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(54) Title: COMPOSITION OF NY-ESO-1-SPECIFIC T CELL RECEPTORS RESTRICTED ON MULTIPLE MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES

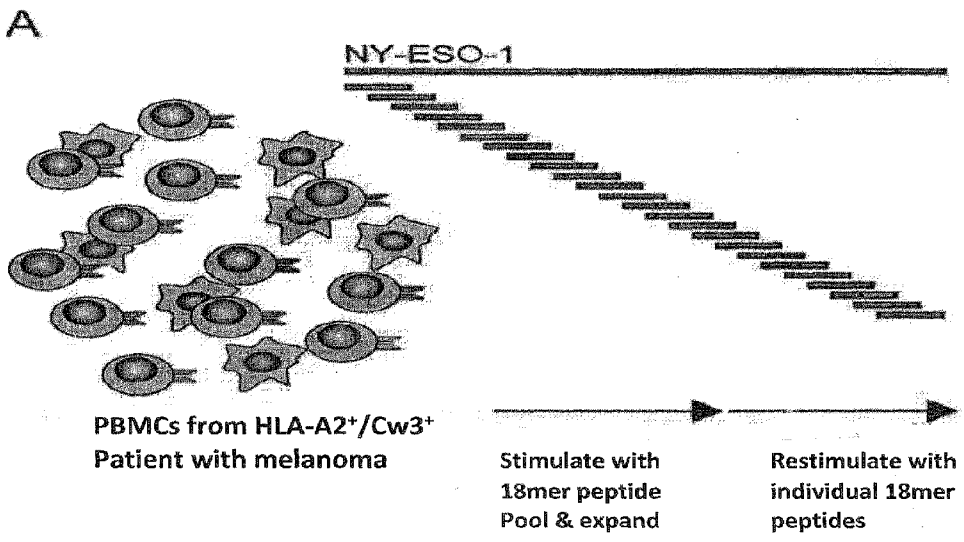

FIG. 1A

(57) Abstract: Tumor-specific T cell receptor (TCR) gene transfer enables specific and potent immune targeting of tumor antigens. The canonical cancer-testis antigen, NY-ESO-1, is not expressed in normal tissues but is aberrantly expressed across a broad array of cancer types. It has also been targeted with A2-restricted TCR gene therapy without adverse events or notable side effects. To enable the targeting of NY-ESO-1 in a broader array of HLA haplotypes, we isolated TCRs specific for NY-ESO-1 epitopes presented by four MHC molecules: HLA-A2, -B07, -B18, and -C03. Using these TCRs, we have developed an approach to extend TCR gene therapies targeting NY-ESO-1 to patient populations beyond those expressing HLA-A2.

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COMPOSITION OF NY-ESO-1-SPECIFIC T CELL RECEPTORS RESTRICTED ON MULTIPLE MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. Section 119(e) of co-pending and commonly-assigned U.S. Provisional Patent Application Serial No 62/727,485, filed on September 5, 2018, and entitled "COMPOSITION OF NY-ESO-1-SPECIFIC T CELL RECEPTORS RESTRICTED ON MULTIPLE MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES" which application is incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with government support under Grant Numbers CA132681 and CA197633, awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 28, 2019, is named 30435_364-WO-U1_SL.txt and is 101,375 bytes in size.

TECHNICAL FIELD

The present invention relates to methods and materials useful in αβ T cell receptor gene therapy.

BACKGROUND OF THE INVENTION

The αβ T cell receptor (TCR) determines the unique specificity of each naïve T cell. Upon assembly with CD3 signaling proteins on the T cell surface, the TCR surveils peptide ligands presented by major histocompatibility complex (MHC) molecules on the surface of nucleated cells. The specificity of the TCR for a peptide-MHC complex is determined by both the presenting MHC molecule and the presented peptide. The MHC locus (also known as the human leukocyte antigen (HLA) locus in humans) is the most multi-allelic locus in the human genome, comprising >18,000 MHC class I and II alleles that vary widely in frequency across ethnic subgroups (1, 2). Ligands presented by MHC class I molecules are derived primarily from proteasomal cleavage of endogenously expressed antigens. Infected and cancerous cells present peptides that are recognized by CD8+ T cells as foreign or aberrant, resulting in T cell-mediated killing of the presenting cell.

T cells can be engineered to kill tumor cells through the transfer of tumor-reactive αβ TCR genes (3). Key to this approach is that the patient expresses the MHC allele on which the therapeutic TCR is restricted and that the targeted peptide is derived from a tumor-associated or tumor-specific antigen. Private (patient-specific) neoantigens resulting from tumor-specific mutations are a potential source of such targets (4). However, implementation of personalized TCR gene therapy is complicated by the need to identify mutations through sequencing, to isolate mutation-reactive, patient-specific TCRs, and to genetically modify patient T cells on-demand. This is still more challenging for tumors that cannot be accessed for sequencing and for low mutational burden tumors with few or no neoantigens (5). Particularly for these last tumor types, targeting public (non-patient specific), tumor-restricted antigens with off-the-shelf TCRs remains an attractive option.

The first public antigen targeted with TCR gene therapy in the clinic was melanocyte antigen MART1/Melan-A, yielding objective responses in 2/15 patients with metastatic melanoma (6). Use of a higher affinity MART1-reactive TCR (F5) increased the response rate to 30% but also produced a variety of side effects including vitiligo, uveitis, and transient hearing loss due to MART1 expression on healthy melanocytes in the skin, eye, and middle ear (7). T cell therapies targeting other public antigens have similarly resulted in morbidity or other serious adverse events due to on-target/off-tumor reactivity. For example, targeting carcinoembryonic antigen produces severe colitis in patients with metastatic colorectal cancer due to reactivity with normal colorectal tissue (8). More seriously, T cell therapies targeted at ERBB2 or MAGE-A3 each resulted in deaths due to unappreciated expression of the target antigen (or similar variant) on vital organs (9, 10). Thus, these studies underscore the importance of identifying stringently tumor-specific public antigens (11), particularly when well-expressed, high-affinity targeting receptors necessary for therapeutic success are employed (7, 12).

NY-ESO-1 – the product of the CTAG1B gene – is an attractive target for off-the-shelf TCR gene therapy. As the prototypical cancer-testis antigen, NY-ESO-1 is not expressed in normal, non-germline tissue, but it is aberrantly expressed in many tumors (13). The frequency of aberrant expression ranges from 10-50% among solid tumors, 25-50% of melanomas, and up to 80% of synovial sarcomas (13-18), with increased expression observed in higher-grade metastatic tumor tissue (14, 15, 19). Moreover, NY-ESO-1 is highly immunogenic, precipitating spontaneous and vaccine-induced T cell immune responses against multiple epitopes presented by various MHC alleles (20-23). As a result, the epitope NY-ESO-1157-165 (SLLMWITQC, (SEQ ID NO. 36)) presented by HLA-A*02:01 has been targeted with cognate 1G4 TCR in gene therapy trials, yielding an objective response rate of 55% and 61% of patients with metastatic melanoma and synovial sarcoma, respectively, and producing no adverse events related

to targeting (24, 25). Targeting this same A2-restricted epitope with lentiviral-mediated TCR gene therapy in patients with multiple myeloma similarly resulted in 70% complete or near-complete responses without significant safety concerns (26). Unfortunately however, the majority of patients who respond to therapy relapse within months, and loss of heterozygosity at the MHCI locus has been reported as a mechanism by which tumors escape adoptive T cell therapy targeting HLA-A*02:01/NY-ESO-1157-165 (27). Thus, NY-ESO-1 is a tumor-specific, immunogenic public antigen that is expressed across an array of tumor types, that is safe to target in the clinic, but that is susceptible to escape when targeted through a single HLA subtype.

For the reasons noted above, there is a need in the art for additional methods and materials useful for NY-ESO-1 TCR gene therapy.

SUMMARY OF THE INVENTION

As noted above, T lymphocytes can be engineered to express tumor-specific T cell receptor (TCR) genes and thereby kill cancer cells. This approach – termed TCR gene therapy – is effective but can cause serious adverse events if the target is also expressed in healthy, non-cancerous tissue. NY-ESO-1 is a tumor-specific antigen that has been targeted successfully and safely through TCR gene therapies for melanoma, synovial sarcoma, and myeloma. However, trials to date have focused exclusively on a single NY-ESO-1-derived epitope presented on HLA-A*02:01, limiting application to patients expressing that allele. As disclosed below, we have developed new TCRs that collectively recognize multiple NY-ESO-1-derived epitopes presented by multiple MHC alleles. We thereby provide a general approach for expanding targeted immunotherapies to more diverse MHC haplotypes.

Embodiments of the present invention include methods and materials for making and using modified CD 8^+ T cells comprising nucleic acids encoding certain αβ T cell

receptor polypeptides. Embodiments of the invention include, for example, a polynucleotide disposed in a vector, wherein the polynucleotide encodes a Vα T cell receptor polypeptide and/or a Vβ T cell receptor polypeptide. In typical embodiments, when a Vα/Vβ T cell receptor comprising the Vα T cell receptor polypeptide and/or the Vβ T cell receptor polypeptide is expressed in a CD 8^+ T cell, the heterologous Vα/Vβ T cell receptor expressed on the surface of the CD 8^+ T cell recognizes a NY-ESO-1 peptide associated with human leukocyte antigen A2, human leukocyte antigen B07, human leukocyte antigen B18, or human leukocyte antigen C03. In the illustrative working embodiments of this invention disclosed herein, the heterologous T cell receptor comprises a Vα/Vβ T cell receptor designated "3A1", "4A2", "5G6", 9D2", "1E4", "2B8" or "3C7".

Embodiments of the invention also include a number of different TCR nucleic acids and polypeptides that are disclosed herein (e.g. αβ TCR nucleic acids and encoded polypeptides for TCRs designated "3A1", "4A2", "5G6", 9D2", "1E4", "2B8" and "3C7"). For example, embodiments of the invention include a composition of matter comprising one or more polynucleotides (polynucleotides typically disposed in one or more vectors) encoding TCR Vα and/or TCR Vβ polynucleotides including: a polynucleotide encoding at least a 3A1 TCR Vα polypeptide (SEQ ID NO: 3); a polynucleotide encoding at least a 3A1 TCR Vβ polypeptide (SEQ ID NO: 4); a polynucleotide encoding at least a 4A2 TCR Vα polypeptide (SEQ ID NO: 7); a polynucleotide encoding at least a 4A2 TCR Vβ polypeptide (SEQ ID NO: 37); a polynucleotide encoding at least a 5G6 TCR Vα polypeptide (SEQ ID NO: 10); a polynucleotide encoding at least a 5G6 TCR Vβ polypeptide (SEQ ID NO: 11); a polynucleotide encoding at least a 9D2 TCR Vα polypeptide (SEQ ID NO: 14); a polynucleotide encoding at least a 9D2 TCR Vβ polypeptide (SEQ ID NO: 15); a polynucleotide encoding at least a 1E4 TCR Vα polypeptide (SEQ ID NO: 18); a

polynucleotide encoding at least a 1E4 TCR Vβ polypeptide (SEQ ID NO: 19); a polynucleotide encoding at least a 2B8 TCR Vα polypeptide (SEQ ID NO: 22); a polynucleotide encoding at least a 2B8 TCR Vβ polypeptide (SEQ ID NO: 23); a polynucleotide encoding a at least 3C7 TCR Vα polypeptide (SEQ ID NO: 26); or a polynucleotide encoding at least a 3C7 TCR Vβ polypeptide (SEQ ID NO: 27). In typical embodiments of the invention, these polynucleotides further encode additional amino acids such as a constant region of an alpha and/or beta polypeptide, a TM domain, a short cytoplasmic tail, or the like. In illustrative embodiments of the invention, the composition comprises a polynucleotide encoding a TCR Vα polypeptide in combination with a polynucleotide encoding a TCR Vβ polypeptide, wherein such polynucleotides are disposed within one or more vectors such that a Vα/Vβ TCR can be expressed on the surface of a mammalian cell (e.g. a $CD8^+$ T cell) transduced with the vector(s), with this expressed heterologous Vα/Vβ TCR recognizing a NY-ESO-1 peptide associated with a human leukocyte antigen.

In another aspect, the invention includes methods for generating a modified $CD8^+$ T cell comprising introducing nucleic acids encoding a TCR polypeptide disclosed herein into a T cell (e.g. a $CD8^+$ T cell obtained from an individual diagnosed with a cancer that expresses a NY-ESO-1 antigen). In another aspect, the invention includes a composition comprising the modified $CD8^+$ T cell generated according to the methods described herein. In another aspect, the invention includes methods of treating a disease or condition characterized by the expression of NY-ESO-1. The treatment methodology comprises administering an effective amount of the modified $CD8^+$ T cell(s) described herein to a subject in need thereof. In typical embodiments of the invention, the subject has a cancer. In certain embodiments of the invention, the cancer cells form solid tumors. In some embodiments of the invention, the cancer is a melanoma, neuroblastoma, a myeloma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, a esophageal

cancer, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, a ovarian cancer, a prostate cancer, or a breast cancer.

Other objects, features and advantages of the present invention will become apparent to those skilled in the art from the following detailed description. It is to be understood, however, that the detailed description and specific examples, while indicating some embodiments of the present invention, are given by way of illustration and not limitation. Many changes and modifications within the scope of the present invention may be made without departing from the spirit thereof, and the invention includes all such modifications.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1E show disclosure relating to the expansion and isolation of NY-ESO-1-specific T cell clones. PBMCs were obtained from patients with metastatic melanoma. T cell cloning strategy for a representative HLA-$A2^+$, HLA-$Cw3^+$ donor is shown. **Figure 1(A)** provides a schematic outlining the expansion and testing strategy to identify NY-ESO-1-reactive T cell clones. PBMCs were incubated with 28 NY-ESO-1 18mer peptides (overlapping by 12 amino acids) and then expanded for 10 days before restimulation with individual peptides in the presence of BFA. Epitopes presented by patient MHC alleles are colored red, blue, and green in those peptides containing the full epitope sequence. **Figure 1(B)** provides a representative flow cytometry measurement of intracellular staining for IFN-γ in expanded PBMCs restimulated with individual NY-ESO-1-derived 18-mer peptides. **Figure 1(C)** provides a schematic outlining the re-expansion strategy using individual 9-mer or 10-mer peptides verified to elicit a T cell response. **Figure 1(D)** provides representative flow cytometry data showing an NY-ESO-1 reactive subpopulation of $CD3^+CD8^+$ T cells prior to sorting. Sorted cells were expanded in the presence of IL-2 and irradiated autologous PBMCs. **Figure 1(E)**

provides representative flow cytometry data showing an NY-ESO-1 reactive subpopulation of $CD3^+CD8^+$ T cells following sorting.

Figures 2A-2D provide disclosure relating to cloning and functional screening of NY-ESO-1-specific T cell receptors. **Figure 2(A)** provides a schematic of functional TCR cloning strategy. For each TCR, two constructs were prepared incorporating either human or murine TCR constant domains. **Figure 2(B)** provides protein sequence of NY-ESO-1 with epitopes relevant to this study delineated (SEQ ID NO: 28). **Figure 2(C)** provides flow cytometry histograms comparing HLA-A2/$NY_{157-165}$ dextramer binding by HEK 293T cells transfected with vector backbone only, previously reported 1G4 TCR, and novel A2-restricted, NY-ESO-1-specific TCRs. **Figure 2(D)** provides flow cytometry histograms comparing indicated peptide-MHC dextramer binding by HEK 293T cells transfected with vector backbone only or the indicated novel NY-ESO-1-specific TCR restricted on MHC alleles other than HLA-A2. Transfection experiments were performed twice, each in duplicate. Representative histograms are presented.

Figures 3A-3F provide disclosure relating to the function of A2-restricted, NY-ESO-1-specific TCRs. **Figure 3(A)** provides an overlay of representative flow cytometry plots comparing A2/NY-ESO-1 $_{157-165}$ dextramer binding by Jurkat and $CD8^+$ Jurkat cells expressing A2-restricted TCRs with human or murine constant domains. **Figure 3(B)** shows dextramer binding mean fluorescence intensity measurements from two independent experiments as in A. **Figure 3(C)** shows the ratio of dextramer binding mean fluorescent intensity measurements from two independent experiments in B. **Figure 3(D)** shows ELISA measuring secretion of IL-2 from TCR-transduced Jurkat cells following 48 hours coincubation with K562 target cells expressing A2/$MART_{26-35}$ or A2/NY-ESO-$1_{157-165}$ single-chain trimer. Experiment was repeated 3 times, each with two technical replicates. Means ± SD for a representative experiment are shown. **Figure**

3(E) shows ELISA measuring secretion of IFN-γ from TCR-transduced PBMCs following 48 hours coincubation with the melanoma cell line M257 or an $A2^+$ derivative. Experiment was repeated at least three times, each with two technical replicates. Means ± SD for a representative experiment are shown. **Figure 3(F)** shows IncuCyte measurements of total green object area over time as a measurement of TCR-transduced T cell-mediated killing of GFP^+ $A2^+$ M257 cells. Means ± SD for four technical replicates are shown.

