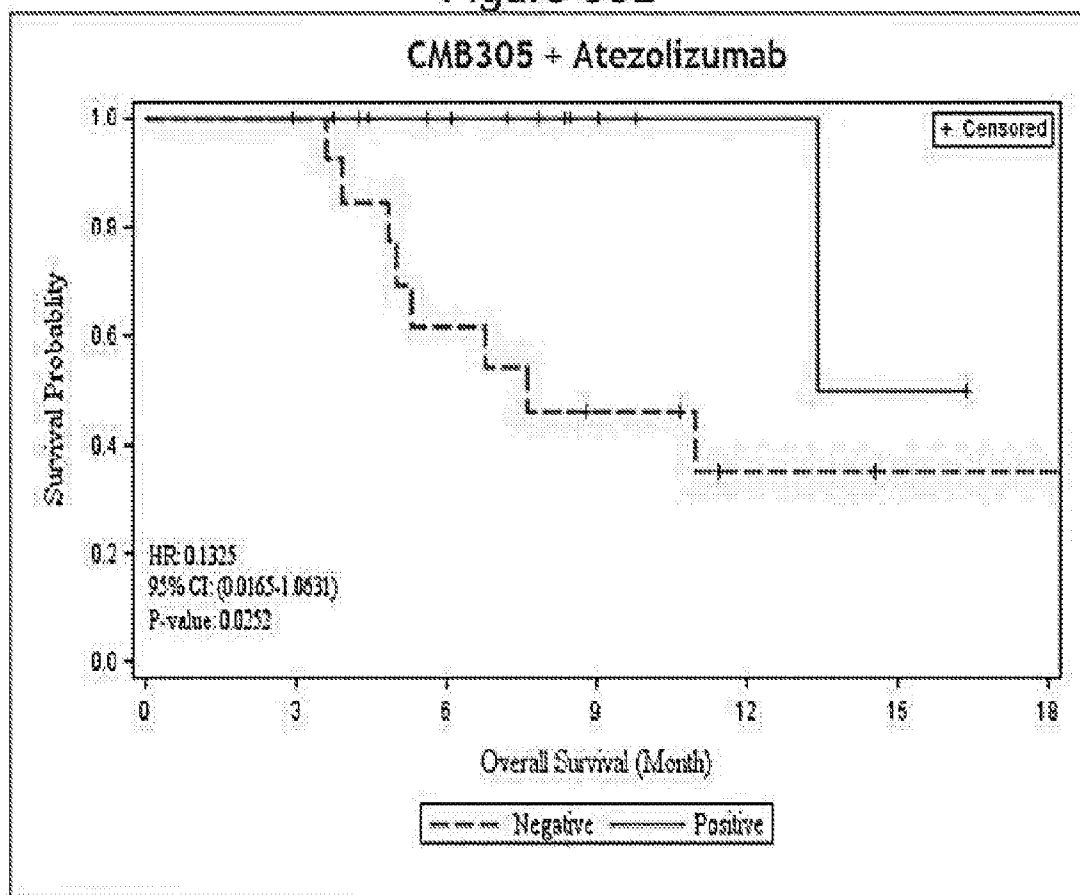


Figure 34