Figures 4A-4J provide disclosure relating to the *in vivo* anti-tumor efficacy of NY-ESO-1 TCR-engineered human T cells. Figures 4(A and B) show schematics of the experimental designs to **Figure 4(A)** generate NY-ESO-1 TCR-engineered human T cells, and to **Figure 4(B)** study anti-tumor efficacy of these engineered T cells in an NSG mouse human prostate tumor xenograft model. PBMCs: peripheral blood mononuclear cells; NSG: immunodeficient NOD/SCID/$\gamma c^{-/-}$ mice. **Figure 4(C)** shows representative flow cytometry plots characterizing engineered human T cells present in the peripheral blood of experimental mice on day 14 post adoptive T cell transfer. **Figure 4(D)** provides a time course showing persistence of engineered human T cells (gated as $LNGFR^+$ $hCD45^+$) in the peripheral blood of experimental mice. **Figures 4(E and F)** provide mean fluorescence intensity measurements for **Figure 4(E)** murine TCR and **Figure 4(F)** HLA-A2/NY-ESO-1 dextramer for engineered human T cells in the peripheral blood of experimental mice on day 14 post adoptive T cell transfer. **Figures 4(G and H)** provide measurements of cross-sectional area for **Figure 4(G)** PC-3/HLA-A2 and **Figure 4(H)** PC-3/HLA-A2/NY-ESO-1 tumors. **Figure 4(I)** provides immunohistology images showing representative tumor sections. $CD3^+$ cells are stained in red. Upper panel scale bar: 500 μm; lower panel scale bar: 50 μm. **Figure 4(J)** shows the percentage of $CD3^+$ cell area over whole tumor section area. Representative of two

experiments. Data are presented as the mean ± SEM (n = 4-5). ns, not significant, * P < 0.05; ** P < 0.01, *** P < 0.001, ****P < 0.0001, by one-way ANOVA.

Figures 5A-5E provide disclosure relating to the function of NY-ESO-1-specific TCRs restricted on MHC alleles other than HLA-A2. Figure 5(A) provides an overlay of representative flow cytometry plots comparing specified dextramer binding by Jurkat and $CD8^+$ Jurkat cells expressing novel TCRs with human or murine constant domains. **Figure 5(B)** provides indicated dextramer binding mean fluorescence intensity measurements from two independent experiments as in (A). **Figure 5(C)** shows the ratio of respective dextramer binding mean fluorescent intensity measurements from two independent experiments in (B). **Figures 5(D and E)** provide ELISAs measuring **Figure 5(D)** secretion of IL-2 from TCR-transduced Jurkat cells or **Figure 5(E)** secretion of IFN-γ from TCR-transduced PBMCs following 48 hours coincubation with K562 target cells expressing indicated single-chain trimer. Experiments were repeated three times, each with two technical replicates. Means ± SD for a representative experiment are shown.

Figures 6A-6D provide disclosure relating to targeting NY-ESO-1 epitopes restricted on multiple MHC alleles broadens the application of TCR gene therapy and makes it robust toward loss of heterozygosity at the MHC locus. T cells transduced with LNGFR only, A2-restricted 3A1 TCR, or B7-restricted 1E4 TCR – or a 1:1 mixture of 3A1-transduced and 1E4-transduced T cells – were co-incubated for 48 hours with HLA-$A2^+eGFP^+$ target cells, HLA-$B7^+eGFP^+$ target cells, or a 1:1 target cell mixture. **Figures 6(A and B)** provide ELISAs measuring secretion of IFN-γ from TCR-transduced PBMCs following 48 hours coincubation with **Figure 6(A)** M257 or **Figure 6(B)** PC-3 tumor cell lines engineered to express eGFP and HLA-A*02:01 or HLA-B*07:02. PC-3 lines were additionally engineered to express NY-ESO-1. M257 lines express endogenous NY-ESO-1. Experiments were repeated three times, each with four

or eight replicates. Means ± SD for a representative experiment are shown. **Figures 6(C and D)** show T-cell mediated killing of **Figure 6(C)** M257 and **Figure 6(D)** PC-3 tumor cell line derivatives measured over time using IncuCyte live-cell analysis. Total green object area (indicative of tumor cell density) at each time point measured over 48 hours was normalized for each treatment relative to treatment with LNGFR-transduced T cells. Experiments were repeated three times, each with four or eight replicates. Results from a representative 8-replicate experiment are shown.

Figures 7A-7E provide disclosure relating to determinations of EC50 for NY-ESO-1-specific TCRs. Figures 7(A and B) provide ELISAs measuring secretion of IFN-γ from TCR-transduced PBMCs following 48 hours coincubation with K562 engineered to express HLA-A*02:01 and pulsed with varied concentrations of **Figure 7(A)** MART126-35 or (B) NYESO1157-165 peptide. **Figure 7(C-E)** provide ELISAs measuring secretion of IFN-γ from TCR-transduced PBMCs following 48 hours coincubation with K562 engineered to express **Figure 7(C)** HLA-B*07:02, **Figure 7(D)** HLA-B*18:01, or **Figure 7(E)** HLA-C*03:04 and pulsed with varied concentrations of indicated peptides. Means ± SD for two technical replicates are shown. EC50 values and associated errors determined by non-linear curve fitting are indicated.

Figures 8A-8C provide disclosure relating to the establishment of xenograft tumor line and function of input T cells for *in vivo* experiments. Figure 8(A) provides an ELISA measuring secretion of IFN-γ from TCR-transduced PBMCs following 48 hours coincubation with derivatives of the PC-3 prostate cancer cell line engineered to express (left) HLA-A*02:01 and NY-ESO-1 full protein, (middle) HLA-A*02:01 alone, or (right) NY-ESO-1 full protein alone. Means ± SD for two technical replicates are shown. **Figure 8(B)** provides an ELISA comparing secretion of IFN-γ from TCR-transduced PBMCs following 48 hours coincubation with indicated M257 or PC-3 target cells. On the 4th day post transduction, TCR-transduced PBMCs were sorted for

CD3+/LNGFR+ and then expanded for 13 additional days prior to the co-culture/ELISA assay and the *in vivo* experiment. Means ± SD for a representative experiment with two technical replicates is shown. **Figure 8(C)** provides flow cytometry contour plots comparing the transduction (LNGFR+) levels of TCR transduced PBMCs used for the *in vivo* experiment.

DETAILED DESCRIPTION OF THE INVENTION

In the description of embodiments, reference may be made to the accompanying figures which form a part hereof, and in which is shown by way of illustration a specific embodiment in which the invention may be practiced. It is to be understood that other embodiments may be utilized, and structural changes may be made without departing from the scope of the present invention. Many of the techniques and procedures described or referenced herein are well understood and commonly employed by those skilled in the art. Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

NY-ESO-1 is an archetypical example of a cancer-testis antigen with restricted expression to germ cells and placental cells and re-expression in tumor cells. NY-ESO-1 expression has been reported in a wide range of tumor types, including neuroblastoma, myeloma, metastatic melanoma, synovial sarcoma, bladder cancer, esophageal cancer, hepatocellular cancer, head and neck cancer, non-small cell lung cancer, ovarian cancer, prostate cancer, and breast cancer. Its ability to elicit spontaneous humoral and cellular immune responses, together with its restricted expression pattern, render it a good

candidate target for cancer immunotherapy. See, e.g., Thomas et al., Front Immunol. 2018; 9: 947. doi: 10.3389/fimmu.2018.00947.

The disclosure herein demonstrates the accomplishment of two significant goals relating to methods and materials useful in NY-ESO-1 TCR gene therapy. First, since TCRs of higher strength and affinity are more effective, we sought to identify new TCRs that target A2/NY-ESO-$1_{157-165}$ with comparable or better sensitivity than the clinically-employed 1G4 TCR. As affinity-enhanced TCRs can be cross-reactive (28-30), we established a protocol for isolating antigen-reactive TCRs directly from patient blood. Two of these novel TCRs demonstrated comparable or greater sensitivity than 1G4 both *in vitro* and *in vivo* in tumor killing assays. Second, to broaden the clinical utility of NY-ESO-1 as a TCR gene therapy target, we used our isolation protocol to identify TCRs that target NY-ESO-1 epitopes presented by common MHC alleles other than HLA-A*02:01. Targeting multiple NY-ESO-1 epitopes will enable treatment of a larger patient set and may render treatment more robust toward tumor escape.

As described herein, the present invention provides methods and materials for making and using modified T cells comprising nucleic acids encoding certain T cell receptor polypeptides. As used herein, the term "T cell receptor" or "TCR" refers to a complex of membrane proteins that participate in the activation of T cells in response to the presentation of antigen. The TCR is responsible for recognizing antigens bound to major histocompatibility complex molecules. TCR is composed of a heterodimer of an alpha (α) and beta (β) chain, although in some cells the TCR consists of gamma and delta chains. TCRs may exist in alpha/beta and gamma/delta forms, which are structurally similar but have distinct anatomical locations and functions. Each chain is composed of two extracellular domains, a variable and constant domain. Embodiments of the invention include a number of different TCR alpha/beta nucleic acids and their encoded

polypeptides (e.g. TCR nucleic acids and encoded polypeptides for the TCRs designated "3A1", "4A2", "5G6", "9D2", "1E4", "2B8" and "3C7").

Embodiments of the invention include compositions of matter comprising one or more vectors comprising the TCR polynucleotides disclosed herein. A "vector" is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, Sendai viral vectors, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

Typically, the vector is an expression vector. The term "expression" as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter. In this context, the term "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an *in vitro* expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., Sendai viruses, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

Embodiments of the invention include, for example, a polynucleotide disposed in an expression vector, wherein the polynucleotide encodes a Vα T cell receptor

polypeptide and/or a Vβ T cell receptor polypeptide. In such embodiments, when a Vα/Vβ T cell receptor comprising the Vα T cell receptor polypeptide and/or the Vβ T cell receptor polypeptide is expressed in a CD 8^+ T cell, the Vα/Vβ T cell receptor recognizes a NY-ESO-1 peptide associated with human leukocyte antigen A2, human leukocyte antigen B07, human leukocyte antigen B18 or human leukocyte antigen C03. In the working embodiments of the invention disclosed herein, the modified CD 8^+ T cell receptor comprises a 3A1 T cell receptor, a 4A2 T cell receptor, a 5G6 T cell receptor, a 9D2 T cell receptor, a 1E4 T cell receptor, a 2B8 T cell receptor, or a 3C7 T cell receptor.

In typical embodiments of the invention, the vector comprises at least one of: a polynucleotide encoding a 3A1 TCR Vα polypeptide (SEQ ID NO: 3); a polynucleotide encoding a 3A1 TCR Vβ polypeptide (SEQ ID NO: 4); a polynucleotide encoding a 4A2 TCR Vα polypeptide (SEQ ID NO: 7); a polynucleotide encoding a 4A2 TCR Vβ polypeptide (SEQ ID NO: 37); a polynucleotide encoding a 5G6 TCR Vα polypeptide (SEQ ID NO: 10); a polynucleotide encoding a 5G6 TCR Vβ polypeptide (SEQ ID NO: 11); a polynucleotide encoding a 9D2 TCR Vα polypeptide (SEQ ID NO: 14); a polynucleotide encoding a 9D2 TCR Vβ polypeptide (SEQ ID NO: 15); a polynucleotide encoding a 1E4 TCR Vα polypeptide (SEQ ID NO: 18); a polynucleotide encoding a 1E4 TCR Vβ polypeptide (SEQ ID NO: 19); a polynucleotide encoding a 2B8 TCR Vα polypeptide (SEQ ID NO: 22); a polynucleotide encoding a 2B8 TCR Vβ polypeptide (SEQ ID NO: 23); a polynucleotide encoding a 3C7 TCR Vα polypeptide (SEQ ID NO: 26); or a polynucleotide encoding a 3C7 TCR Vβ polypeptide (SEQ ID NO: 27). Table 1 below discloses illustrative polynucleotide sequences that encode these TCR polypeptides.

Typically, a composition of the invention comprises one or more Vα/Vβ polynucleotides, for example a polynucleotide encoding a TCR Vα polypeptide in combination with a polynucleotide encoding a TCR Vβ polypeptide such that a Vα/Vβ

TCR can be expressed on the surface of a mammalian cell (e.g. a $CD8^+$ T cell) transduced with the vector(s), wherein the Vα/Vβ TCR recognizes a NY-ESO-1 peptide associated with a HLA. The term "transduced" or "transfected" or "transformed" as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

In another aspect, the invention includes a method for generating a modified T cell comprising introducing one or more nucleic acids (e.g., nucleic acids disposed within a lentiviral vector) encoding a TCR disclosed herein into a T cell (e.g. a $CD8^+$ T cell obtained from an individual diagnosed with a cancer that expresses a NY-ESO-1 antigen). The present invention also includes modified T cells with downregulated or knocked out gene expression (e.g., a modified T cell having a knocked out endogenous T cell receptor and an exogenous/introduced T cell receptor that recognizes a NY-ESO-1 peptide associated with a HLA). The term "knockdown" as used herein refers to a decrease in gene expression of one or more genes. The term "knockout" as used herein refers to the ablation of gene expression of one or more genes.

The modified T cells described herein may be included in a composition for use in a therapeutic regimen. The composition may include a pharmaceutical composition and further include a pharmaceutically acceptable carrier. A therapeutically effective amount of the pharmaceutical composition comprising the modified T cells may be administered. Pharmaceutical compositions of the present invention may comprise the modified T cell as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids

such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration.

Adoptive immunotherapy with T cells harboring antigen-specific TCRs have therapeutic potential in the treatment of cancers. Gene-engineering of CD 8^+ T cells with a specific TCR has the advantage of redirecting the T cell to a selected antigen such as an NY-ESO-1 antigen. In this context, in one aspect, the invention includes methods for stimulating a T cell-mediated immune response to a target cell or tissue in a subject comprising administering to a subject an effective amount of a modified CD 8^+ T cell. In this embodiment, the $CD8^+$ T cell is modified as described elsewhere herein. Embodiments of the invention also include administering multiple modified CD 8^+ T cells that target multiple NY-ESO-1 epitopes. For example, embodiments of the invention include administering at least two different modified $CD8^+$ T cells, for example a first modified $CD8^+$ T cell that targets a NY-ESO-1 peptide associated with a first human leukocyte antigen human leukocyte antigen in combination with a second $CD8^+$ T cells that targets a NY-ESO-1 peptide associated with second human leukocyte antigen.

Embodiments of the invention encompass methods of treating a disease or condition characterized by the expression of NY-ESO-1, a prototypical cancer-testis antigen. The treatment methodology comprises comprising administering an effective amount of a pharmaceutical composition comprising the modified T cell described herein to a subject in need thereof. The term "subject" is intended to include living organisms in which an immune response can be elicited (e.g., mammals). A "subject" or "patient", as used therein, may be a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. Preferably, the subject is human. In typical embodiments of the invention, the human has a cancer expressing NY-ESO-1 antigen. In some embodiments of the

invention, the cells of the cancer form solid tumors. In illustrative embodiments of the invention, the cancer cells are neuroblastoma cells, myeloma cells, metastatic melanoma cells, synovial sarcoma cells, bladder cancer cells, esophageal cancer cells, hepatocellular cancer cells, head and neck cancer cells, non-small cell lung cancer cells, ovarian cancer cells, prostate cancer cells, or breast cancer cells.

A related embodiment of the invention includes a method for prophylaxis and/or therapy of an individual diagnosed with, suspected of having or at risk for developing or recurrence of a cancer, wherein the cancer comprises cancer cells which express NY-ESO-1 antigen. This approach comprises administering to the individual modified human T cells comprising a recombinant polynucleotide encoding a TCR, wherein the T cells are capable of direct recognition of the cancer cells expressing the NY-ESO-1 antigen, and wherein the direct recognition of the cancer cells comprises HLA class II-restricted binding of the TCR to the NY-ESO-1 antigen expressed by the cancer cells.

With respect to use of the engineered $CD8^+$ T cells of the present invention, the method generally comprises administering an effective amount (e.g. by intravenous or intraperitoneal injections) of a composition comprising the $CD8^+$ T cells to an individual in need thereof. An appropriate pharmaceutical composition may be adapted for administration by any appropriate route, such as parenteral (including subcutaneous, intramuscular, or intravenous), enteral (including oral or rectal), inhalation or intranasal routes. Such compositions may be prepared by any method known in the art of pharmacy, for example by mixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

In another aspect, the invention includes use of a polynucleotide or a modified $CD8^+$ T cell described herein in the manufacture of a medicament for the treatment of a disease or condition characterized by the expression of NY-ESO-1, in a subject in need thereof. In illustrative embodiments of the invention, the disease is a cancer expressing

NY-ESO-1 antigen, for example a melanoma, a neuroblastoma, a myeloma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer, a prostate cancer, or a breast cancer.

The technology in this area is fairly developed and a number of methods and materials know in this art can be adapted for use with the invention disclosed herein. Such methods and materials are disclosed, for example in U.S. Patent Publication Nos. 20190247432, 20190119350, 20190002523, 20190002522, 20180371050, 20180057560, 20170029483, 20160024174, and 20150141347, the contents of which are incorporated by reference.

Further aspects and embodiments of the invention are provided in the examples below.

EXAMPLES

EXAMPLE 1: Expansion and isolation of NY-ESO-1-specific T cell clones.

We previously reported the presence of T cells reactive with various NY-ESO-1-derived epitopes in the blood of patients with metastatic melanoma (22). To enrich for these reactive T cells, we stimulated expansion of patient peripheral blood mononuclear cells (PBMCs) with a panel of 28 overlapping 18-mers collectively constituting the full NY-ESO-1 protein sequence (**Fig. 1A**). We then re-stimulated the expanded cells with individual peptides, performed intracellular staining for IFN-γ to determine which peptides drove expansion, and analyzed stimulatory peptides with a predictive algorithm to identify minimal epitopes relevant to each patient's MHC haplotype (31) (**Fig. 1B**). Reactive T cells were re-expanded in the presence of individual 9-10-mer peptides corresponding to immunostimulatory epitopes (**Fig. 1C**) and sorted via fluorescence-activated cell sorting (FACS) using cognate peptide-MHC tetramers (**Fig. 1D**). The cell lines grown from these single cell sorts were clonal and reactive with their cognate

epitopes (**Fig. 1E**). In total, 4 cell lines reactive with HLA-A*02:01/NY-ESO-$1_{157-165}$ and 4 cell lines reactive with epitopes presented by HLA-B and HLA-C alleles were selected for further study.

EXAMPLE 2: Cloning and screening of NY-ESO-1-specific TCRs

We cloned paired TCRα and TCRβ genes from sorted single cells using a commercial RT-PCR kit with custom multiplexed primers targeting all human TRAV and TRBV gene segments. The resulting V_α and V_β cDNAs were sub-cloned into a retroviral vector backbone with either human or murine TCR constant regions (**Fig. 2A**). To verify the specificity of cloned TCRs, we transfected $CD3^+$ HEK 293T cells with each fully human TCR, and stained the transfected cells with peptide-MHC dextramer reagents for each of the targeted NY-ESO-1 epitopes (**Fig. 2B**). All 4 HLA-A2-restricted TCRs exhibited the expected reactivity (**Fig. 2C**). Although analyzed events were gated for similar transfection level, novel TCRs exhibited highly variable dextramer binding. Dextramer binding for the 9D2 TCR was barely discernible from background, whereas the 3A1 TCR exhibited superior dextramer binding compared to the clinically-employed 1G4 TCR. Dextramer binding for 4A2 and 5G6 TCRs were intermediate between 9D2 and 1G4.

Additionally, 3 of 4 of the TCRs restricted on MHC alleles other than HLA-A2 were verified to bind their targets specifically (**Fig. 2D**). Transfected 293T cells expressing the B7/NY-ESO-1_{60-72}-specific 1E4 TCR, the B18/NY-ESO-1_{88-96}-specific 2B8 TCR, or the Cw3/NY-ESO-1_{96-104}-specific 3C7 TCR each bound their respective dextramers, whereas untransfected cells did not. Cells transfected with the 9G2 TCR – cloned from T cells that were reactive with Cw3/NY-ESO-1_{92-100} – did not detectably bind cognate dextramer relative to untransfected cells. A possible reason for this was that HEK 293T cells do not express the CD8 co-receptor. CD8 increases the avidity of the

TCR-pMHC interaction by binding to MHCI directly, enabling lower affinity TCRs to engage (32). We therefore included this TCR for further analysis of CD8 dependency in Jurkat T cells.

EXAMPLE 3: Functional characterization of A2-restricted, NY-ESO-1-specific TCRs

The sensitivity of a TCR-transduced T cell is a function of the monomeric affinity of the TCR for its cognate peptide-MHC (K_d ~ 0.1 - 400 μM) (33) as well as the density of the TCR on the cell surface (12). Transduced TCRs express on the T cell surface at widely varying levels due to variation in the efficiency with which they fold, dimerize, and compete with endogenous TCRs for assembly with limiting CD3 chains (a property termed TCR "strength") (34, 35). Therefore, optimal cytotoxic function of TCR-transduced T cells correlates with TCR affinity and surface expression (3, 12), underscoring the importance of selecting high affinity, efficiently exported TCRs for gene therapy(7).

As higher affinity TCR-pMHC interactions are less dependent on CD8 participation, we reasoned that high affinity TCRs can be identified by comparing dextramer binding of TCR-transduced Jurkat T cells with or without co-expression of CD8. Additionally, because the strength of surface expression for human TCRs can be increased through substitution with murine constant domains (36), we expressed each TCR as a fully human or murinized derivative to assess each TCR's strength. Cells transduced with vehicle only or with a mismatched TCR (MART1-specific F5 TCR) did not exhibit any binding to A2/NY-ESO-$1_{157\text{-}165}$ dextramer (**Fig. 3A, 3B**). By contrast, cells transduced with the well-established 1G4 TCR (K_D = 9.3 μM) (37) bound cognate dextramer whether 1G4 was fully human or murinized, and whether or not CD8 was present. Murinization of 1G4 increased the intensity of dextramer binding by the muTCR

1.4-fold over the parental huTCR, indicating a modest improvement in strength (**Fig. 3B, 3C**). The presence of CD8 increased dextramer binding 3.8-fold for 1G4 muTCR. Dextramer binding for novel TCRs 4A2 and 5G6 was similar in both magnitude and comparative indices to 1G4 (**Fig. 3A-3C**). The 3A1 TCR exhibited only a 1.9-fold increase in dextramer binding in the presence of CD8, indicating that this TCR binds A2/NY-ESO-$1_{157-165}$ with higher affinity than 1G4. This is further supported by the reduced dependence of dextramer binding on CD8 level among $CD8^+$ cells transduced with 3A1 muTCR relative to $CD8^+$ cells transduced with 1G4, 4A2, and 5G6 muTCRs (compare slopes of green populations in **Fig. 3A**). Finally, 9D2 exhibited no detectable binding to dextramer on Jurkat cells in the absence of CD8 and only weak binding upon co-expression of CD8. Murinization of 9D2 did not increase its binding to dextramer.

To compare the functional sensitivity of T cells expressing novel, A2/NY-ESO-1-specific TCRs, we co-incubated TCR-transduced Jurkat T cells with K562 cells expressing either A*02:01/NY-ESO-$1_{157-165}$ or A*02:01/MART1_{27-35} single-chain trimers (38) and measured secreted interleukin-2 (IL-2). All TCRs exhibited their expected peptide specificity: the control MART1-specific F5 TCR mediated IL-2 release only in response to MART1 presentation and all NY-ESO-1-specific TCRs mediated IL-2 release only in response to NY-ESO-1 presentation (**Fig. 3D**). Murinization improved functional sensitivity for all TCRs except for 1G4. Consistent with dextramer staining results, 1G4 and 3A1 muTCRs outperformed 4A2 and 5G6 muTCRs. By contrast, despite its weak binding to dextramer, 9D2 exhibited high functional sensitivity to cognate ligand, comparable to 3A1. To quantify this observation, we pulsed $A2^+$K562 cells with varied concentrations of NY-ESO-$1_{157-165}$ or MART1_{27-35} peptide, and then measured IFN-γ secretion from TCR-transduced primary T cells co-incubated with peptide-pulsed target cells (**Fig. 7A, 7B**). As observed with single-chain trimer targets, 3A1, 9D2, and 1G4 exhibited highest sensitivity to NY-ESO-$1_{157-165}$ peptide. The functional sensitivity of

9D2 was 10-fold higher than 4A2, despite 4A2 binding dextramer with 18-fold higher MFI than 9D2 (**Fig. 3A, 3B**). To evaluate responses to endogenously-processed and presented antigen, TCR-transduced primary T cells were co-incubated with the human melanoma cell line, $A2^+$M257 (**Fig. 3E**). Again, T cells transduced with 3A1, 9D2, and 1G4 responded comparably to one another, and with higher sensitivity than did those transduced with 5G6 and 4A2. TCR-transduced T cells did not respond to the M257 line lacking HLA-A*02:01. Finally, *in vitro* cytotoxicity tracked closely with cytokine release: T cells expressing 9D2 or 3A1 killed $A2^+$M257 tumor cells most efficiently, followed by T cells transduced with 1G4, 5G6, and, least efficiently, 4A2 (**Fig. 3F**).

To enable evaluation of TCR function in a tumor xenograft model, we engineered the PC-3 human prostate cancer cell line to express NY-ESO-1 and HLA-A*02:01 and then verified that this line elicited functional responses from TCR-transduced T cells in an antigen-dependent and MHC-restricted manner (**Fig. 8A**). The relative responses to $A2^+NY^+$PC-3 from our panel of novel NY-ESO-1-reactive TCRs were consistent with those elicited by $A2^+$M257 (**Fig. 3E and Fig. 8B**). Based on these results, we selected 1G4, 3A1, and 9D2 muTCRs for further functional characterization *in vivo*. We transduced activated human PBMCs with a vector encoding each murinized TCR and a transduction marker (low-affinity nerve growth factor receptor (LNGFR) (**Fig. 4A**). We sorted transduced ($CD3^+LNGFR^+$) T cells (**Fig. 8C**) and retro-orbitally i.v. injected these T cells into irradiated NOD/SCID/$\gamma c^{-/-}$ (NSG) mice pre-inoculated with PC-3/HLA-A2 (control) and PC-3/HLA-A2/NYESO (target) tumors on opposing flanks (**Fig. 4B**). We then monitored T cell engraftment and tumor size until the conclusion of the experiment two weeks after T cell injection.

T cells transduced with 1G4 or 9D2 TCRs persisted or minimally expanded in the peripheral blood, while 3A1-transduced T cells expanded significantly (**Fig. 4C and 4D**). By contrast, T cells transduced only with LNGFR contracted over the course of the

experiment, suggesting the expansion of TCR-transduced T cells was antigen-driven. The expression level of murine TCRβ (mTCRβ) was stable over the experimental time course and comparable between T cells transduced with different murinized TCRs (**Fig. 4C and 4E**). The respective staining levels of each TCR-transduced T cell cohort with A*02:01/$NY_{157-165}$ dextramer$^+$ were also stable over time, but, as expected from results *in vitro*, were significantly different between TCRs. Approximately 90% of human T cells transduced with 1G4 or 3A1 were dextramer$^+$ with high MFI. By contrast, only ~1% of 9D2-transduced T cells were dextramer$^+$ and the MFI of staining was not significantly different from LNGFR-transduced controls (**Fig. 4C and 4F**). Nonetheless, T cells transduced with 1G4, 3A1, or 9D2 reduced tumor size comparably and in an antigen-specific manner, while LNGFR-transduced T cells failed to control tumor growth (**Fig. 4G and 4H**).

At the conclusion of the experiment, we sacrificed the mice and analyzed tumors for T cell infiltration by immunohistochemistry. Immunohistochemical staining revealed antigen-specific T cell infiltration only into target tumors in all cohorts receiving TCR-transduced T cells (**Fig. 4I and 4J**). Infiltration was significantly higher in mice receiving 3A1-transduced T cells relative to mice receiving 1G4- or 9D2-transduced T cells.

Example 4: Functional characterization of NY-ESO-1-specific TCRs restricted on HLA-B and HLA-C alleles

The majority of immunotherapies targeting NY-ESO-1 have focused on the A2-restricted NY-ESO-$1_{157-165}$ epitope. To enable broader application of NY-ESO-1-targeted immunotherapies, we cloned TCRs from four non-A2-restricted T cell clones and verified NY-ESO-1-reactivity for three of these in transfected CD3$^+$ 293T (**Fig. 2D**). The fourth TCR – 9G2, cloned from Cw3/NY-ESO-1_{92-100}-reactive T cells – did not impart specificity for Cw3/NY-ESO-1_{92-100} on transduced Jurkat T cells even with co-expressed

CD8 (**Fig. 5A, 5B**) and was not studied further. Comparisons of dextramer binding by the three validated TCRs expressed in Jurkat or $CD8^+$ Jurkat as human or murine TCRs revealed differences in strength and affinity (**Fig. 5A, 5B, 5C**). The B7/NY-ESO-1_{60-72}-specific 1E4 TCR exhibited high strength but low affinity, expressing comparably on the Jurkat cell surface as either a huTCR or a muTCR but binding dextramer only in the presence of $CD8^+$. Dextramer binding to these $CD8^+$, 1E4-transduced cells was steeply dependent on the level of CD8 expressed. By contrast, the B18/NY-ESO-1_{88-96}-specific 2B8 TCR bound dextramer in the absence of $CD8^+$, but binding was substantially higher for the murinized TCR. Finally, the Cw3/NY-ESO-1_{96-104}-specific 3C7 TCR exhibited intermediate strength of surface expression and an affinity index comparable to 2B8.

These differences in TCR strength and affinity were reflected in functional assays. For all three TCRs, murinization of the TCR constant regions increased production of IL-2 from TCR-transduced Jurkat cells co-incubated with cognate target cells. However, this increase was only 1.6- and 3.0-fold over the respective fully human TCRs for 1E4 and 3C7, but was 18.6-fold for 2B8, consistent with the latter's lower strength (**Fig. 5D**). In peptide titration assays, 1E4 TCR imparted lower sensitivity for cognate peptide on transduced $CD8^+$ T cells than did 3C7 or 2B8 (**Fig. 7C, 7D, 7E**), consistent with the presumed lower affinity of 1E4 based on its strictly CD8-dependent dextramer binding.

Primary PBMCs transduced with each TCR responded to the presentation of NY-ESO-1-derived epitopes in a peptide-specific and MHC-restricted manner (**Fig. 5E**). As such, we expect that TCR gene therapies employing NY-ESO-1 specific TCRs restricted on multiple MHCs can be applied more broadly across patient haplotypes and will be more robust toward tumor evasion via loss-of-heterozygosity at the MHCI locus. To test this, we transduced NY-ESO-1-expressing human cancer cells with HLA-A2 or HLA-B7. We then co-incubated one or both of these tumor targets with human T cells transduced with A2-restricted 3A1 TCR, with T cells transduced with B7-restricted 1E4 TCR, or

with a mixture of 3A1- and 1E4-transduced T cells (**Fig. 6**). As expected, combination targeting using a mixture of 3A1- and 1E4-transduced T cells enabled recognition of tumor cell populations expressing both MHC alleles or either MHC allele alone (**Fig. 6A, 6B**). By contrast, T cells targeting a single NY-ESO-1 epitope did not respond to NY-ESO-1-expressing tumor cells that lacked the cognate MHC allele. Moreover, when tumor targets comprised a mixture of cells expressing different MHC alleles (simulating tumor heterogeneity arising from haploinsufficiency), T cells targeting both NY-ESO-1 epitopes killed tumor cells more completely than did T cells targeting either single epitope (**Fig. 6C, 6D**).

Discussion

T cell-mediated immunotherapies are making clinical inroads for previously refractory cancers. Two of the most successful immunotherapy modalities are checkpoint blockade and adoptive transfer of cancer-specific T cells. Checkpoint blockade elicits better clinical responses as tumor mutational burden increases (39-41), suggesting that non-synonymous mutations go undetected by the immune system unless, fortuitously, they generate neoepitopes that are presented by the patient's complement of MHC molecules. This interpretation is bolstered by the recent finding that checkpoint blockade results in higher overall survival for melanoma patients who are heterozygous at the HLA-A, HLA-B, and HLA-C loci and thus present a more diverse array of epitopes than those who are homozygous at one or more of these MHCI loci (42). The importance of a diversely targeted anti-tumor immune response is likewise supported by results from adoptive T cell therapy, which show that loss-of-heterozygosity is a mechanism by which tumors can evade monospecific immune recognition while continuing to express an otherwise immunogenic antigen (43). Thus, a prominent narrative emerging from these studies is that diverse targeting of multiple epitopes presented by multiple MHC alleles is desirable for successful immunotherapy. A second takeaway is that targeting multiple

epitopes derived from a tumor-specific public antigen may be a promising alternative to targeting neoepitopes in cancers with low mutational burden.

It has proven difficult to identify public tumor-associated antigens that mediate tumor regression without also manifesting serious morbidity or deaths resulting from on-target, off-tumor T cell reactivity. We chose to focus on NY-ESO-1 as a public antigenic target based on the criteria that it 1) is expressed exclusively in cancer cells and immunologically privileged germ cells; 2) is expressed in many patients across various tumor types; 3) harbors high-affinity ligands for multiple common MHC alleles; 4) is well-vetted, having yielded objective responses in patients across several tumor types without specificity-related adverse events; and 5) is yet underexploited, as the majority of studies have focused on mobilizing T cell responses solely against the A2-restricted NY-ESO-$1_{157-165}$ epitope.

We employed an antigen-specific expansion protocol to isolate NY-ESO-1-reactive T cells from the peripheral blood of patients with metastatic melanoma. Using this approach, we cloned several HLA-A2-restricted TCRs and compared them in terms of their strength of surface expression, affinity (i.e. dependence of target binding on CD8), and function (antigen-induced cytokine release and tumor target killing). From four candidates, we identified two that recognized and killed NY-ESO-1-expressing cancer cells as well or better than the clinically-employed 1G4 TCR. This expansion-based approach to TCR candidate identification is ideally-suited for targeting public epitopes because the speed of isolation is not a critical parameter; once identified, these TCRs can be used as off-the-shelf targeting receptors for any patient expressing the requisite MHC allele. Antigen-specific expansion of neoantigen-reactive T cells from peripheral blood has also been demonstrated (44, 45). However, on-demand isolation of private neoepitope-targeted TCRs will require more rapid approaches than that used here (e.g. direct capture of antigen-specific T cells from blood or expansion protocols optimized for

rapidity). As the release of IFN-γ is strongly correlated with cytotoxicity (46), candidate evaluation can be accelerated by using IFN-γ release as a surrogate for more involved tumor xenograft assays.

One of the HLA-A2/NY-ESO-1-reactive TCRs isolated – 9D2 – exhibited poor staining with cognate multimer but high functional avidity toward cognate antigen-presenting target cells. This is consistent with the observation that multimer staining underestimates functional T cell subsets (47) and may be explained by the higher affinity threshold for multimer binding relative to that for T cell activation (48). However, another isolated A2-restricted TCR – 4A2 – exhibited robust multimer staining but poor function in cell-based assays, seemingly at odds with this affinity threshold explanation. While we do not have an explanation for this latter result, both results caution against relying overmuch on multimer staining when down-selecting immunotherapy candidates.

The HLA-A*02:01 allele is the most prevalent MHCI allele in Caucasian (45%) and Hispanic (41%) U.S. populations, but it is less common among Asian (15%) and African (16%) U.S. populations (2). These latter populations would be particularly well-served by expanding the targeting of TCR gene therapies beyond HLA-A2 to a more expansive panel of targetable MHC alleles. In addition to HLA-A2-restricted TCRs, we isolated and functionally characterized NY-ESO-1-specific TCRs restricted on various HLA-B and HLA-C alleles. In doing so, we demonstrated in principle that TCR gene therapy can be extended to a greater subset of patients/haplotypes and that, when used in combination, TCRs recognizing multiple epitopes from the same antigen can more robustly kill tumors with heterogeneous MHC expression (e.g. resulting from somatic loss-of-heterozygosity). Over 80% of people across ethnic groups express at least one allele from three MHCI supertypes (A2, A3, and B7, two of which were represented here) and >99% of people express at least one allele from nine MHCI supertypes (49).

Therefore, obtaining a panel of public antigen-specific TCR reagents that enable comprehensive application of TCR gene therapy is a finite and surmountable challenge.

Materials and methods

Materials

Peptides were purchased from Anaspec (Fremont, CA), Thermo Fisher Scientific (Waltham, MA), and Mimotopes (Victoria, Australia). Fluorescent antibodies and 7-AAD used for flow cytometry were purchased from BD Biosciences (San Jose, CA), BioLegend (San Diego, CA) or eBioscience (San Diego, CA). Fluorescent peptide-MHC multimers were purchased from TCMetrix (Epalinges, Switzerland) or prepared in-house as described (50) from biotinylated monomers (obtained from NIH Tetramer Core, Atlanta, GA, or expressed heterologously in *E. coli*, refolded, and biotinylated in-house as described(51)). Primers were purchased from Integrated DNA Technologies (Coralville, IA). KOD polymerase master mix and polybrene were purchased from EMD Millipore (Darmstadt, Germany). Sequencing was performed by Retrogen Inc (San Diego, CA). Anti-CD3 (OKT3) and anti-CD28 (CD28.2) activating antibodies were purchased from eBioscience. Cytokines were purchased from Peprotech, Inc. (Rocky Hill, NJ). BioT transfection reagent was purchased from Bioland Scientific (Paramount, CA). Cell culture media, antibiotics, and fetal bovine serum were purchased from Corning (Corning, NY). Human AB serum was purchased from Omega Scientific (Tarzana, CA). Poly-L-lysine and PHA-L (phytohaemagglutinin-L) were purchased from Sigma (St. Louis, MO).

Cells

Cell lines (293T/17, Jurkat E6-1, and K562) were purchased from the American Type Culture Collection (Manassas, VA). 293T cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with antibiotics (penicillin/streptomycin) and

10% (v/v) fetal bovine serum (FBS). Jurkat and K562 cells were grown in RPMI 1640 medium supplemented with antibiotics, 10% (v/v) FBS, 10 mM HEPES, 50 μM β-mercaptoethanol, 1x MEM NEAA, and 1 mM sodium pyruvate. The cells were split every 2-3 days to maintain adherent cells sub-confluently or non-adherent cells at a density of $<10^6$ cells/mL. Jurkat and K562 cells were transduced with non-replicative viral vectors, analyzed by flow cytometry, and used directly in cell assays or sorted by FACS to establish derivative cell lines as indicated. Primary human PBMCs used in functional assays were purchased from the CFAR Virology Core Lab at the UCLA AIDS Institute, stimulated, transduced, and cultured as previously described (52). T cells were grown from PBMCs in T cell medium (AIM-V mediu supplemented with 5% heat-inactivated human AB serum, 55 μM β-mercaptoethanol and 4 mM L-glutamine) with freshly added cytokines. All cells were grown and assayed at 37 °C with 5% atmospheric CO_2.

Generation and culture of NY-ESO-1 specific CD8$^+$ T-lymphocyte clones

$CD8^+$ T-lymphocyte clones specific for epitopes from NY-ESO-1 with various HLA restrictions (157-165/HLA-A*02:01 (53), 60-72/HLA-B*07:02 (21), 88-96/HLA-B*18:01 (23), 92-100/HLA-C*03:04 (54), 96-104/HLA-C*03:04 (22), 124-133/HLA-C*03:04 (22)) were generated from HLA-typed patients with melanoma. All selected patients had Grade III/IV metastatic melanoma and previously documented NY-ESO-1 responses to relevant T lymphocyte epitopes *ex vivo* (55). Patient PBMCs were stimulated in the presence of 1 μM pooled peptides (Mimotopes), comprising 28 x 18-mers overlapping by 12 amino acids, collectively spanning the NY-ESO-1 protein sequence and then cultured for 10 days in the presence of 25 IU/ml IL-2 (Peprotech).

On day 10, cells were restimulated with 1 μM of each individual peptide in the presence of brefeldin A and activation of $CD8^+$ T cells in response to each peptide was

determined by intracellular cytokine stain (ICS). Briefly, cells were labeled with live/dead fixable violet stain (Invitrogen) according to the manufacturer's instructions, then incubated with antibodies against CD3 and CD8 for 15 min at 4 °C. Samples were washed and fixed with fix/permeabilisation reagent (BD biosciences) for 20 min at 4°C. Cells were stained with anti-IFNγ (eBiosciences) in permeabilisation/wash solution (BD biosciences) for 25 min at 4°C. The gating strategy was: SSC/LD$^-$; CD3$^+$/CD8$^+$; CD8$^+$/IFNγ$^+$. Data from at least 100,000 stained cells were acquired on a FACSCanto and analyzed with FlowJo software. Data collection and analysis was in accordance with the MIATA guidelines (56).

NY-ESO-1-reactive T cells were expanded in the presence of their identified cognate 9-10-mer epitope and then labeled with a fluorescent tetramer comprising the relevant peptide and HLA molecule (TCMetrix, Epalinges, Switzerland) and single-cell sorted using a MoFlo cell sorter. Clones were re-expanded with pooled, allogeneic healthy donor PBMC as feeder cells, 1 µg/ml PHA-L and 600 IU/ml IL-2 (Cetus). After approximately 20 days, 1–10 x 10^3 clones were restimulated in the presence of allogeneic PBMC as feeder cells, PHA-L and IL-2, as described above. Clone specificity was confirmed by tetramer staining.

T-lymphocyte clones/lines were cultured in RPMI 1640 media supplemented with 2 mM Glutamax, 100 IU/ml penicillin, 100 µg/ml streptomycin, 20 mM HEPES, 1% nonessential amino acids, 1 mM sodium pyruvate, 55 µM β-mercaptoethanol, and 10% human serum (TCRPMI). IL-2 (100 IU/ml) was added and replaced every 3 days.

Cloning TCR constructs

Single NY-ESO-1-reactive T cells were sorted for antigenic specificity on a FACS Aria II and were lysed by freeze-thaw in the presence of RNase inhibitor. Novel TCR variable genes were cloned from single, sorted T cells using a custom panel of

human TCR variable region-specific primers with the Qiagen OneStep RT-PCR kit (Redwood City, CA), followed by a nested PCR amplification step. Amplified variable genes were integrated via assembly PCR and restriction enzyme-mediated cloning into a TCR expression cassette with either human or mouse TCR constant domains and a 2A ribosomal skipping peptide linking the alpha and beta genes. A P2A-linked gene encoding a truncated version of the low affinity nerve growth factor receptor (LNGFR) was also included in the cassette as an independent transfection/transduction marker. Antigenic specificity and MHC restriction of cloned TCRs were evaluated in 293T cells co-transfected with TCR and CD3 genes, as previously described (52).

Evaluation of TCR export and dextramer binding on Jurkat T cells

Jurkat T cells were transduced with MSGV-based retroviruses encoding each novel TCR in the format LNGFRΔ-P2A-TCRα-F2A-TCRβ. Viruses were produced in 293T cells as described (52). For transduction, Jurkat T cells were centrifuged (1350xg for 90 minutes at 30 °C) with unconcentrated viral supernatants supplemented with 5 μg/mL polybrene. TCR-transduced Jurkat cells were stained with cognate pMHC dextramer for 15 min at room temperature and then co-stained with antibodies against LNGFR and CD8α for 15 min at 4 °C. Stained cells were analyzed by flow cytometry using a FACSCanto analyzer. Data shown are gated on $LNGFR^+$ (transduced) cells. Transduction efficiency was >95%.

PBMC activation and transduction

Primary human PBMCs were purchased from the CFAR Virology Core Lab at the UCLA AIDS Institute. The same PBMC donor was used in all reported experiments. Primary human PBMCs were transduced with retroviruses encoding novel TCRs as described (52). Briefly, two days prior to viral transduction, 1-2 x 10^6 total thawed

PBMCs were activated per well in 24-well plates with plate-coated anti-CD3 (clone OKT3), T cell medium containing 1 µg/mL soluble anti-CD28 (clone CD28.2), and 300 U/ml IL-2. After 48 hours of activation, the majority of the medium was replaced with unconcentrated retroviral supernatant supplemented with 10 µg/mL polybrene and cells were centrifuged for 90 min at 1350xg at 30 °C. Following spinfection, the majority of retroviral supernatant was replaced with fresh medium containing 300 U/mL IL-2 and 1 µg/mL anti-CD28. The transduction was repeated 24 hours later, after which the cells were washed with 1x PBS and then returned to fresh medium containing final 300 U/mL IL-2 and cultured for an additional 3 to 4 days before being used in antigenic stimulation assays. One day prior to or on the day of co-culturing, PBMCs were analyzed by FACS for assessment of expression levels for LNGFR, TCR, and/or pMHC multimer binding.

Functional co-culture assays – cytokine ELISA

When Jurkat T cells were used as effectors, co-cultures were performed in RPMI supplemented with 10% FBS, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 4 mM L-glutamine. Effector cells (50,000 TCR-transduced Jurat T cells) were co-incubated with target cells (50,000 K562 cells transduced with cognate or control single-chain trimers) in 96-well flat-bottom plates. Supernatants from duplicate wells were collected 44-48h post-co-culturing and analyzed by enzyme-linked immunosorbent assay (ELISA) as described below.

When primary PBMCs were used as effectors, co-cultures were performed in T cell media containing 300 U/mL IL-2. Effector cells (50,000 TCR-transduced PBMCs) were co-incubated with target cells (50,000 M257, PC-3, or K562 cells) in 96-well flat-bottom plates. In some experiments target cells were pulsed with peptide. Supernatants from 2-8-fold replicate wells for each condition were collected 44-48 hours post-co-

culturing and analyzed by enzyme-linked immunosorbent assay (ELISA) as described below.

For experiments in which target cells were titrated with pulsed peptide, lyophilized peptides were dissolved to 10 mM in DMSO and then further diluted in water to 2 mM working stocks. At point of use, the 2 mM stock was diluted to 250 µM in cell media and then 5-fold serially diluted from 250 µM down to 3.2 nM. Target cells were pulsed by adding 25 µL of each serial dilution per well on a 96-well U-bottom plate, followed by addition of 50,000 target cells in 100 µl media, yielding the final peptide concentration ranging from 50 µM to 0.64 nM. Cells were pulsed with peptides for 2 hours at 37 °C, diluted with 100ul of media per well at the end of incubation, centrifuged, and the supernatant was removed. The cells were washed with 200ul of media and then re-suspended in 100 µl of media. Fifty thousand PBMCs prepared in 100 µl of media were then added to each well for co-culturing.

In general, ELISA results were converted to concentration (ng/mL) by interpolation relative to a standard curve and concentrations from replicate ELISA assays were averaged. Supernatants were diluted 50-100-fold for ELISA analysis. Occasionally, higher dilutions were required to place signal within the range of the standard curve. All reagents for ELISA analyses were from BD Biosciences: OptEIA Reagent Set B (550534) was used for diluent and washes and OptEIA human IFN-γ ELISA kit (555142) and OptEIA human IL-2 ELISA kit (555190) were used for measuring IFN-γ and IL-2 release, respectively.

Functional co-culture assays – IncuCyte cell killing assay

Prior to co-culture for IncuCyte killing assays, a 96-well flat-bottom plate was coated with 100 µl of 0.001% poly-L-Lysine in PBS for 1 hour at 37 °C, washed 2 times with 200 µl PBS each, and air-dried briefly. Target cells were added and allowed to

settle at RT for 3 hours before the effector cells were added. Co-cultures typically employed 25,000 PBMCs and 25,000 target cells per well of a 96-well plate. In assays where multiple effector populations (bearing different TCRs) or multiple targets (bearing different MHC) were mixed, 25,000 of each cell type was used to yield a total of 75,000 or 100,000 cells per well (for single/mixed or mixed/mixed, respectively). The total volume for all wells was adjusted to 200 µL. Total green object area (μm^2/well) was quantified and its disappearance interpreted as killing of the GFP^+ target cells. Cells were imaged at two positions per well every 2 hours and these two images were added together for one data point. Data points obtained from 4-8 replicate co-cultures for each effector/target combination were used to plot graph curves and to calculate standard deviation.

Animals

NOD.Cg-PrkdcSCIDIL-2rgtm1Wjl/SzJ (NOD/SCID/IL-$2Rg^{-/-}$, NSG) mice were purchased from the Jackson Laboratory and maintained in the animal facilities at the University of California, Los Angeles (UCLA). Adult (16 weeks old) male mice were used for *in vivo* tumor challenge experiments. All animal experiments were approved by the Institutional Animal Care and Use Committee of UCLA.

Human prostate tumor xenograft mouse model

For xenograft tumor implantation, 10×10^6 PC-3/HLA-A2 cells (PC-3 cell line overexpressing HLA-A2) were s.c. injected on one flank of each mouse and 10×10^6 PC-3/HLA-A2/NY-ESO-1 cells (PC-3 cell line overexpressing HLA-A2 and NYESO) were subcutaneously injected on the other flank. Mice were allowed to develop solid tumors over the course of 1 week. On day 8 post tumor injection, mice were irradiated (100 rad) and then retro-orbitally i.v. injected with 8×10^6 purified T cells that were engineered to

express LNGFR only or together with a NY-ESO-1-specific TCR (1G4, 3A1, or 9D2). Mice were bled on day 3, 7, 10 and day 14 for flow cytometry analysis. On day 14, mice were euthanized and tumors were collected for immunohistology analysis.

Immunohistology

Solid tumors dissected out from the experimental mice were fixed in 10% neutral-buffered formalin and embedded in paraffin for sectioning (4 mm thickness), followed by hematoxylin and eosin (H/E) staining or antibody staining (for human CD3ε) by using standard procedures (UCLA Translational Pathology Core Laboratory). The sections were imaged using an Olympus BX51 upright microscope equipped with an Optronics Macrofire CCD camera (AU Optronics) at 4x and 40x magnifications. The images were analyzed by using Optronics PictureFrame software (AU Optronics) and Image J software (version 1.51J8). With Image J human CD3 antibody stained slides were quantified by measuring $CD3^+$ area through setting color threshold. Parameters used are as follow: thresholding method: default; threshold color: red; color space: HSB; brightness: 168-215.

Statistical analysis

Statistical analysis of tumor xenograft experiments was performed with one-way ANOVA followed by Tukey's multiple comparison test. Data are presented as the mean ± SEM. $P < 0.05$ was considered significant. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****$P < 0.0001$. All statistical analyses were performed with GraphPad PRISM software (version 6.0).

TABLE 1: TCR α/β POLYNUCLEOTIDE AND POLYPEPTIDE SEQUENCES

The following disclosure provides polynucleotide sequences of various embodiments of the invention and the variable region TCR protein sequences that they encode (e.g. the polynucleotide sequence of SEQ ID NO: 1 encodes the variable region TCR protein sequence of SEQ ID NO: 3).

3A1 TCR Vα DNA sequence

GGTCAACAGCTGAATCAGAGTCCTCAATCTATGTTTATCCAGGAAGGAGAAGATGTCTCCATG
AACTGCACTTCTTCAAGCATATTTAACACCTGGCTATGGTACAAGCAGGACCCTGGGGAAGGT
CCTGTCCTCTTGATAGCCTTATATAAGGCTGGTGAATTGACCTCAAATGGAAGACTGACTGCT
CAGTTTGGTATAACCAGAAAGGACAGCTTCCTGAATATCTCAGCATCCATACCTAGTGATGTA
GGCATCTACTTCTGTGCTGGATTTCTGGATAGCAACTATCAGTTAATCTGGGGCGCTGGGACC
AAGCTAATTATAAAGCCAGAT (SEQ ID NO: 1)

3A1 TCR Vβ DNA sequence

GAAGCCCAAGTGACCCAGAACCCAAGATACCTCATCACAGTGACTGGAAAGAAGTTAACAGT
GACTTGTTCTCAGAATATGAACCATGAGTATATGTCCTGGTATCGACAAGACCCAGGGCTGGG
CTTAAGGCAGATCTACTATTCAATGAATGTTGAGGTGACTGATAAGGGAGATGTTCCTGAAGG
GTACAAAGTCTCTCGAAAAGAGAAGAGGAATTTCCCCCTGATCCTGGAGTCGCCCAGCCCCA
ACCAGACCTCTCTGTACTTCTGTGCCAGCGCTAGCGGGTACCGCACAGATACGCAGTATTTTG
GCCCAGGCACCCGGCTGACAGTGCTCGAGGAC (SEQ ID NO: 2)

3A1 TCR Vα protein sequence

GQQLNQSPQSMFIQEGEDVSMNCTSSSIFNTWLWYKQDPGEGPVLLIALYKAGELTSNGRLTAQF
GITRKDSFLNISASIPSDVGIYFCAGFLDSNYQLIWGAGTKLIIKPD (SEQ ID NO: 3)

3A1 TCR Vβ protein sequence

EAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEG
YKVSRKEKRNFPLILESPSPNQTSLYFCASASGYRTDTQYFGPGTRLTVLED (SEQ ID NO: 4)

4A2 TCR Vα DNA sequence

GCTCAGTCAGTGGCTCAGCCGGAAGATCAGGTCAACGTTGCTGAAGGGAATCCTCTGACTGTG
AAATGCACCTATTCAGTCTCTGGAAACCCTTATCTTTTTTGGTATGTTCAATACCCCAACCGAG
GCCTCCAGTTCCTTCTGAAATACATCACAGGGGATAACCTGGTTAAAGGCAGCTATGGCTTTG
AAGCTGAATTTAACAAGAGCCAAACCTCCTTCCACCTGAAGAAACCATCTGCCCTTGTGAGCG
ACTCCGCTTTGTACTTCTGTGCTGTGAGAGACAGTCGGTCTGGGGCTGGGAGTTACCAACTCA
CTTTCGGGAAGGGGACCAAACTCTCGGTCATACCAAAT (SEQ ID NO: 5)

4A2 TCR Vβ DNA sequence

GGTGCTGTCGTCTCTCAACATCCGAGCTGGGTTATCTGTAAGAGTGGAACCTCTGTGAAGATC
GAGTGCCGTTCCCTGGACTTTCAGGCCACAACTATGTTTTGGTATCGTCAGTTCCCGAAACAG
AGTCTCATGCTGATGGCAACTTCCAATGAGGGCTCCAAGGCCACATACGAGCAAGGCGTCGA
GAAGGACAAGTTTCTCATCAACCATGCAAGCCTGACCTTGTCCACTCTGACAGTGACCAGTGC
CCATCCTGAAGACAGCAGCTTCTACATCTGCAGTGCTCCCCAAGGTTATGGGGGCACAGATAC
GCAGTATTTTGGCCCAGGCACCCGGCTGACAGTGCTCGAGGAC (SEQ ID NO: 6)

4A2 TCR Vα protein sequence

AQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYITGDNLVKGSYGFE
AEFNKSQTSFHLKKPSALVSDSALYFCAVRDSRSGAGSYQLTFGKGTKLSVIPN (SEQ ID NO: 7)

4A2 TCR Vβ protein sequence

GAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFWYRQFPKQSLMLMATSNEGSKATYEQGVEK
DKFLINHASLTLSTLTVTSAHPEDSSFYICSAPQGYGGTDTQYFGPGTRLTVLED (SEQ ID NO: 37)

5G6 TCR Vα DNA sequence

GATGCTAAGACCACACAGCCAAATTCAATGGAGAGTAACGAAGAAGAGCCTGTTCACTTGCC
TTGTAACCACTCCACAATCAGTGGAACTGATTACATACATTGGTATCGACAGCTTCCCTCCCA
GGGTCCAGAGTACGTGATTCATGGTCTTACAAGCAATGTGAACAACAGAATGGCCTCTCTGGC
AATCGCTGAAGACAGAAAGTCCAGTACCTTGATCCTGCACCGTGCTACCTTGAGAGATGCTGC
TGTGTACTACTGCATCCTGAGAACCTCTGGGGCTGGGAGTTACCAACTCACTTTCGGGAAGGG
GACCAAACTCTCGGTCATACCAAAT (SEQ ID NO: 8)

5G6 TCR Vβ DNA sequence

AGTGCTGTCATCTCTCAAAAGCCAAGCAGGGATATCTGTCAACGTGGAACCTCCCTGACGATC
CAGTGTCAAGTCGATAGCCAAGTCACCATGATGTTCTGGTACCGTCAGCAACCTGGACAGAGC
CTGACACTGATCGCAACTGCAAATCAGGGCTCTGAGGCCACATATGAGAGTGGATTTGTCATT
GACAAGTTTCCCATCAGCCGCCCAAACCTAACATTCTCAACTCTGACTGTGAGCAACATGAGC
CCTGAAGACAGCAGCATATATCTCTGCAGCGCGGGAGGAGCGGGAGCGTCAGATACGCAGTA
TTTTGGCCCAGGCACCCGGCTGACAGTGCTCGAGGAC (SEQ ID NO: 9)

5G6 TCR Vα protein sequence

DAKTTQPNSMESNEEEPVHLPCNHSTISGTDYIHWYRQLPSQGPEYVIHGLTSNVNNRMASLAIAE
DRKSSTLILHRATLRDAAVYYCILRTSGAGSYQLTFGKGTKLSVIPN (SEQ ID NO: 10)

5G6 TCR Vβ protein sequence

SAVISQKPSRDICQRGTSLTIQCQVDSQVTMMFWYRQQPGQSLTLIATANQGSEATYESGFVIDKFP
ISRPNLTFSTLTVSNMSPEDSSIYLCSAGGAGASDTQYFGPGTRLTVLED (SEQ ID NO: 11)

9D2 TCR Vα DNA sequence

CAGAAGGAGGTGGAGCAGAATTCTGGACCCCTCAGTGTTCCAGAGGGAGCCATTGCCTCTCTC
AACTGCACTTACAGTGACCGAGGTTCCCAGTCCTTCTTCTGGTACAGACAATATTCTGGGAAA
AGCCCTGAGTTGATAATGTTCATATACTCCAATGGTGACAAAGAAGATGGAAGGTTTACAGCA
CAGCTCAATAAAGCCAGCCAGTATGTTTCTCTGCTCATCAGAGACTCCCAGCCCAGTGATTCA
GCCACCTACCTCTGTGCCGTAGATGACAAGATCATCTTTGGAAAAGGGACACGACTTCATATT
CTCCCCAAT (SEQ ID NO: 12)

9D2 TCR Vβ DNA sequence

GATGCTGGAGTTATCCAGTCACCCCGGCACGAGGTGACAGAGATGGGACAAGAAGTGACTCT
GAGATGTAAACCAATTTCAGGACACGACTACCTTTTCTGGTACAGACAGACCATGATGCGGGG
ACTGGAGTTGCTCATTTACTTTAACAACAACGTTCCGATAGATGATTCAGGGATGCCCGAGGA
TCGATTCTCAGCTAAGATGCCTAATGCATCATTCTCCACTCTGAAGATCCAGCCCTCAGAACC
CAGGGACTCAGCTGTGTACTTCTGTGCCAGCAGTTTGGGACAGCCAAGCACAGATACGCAGTA
TTTTGGCCCAGGCACCCGGCTGACAGTGCTCGAGGAC (SEQ ID NO: 13)

9D2 TCR Vα protein sequence

QKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGDKEDGRFTAQLN
KASQYVSLLIRDSQPSDSATYLCAVDDKIIFGKGTRLHILPN (SEQ ID NO: 14)

9D2 TCR Vβ protein sequence

DAGVIQSPRHEVTEMGQEVTLRCKPISGHDYLFWYRQTMMRGLELLIYFNNNVPIDDSGMPEDRF
SAKMPNASFSTLKIQPSEPRDSAVYFCASSLGQPSTDTQYFGPGTRLTVLED (SEQ ID NO: 15)

1E4 TCR Vα DNA sequence

AAACAGGAGGTGACGCAGATTCCTGCAGCTCTGAGTGTCCCAGAAGGAGAAAACTTGGTTCT
CAACTGCAGTTTCACTGATAGCGCTATTTACAACCTCCAGTGGTTTAGGCAGGACCCTGGGAA
AGGTCTCACATCTCTGTTGCTTATTCAGTCAAGTCAGAGAGAGCAAACAAGTGGAAGACTTAA
TGCCTCGCTGGATAAATCATCAGGACGTAGTACTTTATACATTGCAGCTTCTCAGCCTGGTGA
CTCAGCCACCTACCTCTGTGCTGTGAGTACTGCGTATTCAGGAGGAGGTGCTGACGGACTCAC
CTTTGGCAAAGGGACTCATCTAATCATCCAGCCCTAT (SEQ ID NO: 16)

1E4 TCR Vβ DNA sequence

GATACTGGAGTCTCCCAGAACCCCAGACACAAGATCACAAAGAGGGGACAGAATGTAACTTT
CAGGTGTGATCCAATTTCTGAACACAACCGCCTTTATTGGTACCGACAGACCCTGGGGCAGGG
CCCAGAGTTTCTGACTTACTTCCAGAATGAAGCTCAACTAGAAAAATCAAGGCTGCTCAGTGA
TCGGTTCTCTGCAGAGAGGCCTAAGGGATCTTTCTCCACCTTGGAGATCCAGCGCACAGAGCA
GGGGGACTCGGCCATGTATCTCTGTGCCAGCAGCCCCCCGACTGTTCGGGTCTATGGCTACAC
CTTCGGTTCGGGGACCAGGTTAACCGTTGTAGAGGAC (SEQ ID NO: 17)

1E4 TCR Vα protein sequence

KQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLTSLLLIQSSQREQTSGRLNASLD
KSSGRSTLYIAASQPGDSATYLCAVSTAYSGGGADGLTFGKGTHLIIQPY (SEQ ID NO: 18)

1E4 TCR Vβ protein sequence

DTGVSQNPRHKITKRGQNVTFRCDPISEHNRLYWYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFS
AERPKGSFSTLEIQRTEQGDSAMYLCASSPPTVRVYGYTFGSGTRLTVVED (SEQ ID NO: 19)

2B8 TCR Vα DNA sequence

GGACAACAGGTAATGCAAATTCCTCAGTACCAGCATGTACAAGAAGGAGAAGACTTCACCAC
GTACTGCAATTCCTCAACTACTTTAAGCAATATACAGTGGTATAAGCAAAGGCCTGGTGGACA
TCCCGTTTTTTTGATACAGTTAGTGAAGAGTGGAGAAGTGAAGAAGCAGAAAAGACTGACAT
TTCAGTTTGGAGAAGCAAAAAAGAACAGCTCCCTGCACATCACAGCCACCCAGACTACAGAT
GTAGGAACCTACTTCTGTGCGGACCCTAACTTTGGAAATGAGAAATTAACCTTTGGGACTGGA
ACAAGACTCACCATCATACCCAAT (SEQ ID NO: 20)

2B8 TCR Vβ DNA sequence

GAAGCCCAAGTGACCCAGAACCCAAGATACCTCATCACAGTGACTGGAAAGAAGTTAACAGT
GACTTGTTCTCAGAATATGAACCATGAGTATATGTCCTGGTATCGACAAGACCCAGGGCTGGG
CTTAAGGCAGATCTACTATTCAATGAATGTTGAGGTGACTGATAAGGGAGATGTTCCTGAAGG
GTACAAAGTCTCTCGAAAAGAGAAGAGGAATTTCCCCCTGATCCTGGAGTCGCCCAGCCCCA
ACCAGACCTCTCTGTACTTCTGTGCCAGCAGTTTGAATCCCTTTGCAACTAATGAAAAACTGTT
TTTTGGCAGTGGAACCCAGCTCTCTGTCTTGGAGGAC (SEQ ID NO: 21)

2B8 TCR Vα protein sequence

GQQVMQIPQYQHVQEGEDFTTYCNSSTTLSNIQWYKQRPGGHPVFLIQLVKSGEVKKQKRLTFQF
GEAKKNSSLHITATQTTDVGTYFCADPNFGNEKLTFGTGTRLTIIPN (SEQ ID NO: 22)

2B8 TCR Vβ protein sequence

EAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEG
YKVSRKEKRNFPLILESPSPNQTSLYFCASSLNPFATNEKLFFGSGTQLSVLED (SEQ ID NO: 23)

3C7 TCR Vα DNA sequence

GGACAAAACATTGACCAGCCCACTGAGATGACAGCTACGGAAGGTGCCATTGTCCAGATCAA
CTGCACGTACCAGACATCTGGGTTCAACGGGCTGTTCTGGTACCAGCAACATGCTGGCGAAGC
ACCTACATTTCTGTCTTACAATGTTCTGGATGGTTTGGAGGAGAAAGGTCGTTTTTCTTCATTC
CTTAGTCGGTCTAAAGGGTACAGTTACCTCCTTTTGAAGGAGCTCCAGATGAAAGACTCTGCC
TCTTACCTCTGTGCTGTGAGAGGCGACTACAAGCTCAGCTTTGGAGCCGGAACCACAGTAACT
GTAAGAGCAAAT (SEQ ID NO: 24)

3C7 TCR Vβ DNA sequence

GATTCTGGAGTCACACAAACCCCAAAGCACCTGATCACAGCAACTGGACAGCGAGTGACGCT
GAGATGCTCCCCTAGGTCTGGAGACCTCTCTGTGTACTGGTACCAACAGAGCCTGGACCAGGG
CCTCCAGTTCCTCATTCAGTATTATAATGGAGAAGAGAGAGCAAAAGGAAACATTCTTGAACG
ATTCTCCGCACAACAGTTCCCTGACTTGCACTCTGAACTAAACCTGAGCTCTCTGGAGCTGGG
GGACTCAGCTTTGTATTTCTGTGCCAGCAGCTCGATACACGGTGTCTCTGGGGCCAACGTCCT
GACTTTCGGGGCCGGCAGCAGGCTGACCGTGCTGGAGGAC (SEQ ID NO: 25)

3C7 TCR Vα protein sequence

GQNIDQPTEMTATEGAIVQINCTYQTSGFNGLFWYQQHAGEAPTFLSYNVLDGLEEKGRFSSFLSR
SKGYSYLLLKELQMKDSASYLCAVRGDYKLSFGAGTTVTVRAN (SEQ ID NO: 26)

3C7 TCR Vβ protein sequence

DSGVTQTPKHLITATGQRVTLRCSPRSGDLSVYWYQQSLDQGLQFLIQYYNGEERAKGNILERFSA
QQFPDLHSELNLSSLELGDSALYFCASSSIHGVSGANVLTFGAGSRLTVLED (SEQ ID NO: 27)

NY-ESO-1 protein (Homo sapiens): GenBank: CAA05908.1

MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPGGGAPRG
PHGGAASGLNGCCRCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTV
SGNILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRR (SEQ ID NO: 28)

Terminology used in the disclosure such as "A2/NY-ESO-$1_{157-165}$" refers to HLA A2 associated with a NY-ESO-1 peptide comprising amino acids 157-165 of the above protein sequence (i.e. SLLMWITQC (SEQ ID NO: 36)).

The following sequences comprise polynucleotide embodiments of the invention disposed in a vector.

pMTB1328 (MSGV-LNGFR-P2A-GB4A2 TCR Mouse Constant)

tgaaagaccccacctgtaggtttggcaagctagcttaagtaacgccattttgcaaggcatggaaaatacataactgagaatagagaagttcagatcaaggtta
ggaacagagagacagcagaatatgggccaaacaggatatctgtggtaagcagttcctgccccggctcagggccaagaacagatggtccccagatgcgg
tcccgccctcagcagtttctagagaaccatcagatgtttccagggtgccccaaggacctgaaaatgaccctgtgccttatttgaactaaccaatcagttcgctt
ctcgcttctgttcgcgcgcttctgctcccgagctcaataaaagagcccacaacccctcactcggcgcgccagtcctccgatagactgcgtcgcccgggta
cccgtattcccaataaagcctcttgctgtttgcatccgaatcgtggactcgctgatccttgggagggtctcctcagattgattgactgcccacctcgggggtctt

tcatttggaggttccaccgagatttggagacccctgcctagggaccaccgacccccccgccgggaggtaagctggccagcggtcgtttcgtgtctgtctct
gtcttttgtgcgtgtttgtgccggcatctaatgtttgcgcctgcgtctgtactagttagctaactagctctgtatctggcggacccgtggtggaactgacgagttcg
gaacacccggccgcaaccctgggagacgtcccagggacttcgggggccgtttttgtggcccgacctgagtcctaaaatcccgatcgttaggactctttggt
gcacccccctagaggagggatatgtggttctggtaggagacgagaacctaaaacagttcccgcctccgtctgaatttttgctttcggtttgggaccgaagcc
gcgccgcgcgtcttgtctgctgcagcatcgttctgtgttgtctctgtctgactgtgtttctgtatttgtctgaaaatatgggcccgggctagcctgttaccactccc
ttaagtttgaccttaggtcactggaaagatgtcgagcggatcgctcacaaccagtcggtagatgtcaagaagagacgttgggttaccttctgctctgcagaat
ggccaacctttaacgtcggatggccgcgagacggcaccttaacgagacctcatcacccaggttaagatcaaggtcttttcacctggcccgcatggacac
ccagaccaggtcccctacatcgtgacctgggaagccttggcttttgaccccctccctgggtcaagccctttgtacaccctaagcctccgcctcctcttcctcc
atccgcccccgtctctccccctgaacctcctcgttcgacccccgcctcgatcctccctttatccagccctcactccttctctaggcgccccatatggccatatga
gatcttatatggggcaccccccgccccttgtaaacttccctgaccctgacatgacaagagttactaacagcccctctctccaagctcacttacaggctctctact
agtccagcacgaagtctggagacctctggcggcagcctaccaagaacaactggaccgaccggtggtacctcacccttaccgagtcggcgacacagtgtg
ggtccgccgacaccagactaagaacctagaacctcgctggaaaggaccttacacagtcctgctgaccaccccccaccgccctcaaagtagacggcatcgc
agcttggatacacgccgcccacgtgaaggctgccgaccccgggggtggaccatcctctagaccgccatgtcgggggcaggtgccaccggccgcgcca
tggacgggccgcgcctgctgttgctgcttctgggggtgtcccttggaggtgccaaggaggcatgccccacaggcctgtacacacacagcggtgagtg
ctgcaaagcctgcaacctgggcgagggtgtggcccagccttgtggagccaaccagaccgtgtgtgagccctgcctggacagcgtgacgttctccgacgt
ggtgagcgcgaccgagccgtgcaagccgtgcaccgagtgcgtggggctccagagcatgtcggcgccatgcgtggaggccgacgacgccgtgtgccg
ctgcgcctacggctactaccaggatgagacgactgggcgctgcgaggcgtgccgcgtgtgcgaggcgggctcgggcctcgtgttctcctgccaggaca
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PUBLICATIONS

All publications mentioned herein (e.g. those listed numerically herein) are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. Publications cited herein are cited for their disclosure prior to the filing date of the present application. Nothing here is to be construed as an admission that the inventors are not entitled to antedate the publications by virtue of an earlier priority date or prior date of invention. Further, the actual publication dates may be different from those shown and require independent verification. The following references include descriptions of methods and materials in this field of technology.

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55. Davis ID, *et al.* (2004) Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4(+) and CD8(+) T cell responses in humans. *Proc Natl Acad Sci U S A* 101(29):10697-10702.
56. Britten CM, *et al.* (2012) T cell assays and MIATA: the essential minimum for maximum impact. *Immunity* 37(1):1-2.

CONCLUSION

This concludes the description of the illustrative embodiments of the present invention. The foregoing description of one or more embodiments of the invention has been presented for the purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed. Many modifications and variations are possible in light of the above teaching.

CLAIMS:

1. A polynucleotide disposed in a vector, wherein:

the polynucleotide encodes a Vα T cell receptor polypeptide and/or a Vβ T cell receptor polypeptide; and

when a Vα/Vβ T cell receptor comprising the Vα T cell receptor polypeptide and/or the Vβ T cell receptor polypeptide is expressed in a CD 8^+ T cell, the Vα/Vβ T cell receptor recognizes a NY-ESO-1 peptide associated with:

human leukocyte antigen A2;

human leukocyte antigen B07;

human leukocyte antigen B18; or

human leukocyte antigen C03.

2. The polynucleotide of claim 1, wherein the T cell receptor comprises:

a 3A1 T cell receptor;

a 4A2 T cell receptor;

a 5G6 T cell receptor;

a 9D2 T cell receptor;

a 1E4 T cell receptor;

a 2B8 T cell receptor; or

a 3C7 T cell receptor.

3. The polynucleotide of claim 2, wherein the vector comprises at least one of:

(a) a polynucleotide encoding a 3A1 Vα polypeptide (SEQ ID NO: 3);

(b) a polynucleotide encoding a 3A1 Vβ polypeptide (SEQ ID NO: 4);

(c) a polynucleotide encoding a 4A2 Vα polypeptide (SEQ ID NO: 7);

(d) a polynucleotide encoding a 4A2 Vβ polypeptide (SEQ ID NO: 37);

(e) a polynucleotide encoding a 5G6 Vα polypeptide (SEQ ID NO: 10);

(f) a polynucleotide encoding a 5G6 Vβ polypeptide (SEQ ID NO: 11);

(g) a polynucleotide encoding a 9D2 Vα polypeptide (SEQ ID NO: 14);

(h) a polynucleotide encoding a 9D2 Vβ polypeptide (SEQ ID NO: 15);
(i) a polynucleotide encoding a 1E4 Vα polypeptide (SEQ ID NO: 18);
(j) a polynucleotide encoding a 1E4 Vβ polypeptide (SEQ ID NO: 19);
(k) a polynucleotide encoding a 2B8 Vα polypeptide (SEQ ID NO: 22);
(l) a polynucleotide encoding a 2B8 Vβ polypeptide (SEQ ID NO: 23);
(m) a polynucleotide encoding a 3C7 Vα polypeptide (SEQ ID NO: 26); or
(n) a polynucleotide encoding a 3C7 Vβ polypeptide (SEQ ID NO: 27).

4. The polynucleotide of claim 3, wherein the vector comprises a polynucleotide sequence that modulates expression of the polypeptide within CD 8^+ T cells.

5. The polynucleotide of claim 4, wherein the vector is a Sendai viral vector, an adenoviral vector, an adeno-associated virus vector, a retroviral vector, or a lentiviral vector.

6. The polynucleotide of claim 1, wherein the vector comprises a polynucleotide encoding a Vα polypeptide or a polynucleotide encoding a Vβ polypeptide.

7. The polynucleotide of claim 1, wherein the vector comprises a polynucleotide encoding a Vα polypeptide in combination with a polynucleotide encoding a Vβ polypeptide disposed in the vector so that a Vα/Vβ T cell receptor (TCR) is expressed on the surface of a CD 8^+ T cell.

8. A composition of matter comprising a host cell transduced with a vector of any one of claims 1-7.

9. The composition of claim 8, wherein the host cell is a human CD 8^+ T cell.

10. The composition of claim 9, wherein the composition is a pharmaceutical composition comprising one more pharmaceutically acceptable excipients selected from

the group consisting of buffering agents, antimicrobial agents, tonicity adjusting agents, wetting agents, detergents and pH adjusting agents.

11. The composition of claim 10, wherein:

the $CD8^+$ T cell is obtained from an individual diagnosed with a cancer that expresses a NY-ESO-1 antigen; and

the $CD8^+$ T cell is transduced with a vector comprising a polynucleotide encoding a TCR Vα polypeptide in combination with a polynucleotide encoding a TCR Vβ polypeptide such that a heterologous TCR is expressed on a surface of the $CD8^+$ T cell, wherein the heterologous TCR recognizes a NY-ESO-1 peptide associated with a human leukocyte antigen expressed on the surface of cells of the cancer.

12. The composition of claim 11, wherein the vector is a retroviral vector.

13. A method of killing cancer cells that express a NY-ESO-1 antigen, the method comprising combining the cancer cells with $CD8^+$ T cells of claim 9 under conditions that allow a heterologous TCR to be expressed on the surface of the $CD8^+$ T cell and recognize NY-ESO-1 peptides associated with a human leukocyte antigens expressed on the surface of cells of the cancer, so that the cancer cells are recognized and killed.

14. The method of claim 13, wherein the method is performed *in vivo* on a patient infused with the $CD8^+$ T cells.

15. The method of claim 13, wherein the cancer cells form solid tumors.

16. The method of claim 13, wherein the cancer cells are neuroblastoma cells, myeloma cells, metastatic melanoma cells, synovial sarcoma cells, bladder cancer cells, esophageal cancer cells, hepatocellular cancer cells, head and neck cancer cells, non-small cell lung cancer cells, ovarian cancer cells, prostate cancer cells, or breast cancer cells.

17. The method of claim 13, wherein the method comprises administering a first modified $CD8^+$ T cell that targets a NY-ESO-1 peptide associated with a first human leukocyte antigen human leukocyte antigen in combination with a second $CD8^+$ T cell that targets a NY-ESO-1 peptide associated with second human leukocyte antigen.

18. The use of the polynucleotide of claim 1 or the $CD8^+$ T cell of claim 7 for the manufacture of a medicament for the treatment of a cancer.

19. The use of claim 18, wherein the polynucleotide comprises at least one of:
(a) a polynucleotide encoding a 3A1 Vα polypeptide (SEQ ID NO: 3);
(b) a polynucleotide encoding a 3A1 Vβ polypeptide (SEQ ID NO: 4);
(c) a polynucleotide encoding a 4A2 Vα polypeptide (SEQ ID NO: 7);
(d) a polynucleotide encoding a 4A2 Vβ polypeptide (SEQ ID NO: 37);
(e) a polynucleotide encoding a 5G6 Vα polypeptide (SEQ ID NO: 10);
(f) a polynucleotide encoding a 5G6 Vβ polypeptide (SEQ ID NO: 11);
(g) a polynucleotide encoding a 9D2 Vα polypeptide (SEQ ID NO: 14);
(h) a polynucleotide encoding a 9D2 Vβ polypeptide (SEQ ID NO: 15);
(i) a polynucleotide encoding a 1E4 Vα polypeptide (SEQ ID NO: 18);
(j) a polynucleotide encoding a 1E4 Vβ polypeptide (SEQ ID NO: 19);
(k) a polynucleotide encoding a 2B8 Vα polypeptide (SEQ ID NO: 22);
(l) a polynucleotide encoding a 2B8 Vβ polypeptide (SEQ ID NO: 23);
(m) a polynucleotide encoding a 3C7 Vα polypeptide (SEQ ID NO: 26); or
(n) a polynucleotide encoding a 3C7 Vβ polypeptide (SEQ ID NO: 27).

20. The use of claim 19, wherein the cancer is a melanoma, neuroblastoma, a myeloma, a metastatic melanoma, a synovial sarcoma, a bladder cancer, an esophageal cancer, a hepatocellular cancer, a head and neck cancer, a non-small cell lung cancer, an ovarian cancer, a prostate cancer, or a breast cancer.

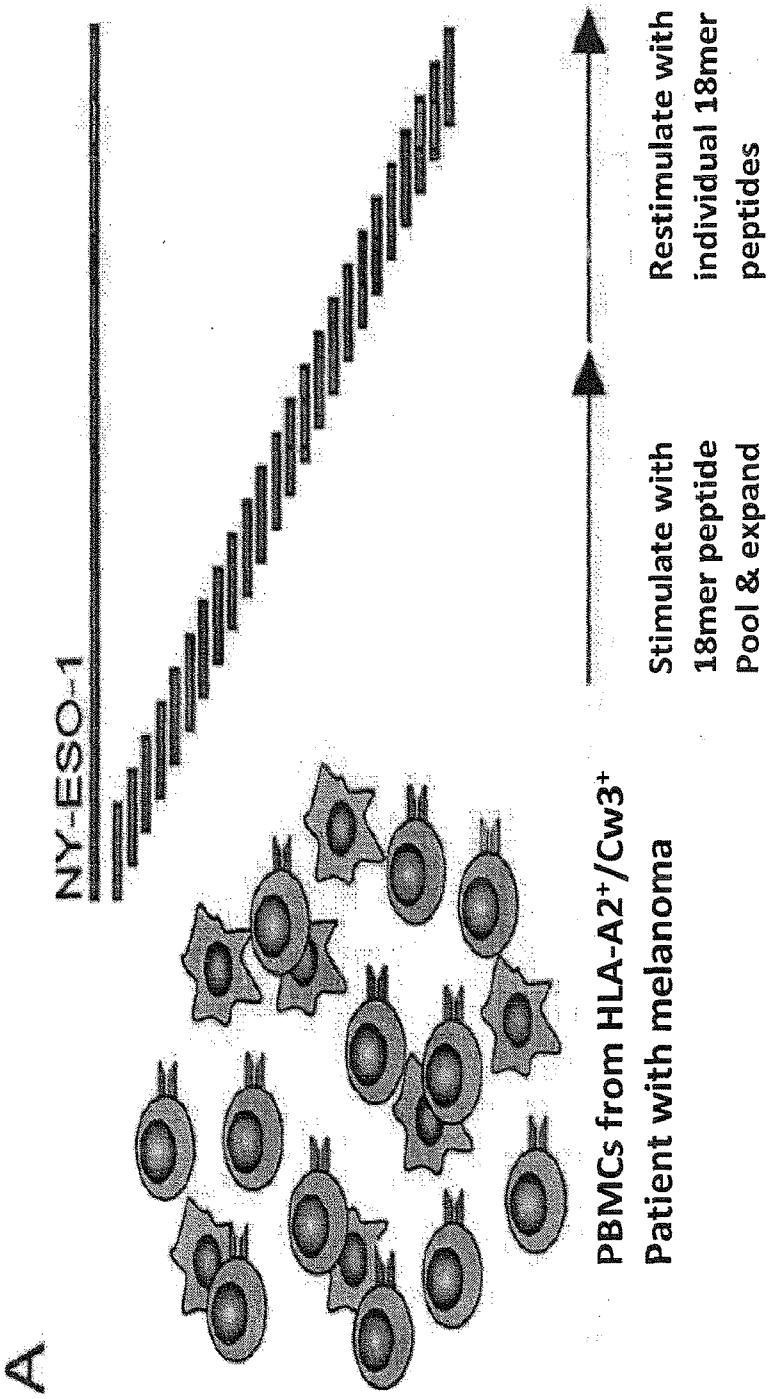

A
NY-ESO-1
PBMCs from HLA-A2+/Cw3+
Patient with melanoma
Stimulate with
18mer peptide
Pool & expand
Restimulate with
individual 18mer
peptides

FIG. 1A

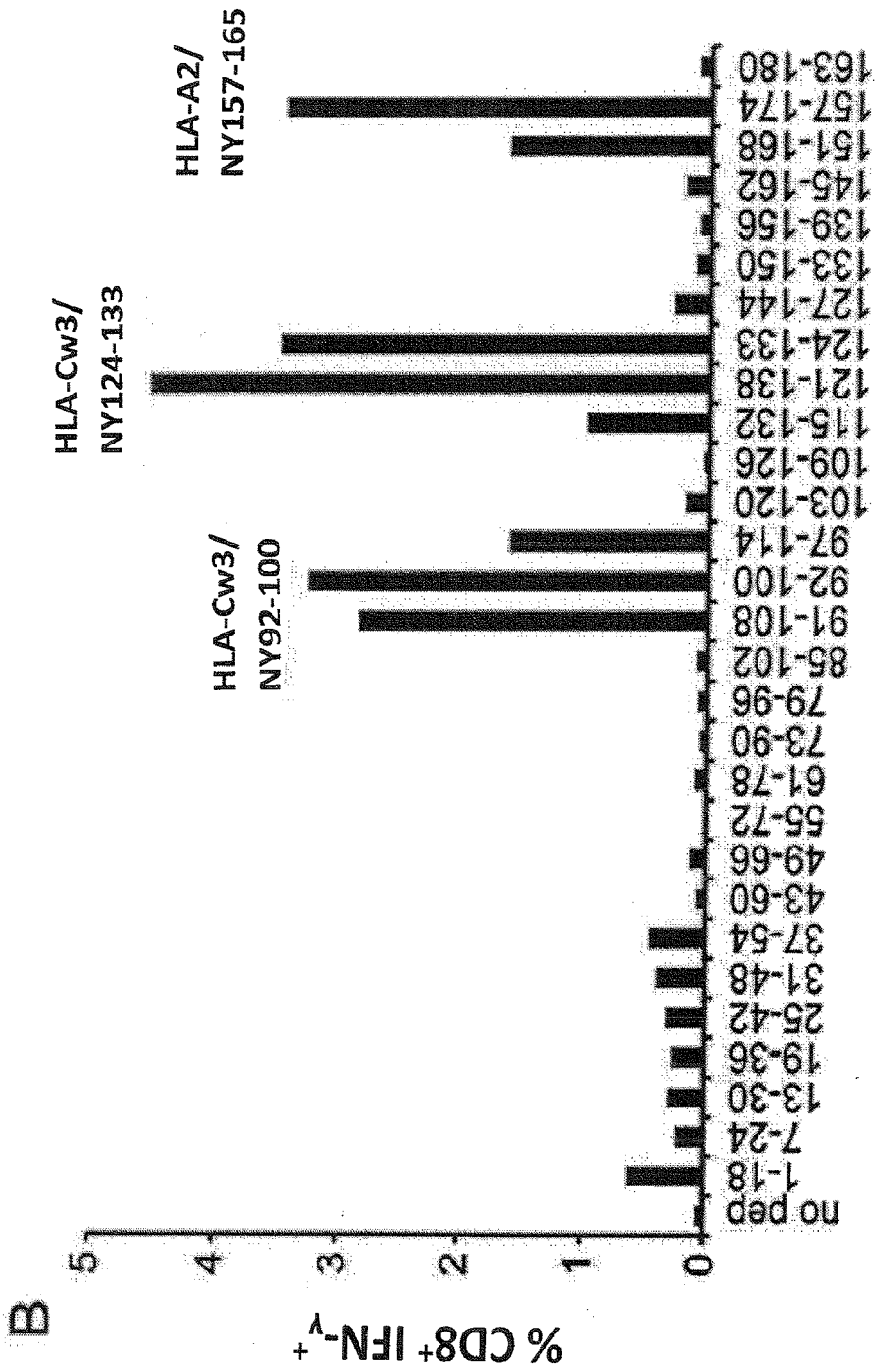
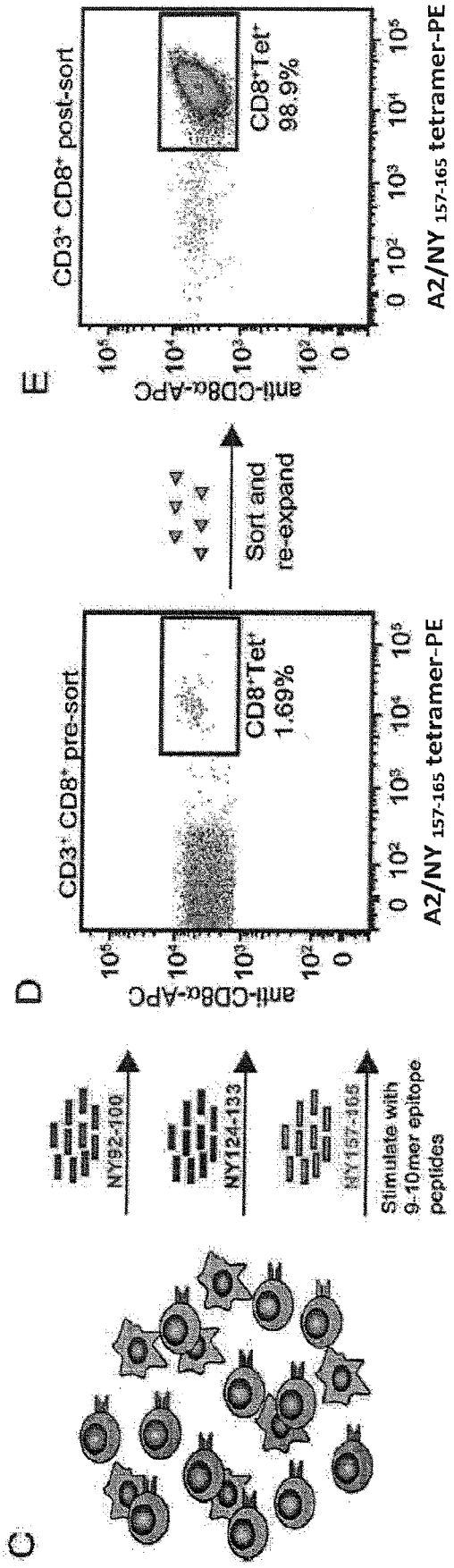

B
% CD8+ IFN-γ+
0
1
2
3
4
5
no pep
1-18
7-24
13-30
19-36
25-42
31-48
37-54
43-60
49-66
55-72
61-78
73-90
79-96
85-102
91-108
92-100
97-114
103-120
109-126
115-132
121-138
124-133
127-144
133-150
139-156
145-162
151-168
157-174
163-180
HLA-A2/ NY157-165
HLA-Cw3/ NY124-133
HLA-Cw3/ NY92-100

FIG. 1B


C
NY92-100
NY124-133
NY157-165
Stimulate with 9-10mer epitope peptides
D
CD3+ CD8+ pre-sort
anti-CD8α-APC
A2/NY 157-165 tetramer-PE
CD8+Tet+ 1.69%
Sort and re-expand
E
CD3+ CD8+ post-sort
anti-CD8α-APC
A2/NY 157-165 tetramer-PE
CD8+Tet+ 98.9%

FIGS. 1C-1E

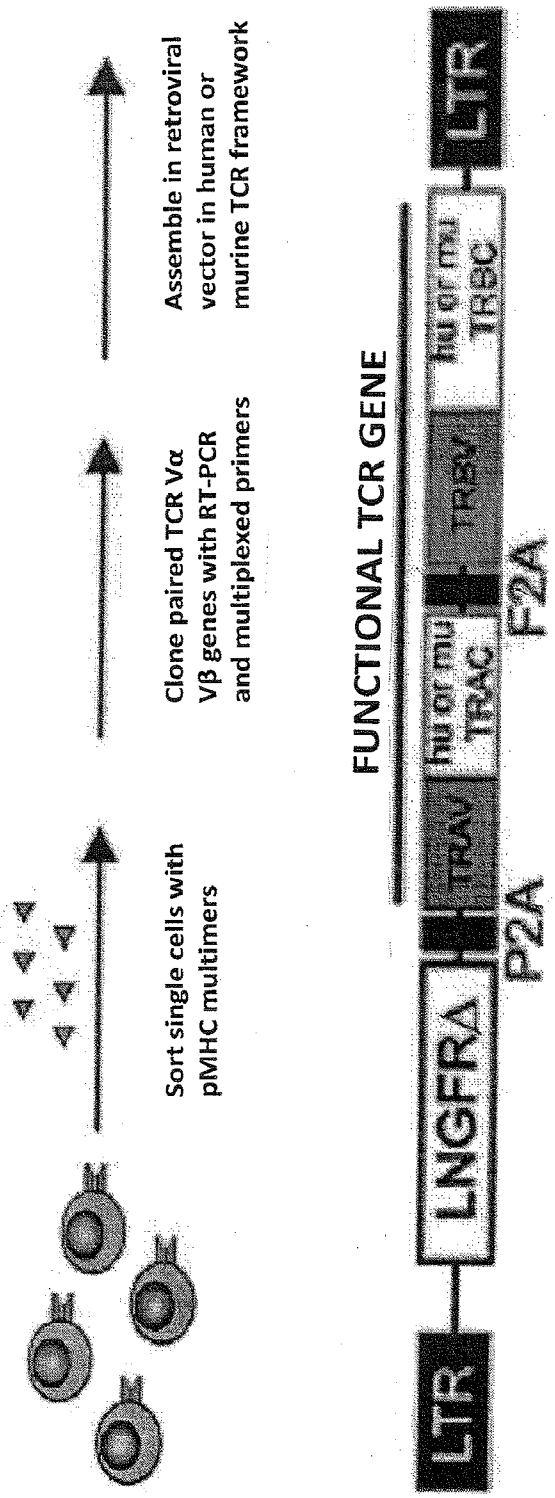
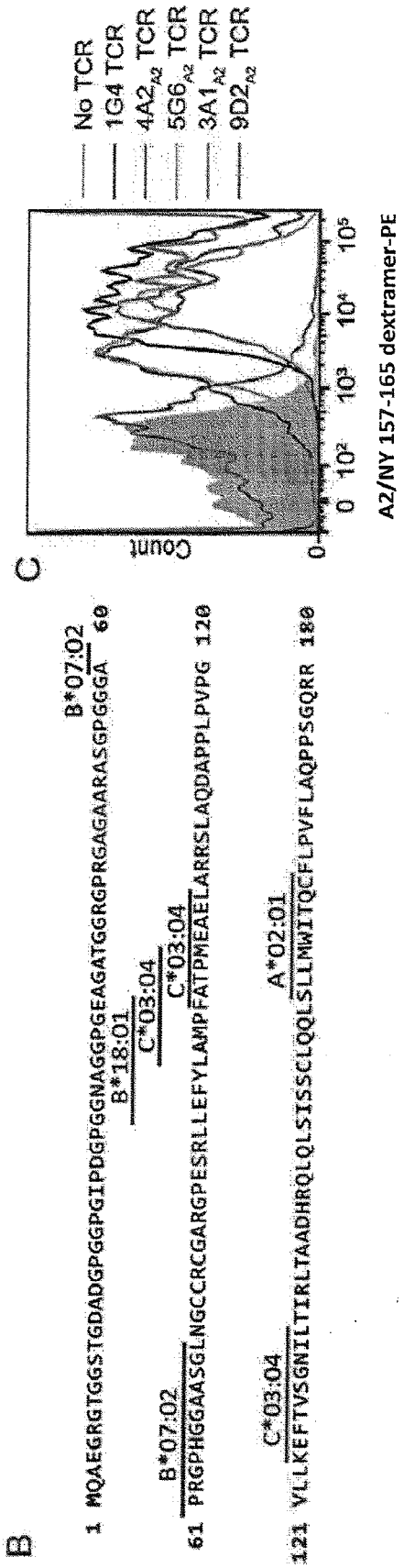

Sort single cells with pMHC multimers
Clone paired TCR Vα Vβ genes with RT-PCR and multiplexed primers
Assemble in retroviral vector in human or murine TCR framework
FUNCTIONAL TCR GENE
LTR
LNGFRΔ
P2A
TRAV
hu or mu TRAC
F2A
TRBV
hu or mu TRBC
LTR

FIG. 2A


B
B*07:02
1 MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPGGGA 60
B*18:01
C*03:04
B*07:02
C*03:04
61 PRGPHGGAASGLNGCCRCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPG 120
A*02:01
C*03:04
121 VLLKEFTVSGNILTIRLTAADHRQLQLSISSCLQQLSLLMWITQCFLPVFLAQPPSGQRR 180
C
No TCR
1G4 TCR
4A2A2 TCR
5G6A2 TCR
3A1A2 TCR
9D2A2 TCR
Count
0
0 10² 10³ 10⁴ 10⁵
A2/NY 157-165 dextramer-PE

FIGS. 2B-2C

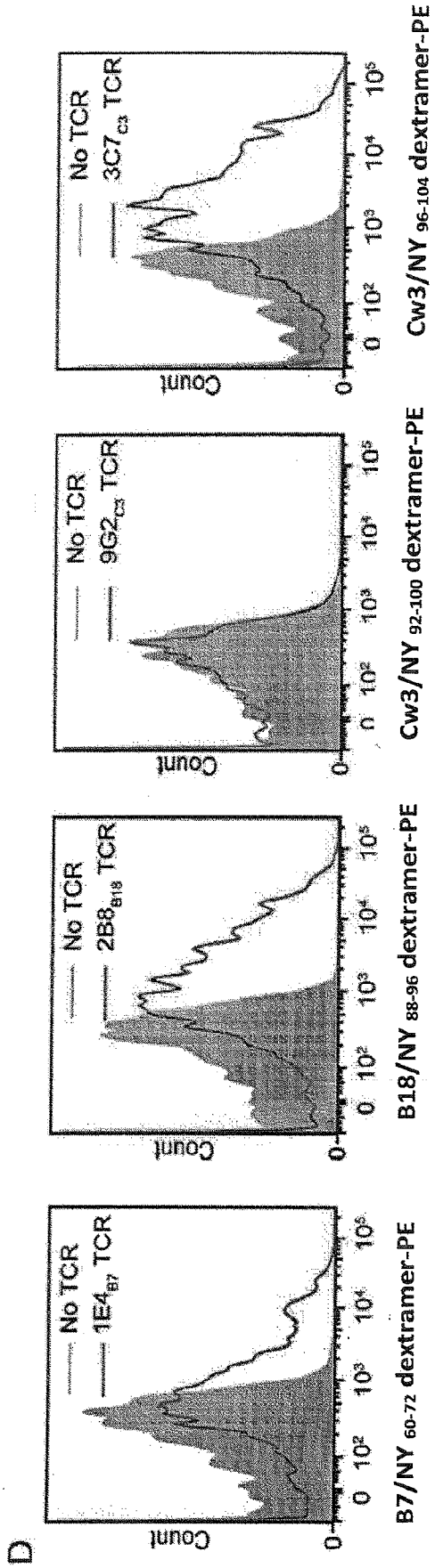

D
No TCR
1E4B7 TCR
Count
0 10² 10³ 10⁴ 10⁵
B7/NY 60-72 dextramer-PE
No TCR
2B8B18 TCR
Count
0 10² 10³ 10⁴ 10⁵
B18/NY 88-96 dextramer-PE
No TCR
9G2C3 TCR
Count
0 10² 10³ 10⁴ 10⁵
Cw3/NY 92-100 dextramer-PE
No TCR
3C7C3 TCR
Count
0 10² 10³ 10⁴ 10⁵
Cw3/NY 96-104 dextramer-PE

FIG. 2D

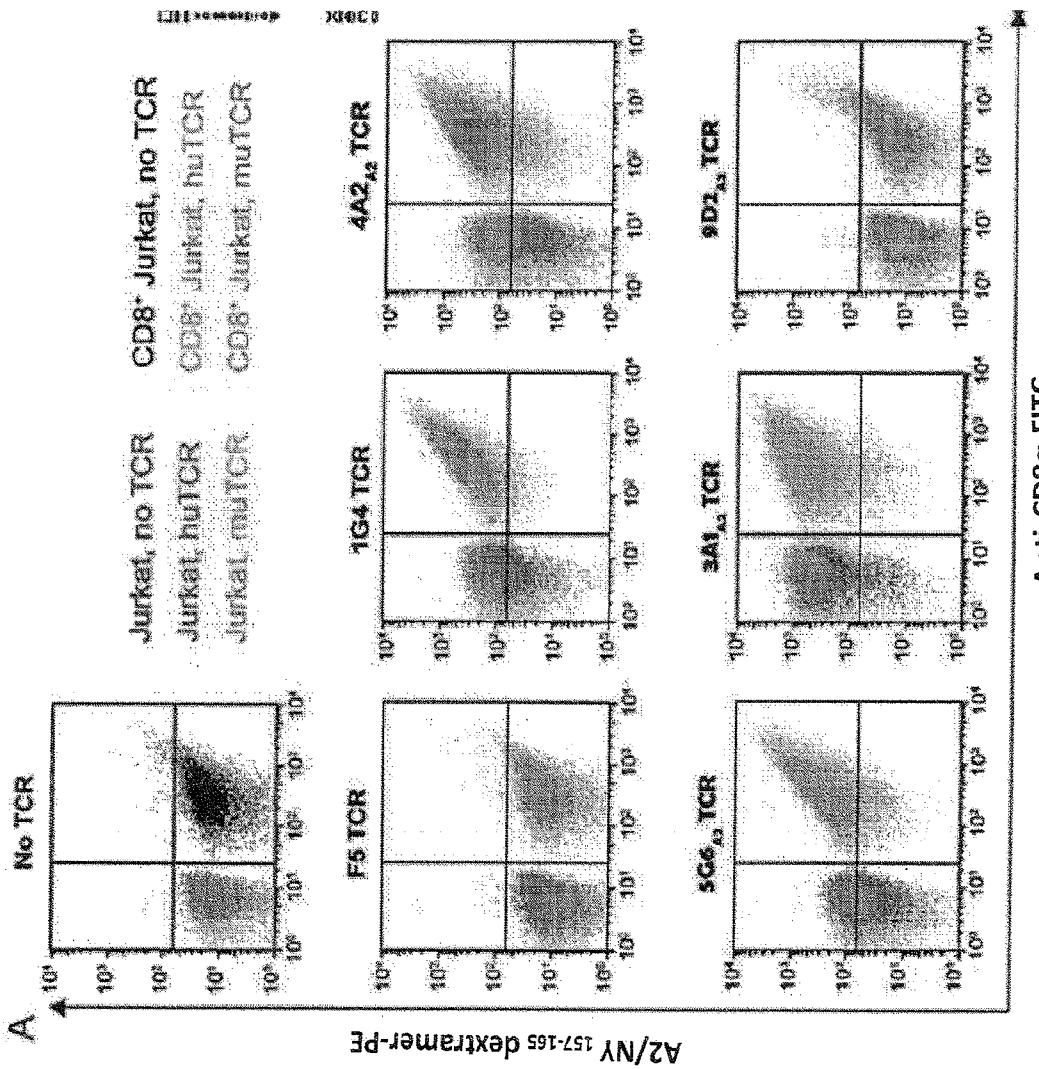

A
No TCR
F5 TCR
1G4 TCR
4A2$_{A2}$ TCR
3G6$_{A3}$ TCR
3A1$_{A3}$ TCR
9D2$_{A3}$ TCR
Jurkat, no TCR
Jurkat, huTCR
Jurkat, muTCR
CD8$^+$ Jurkat, no TCR
CD8$^+$ Jurkat, huTCR
CD8$^+$ Jurkat, muTCR
A2/NY$_{157-165}$ dextramer-PE
Anti-CD8α-FITC

FIG. 3A

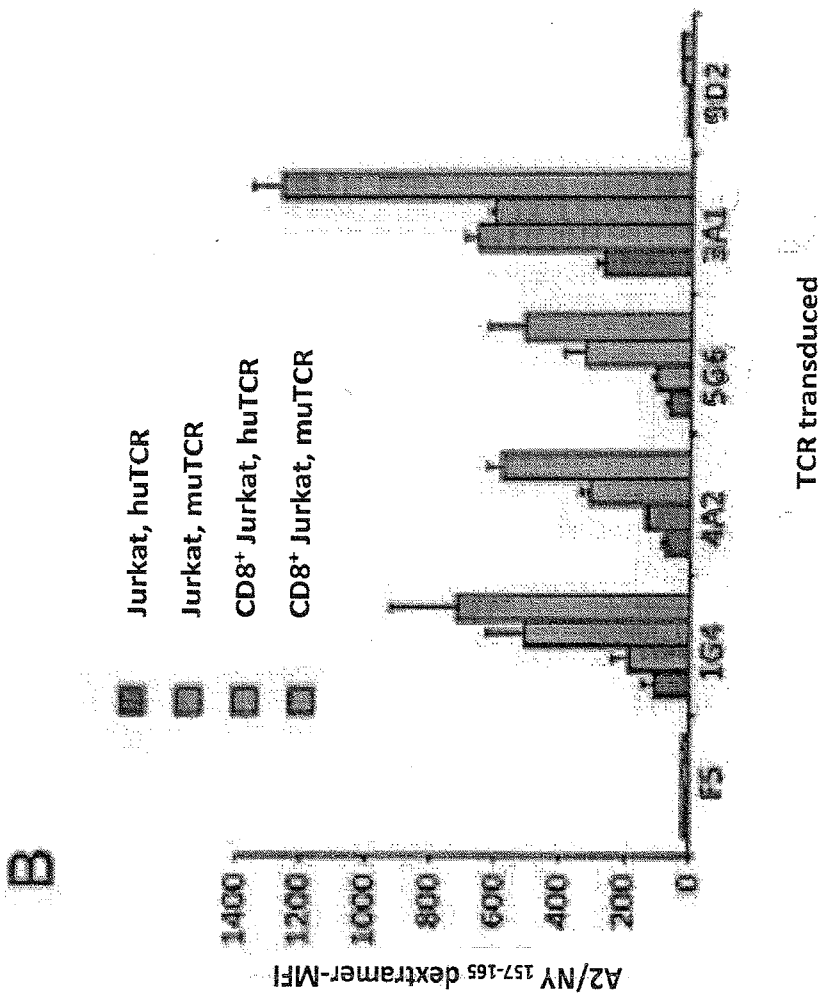

B
Jurkat, huTCR
Jurkat, muTCR
CD8+ Jurkat, huTCR
CD8+ Jurkat, muTCR
A2/NY 157-165 dextramer-MFI
1400
1200
1000
800
600
400
200
0
F5
1G4
4A2
5G6
3A1
9D2
TCR transduced

FIG. 3B

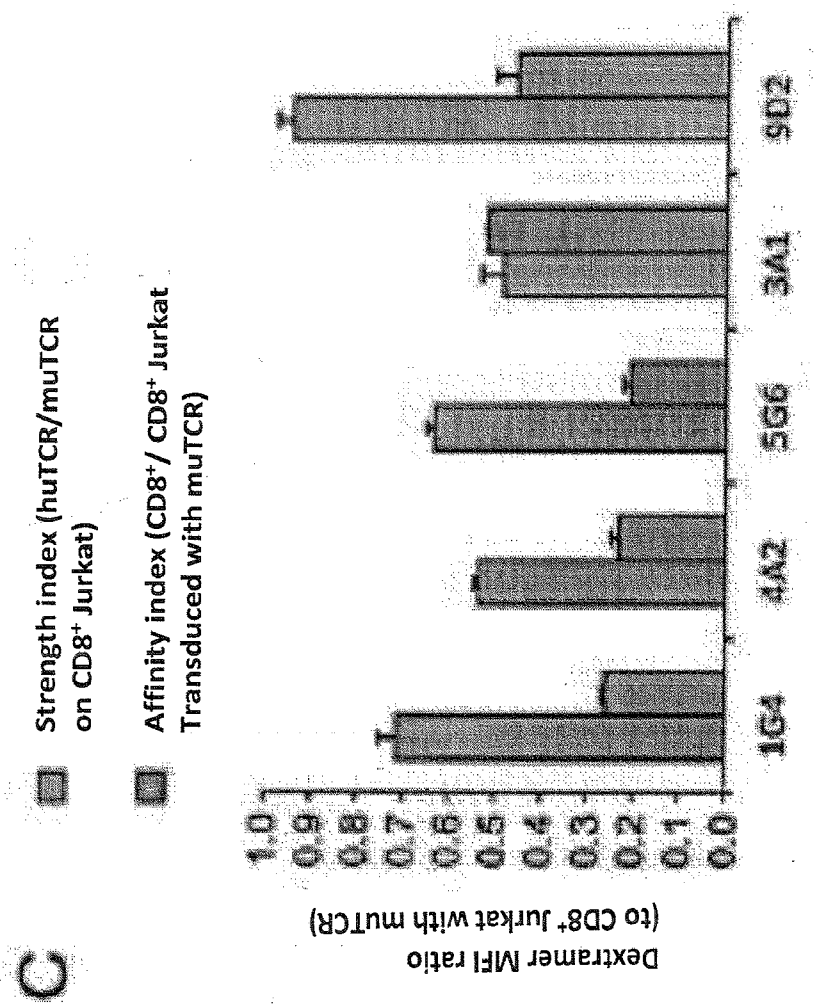
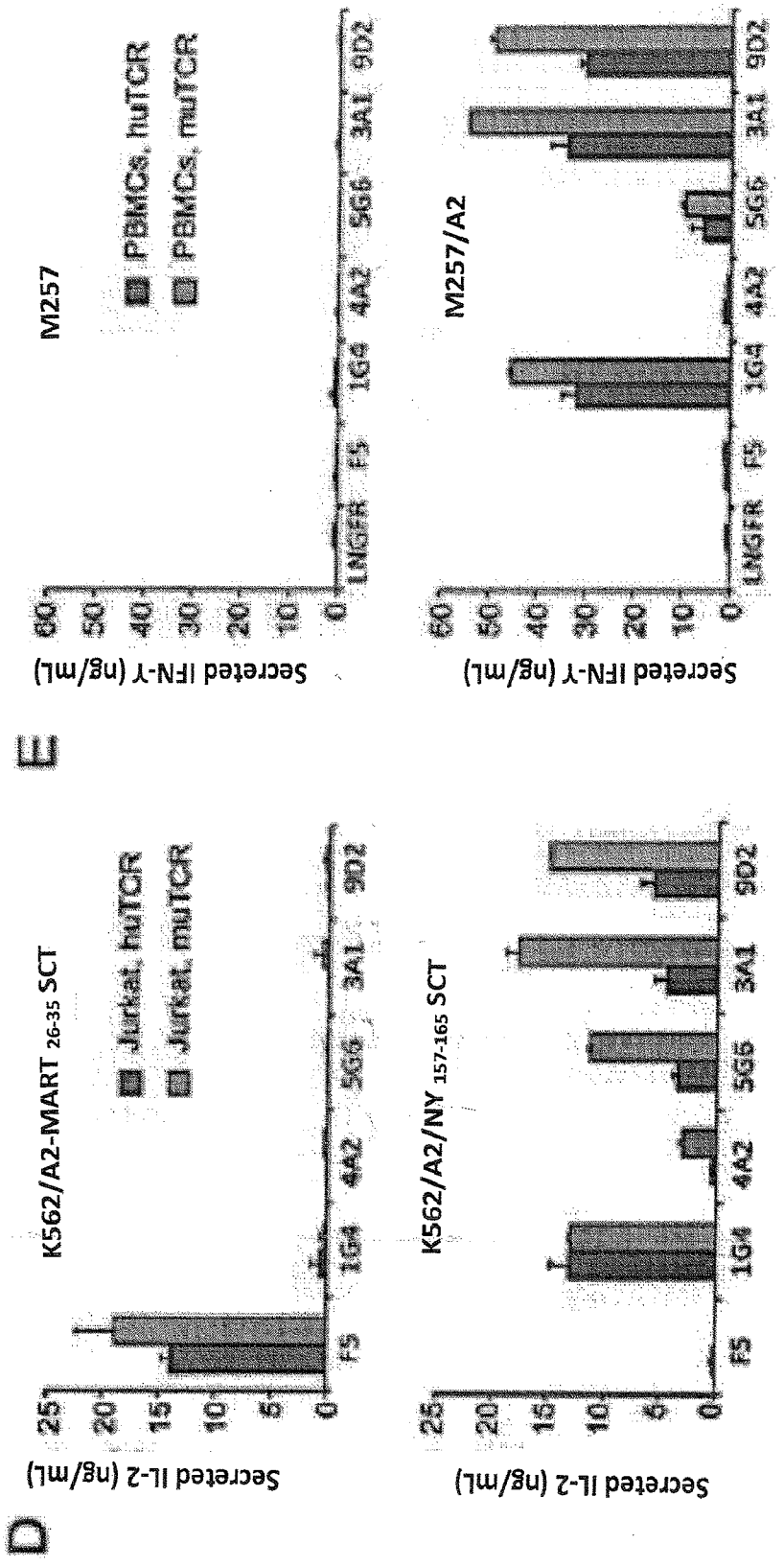

C
Strength index (huTCR/muTCR on CD8+ Jurkat)
Affinity index (CD8+/ CD8+ Jurkat Transduced with muTCR)
Dextramer MFI ratio (to CD8+ Jurkat with muTCR)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1G4
4A2
5G6
3A1
9D2
TCR transduced

FIG. 3C


D
K562/A2-MART 26-35 SCT
Jurkat, huTCR
Jurkat, muTCR
Secreted IL-2 (ng/mL)
0
5
10
15
20
25
F5
1G4
4A2
5G6
3A1
9D2
K562/A2/NY 157-165 SCT
Secreted IL-2 (ng/mL)
E
M257
PBMCs, huTCR
PBMCs, muTCR
Secreted IFN-γ (ng/mL)
0
10
20
30
40
50
60
LNGFR
F5
1G4
4A2
5G6
3A1
9D2
M257/A2
Secreted IFN-γ (ng/mL)

FIGS. 3D-3E

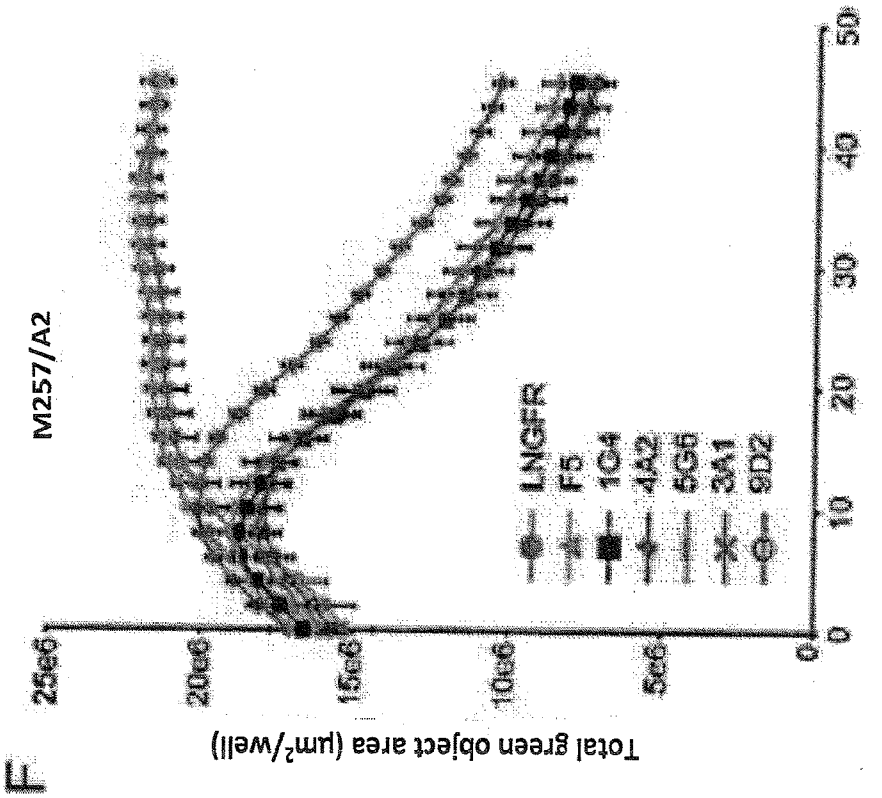

F
M257/A2
Total green object area (μm²/well)
25e6
20e6
15e6
10e6
5e6
0
10
20
30
40
50
Time (hours)
LNGFR
F5
1C4
4A2
6G6
3A1
9D2

FIG. 3F

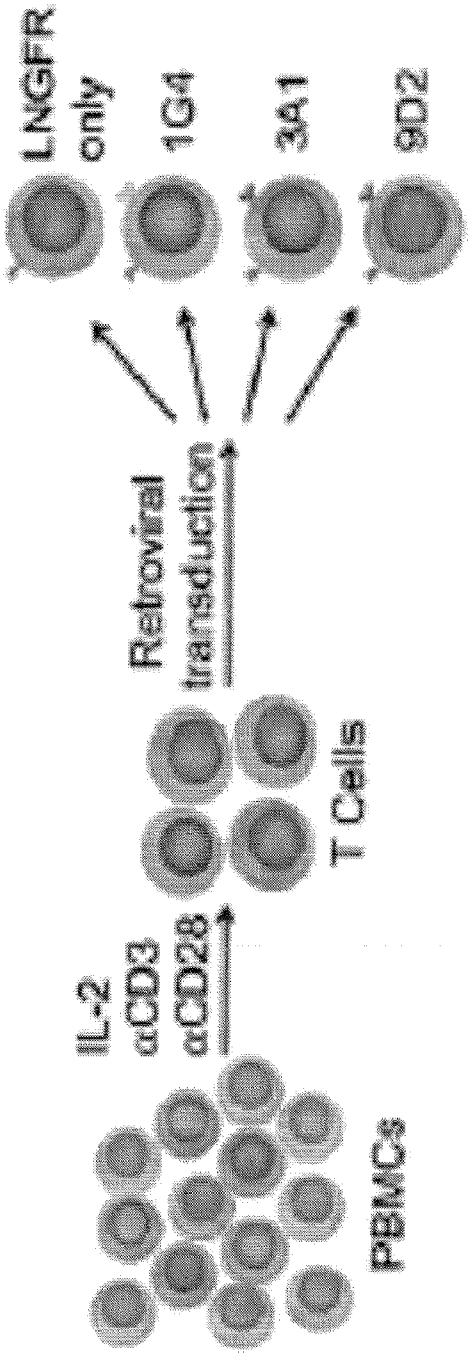

LNGFR only
1G4
3A1
9D2
Retroviral transduction
T Cells
IL-2
αCD3
αCD28
PBMCs

FIG. 4A

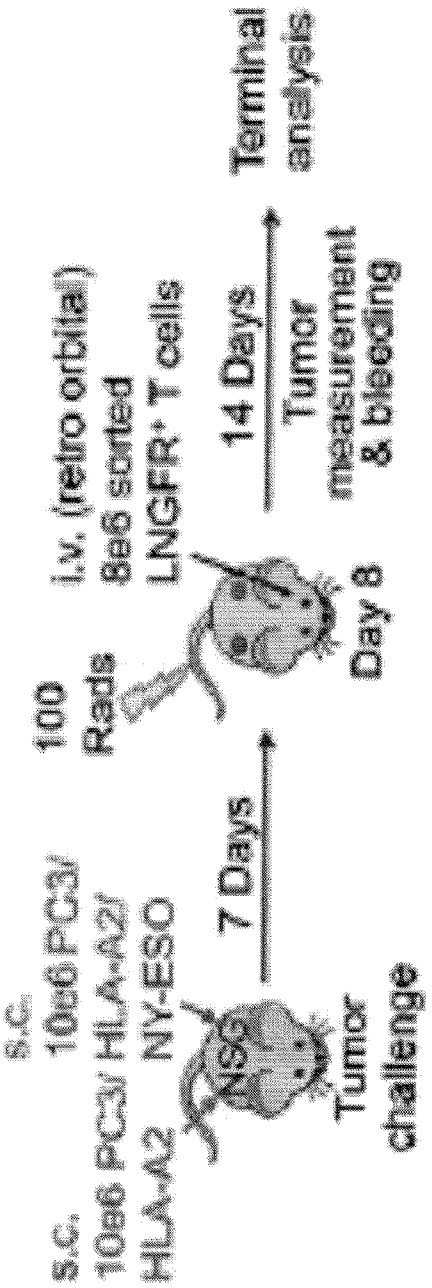

s.c. 10e6 PC3/ HLA-A2/ NY-ESO
s.c. 10e6 PC3/ HLA-A2
7 Days
NSG
Tumor challenge
100 Rads
i.v. (retro orbital) 8e6 sorted LNGFR+ T cells
Day 8
14 Days
Tumor measurement & bleeding
Terminal analysis

FIG. 4B

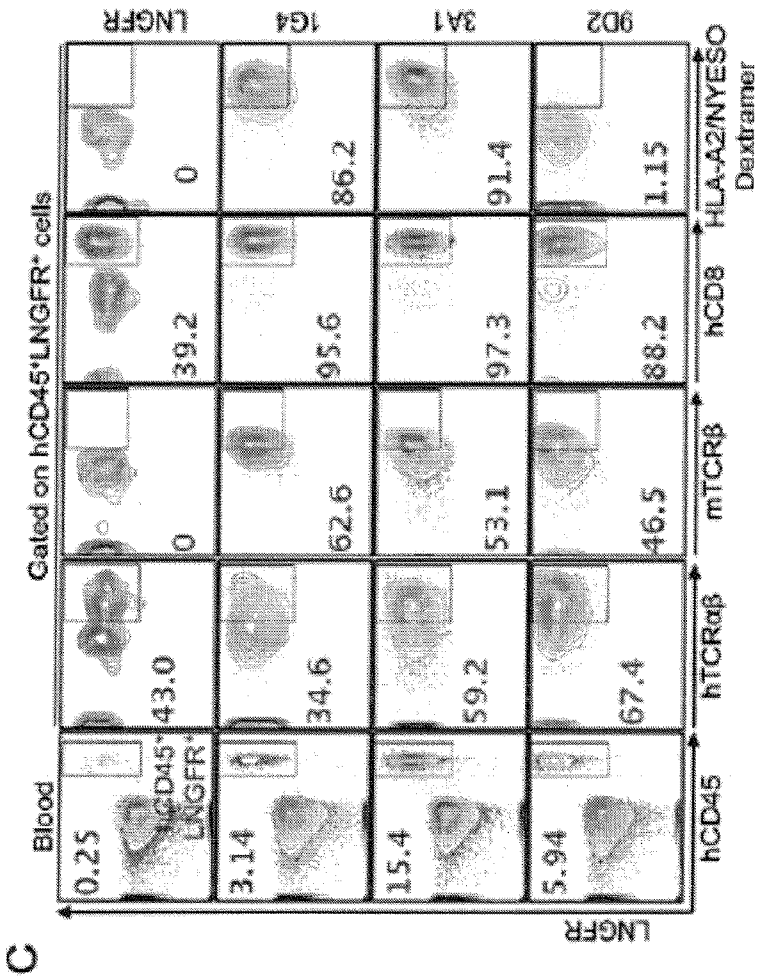

C
Blood
Gated on hCD45+LNGFR+ cells
LNGFR
hCD45
hTCRαβ
mTCRβ
hCD8
HLA-A2/NYESO Dextramer
9D2
3A1
1G4
LNGFR
0.25
43.0
0
39.2
0
3.14
34.6
62.6
95.6
86.2
15.4
59.2
53.1
97.3
91.4
5.94
67.4
46.5
88.2
1.15

FIG. 4C

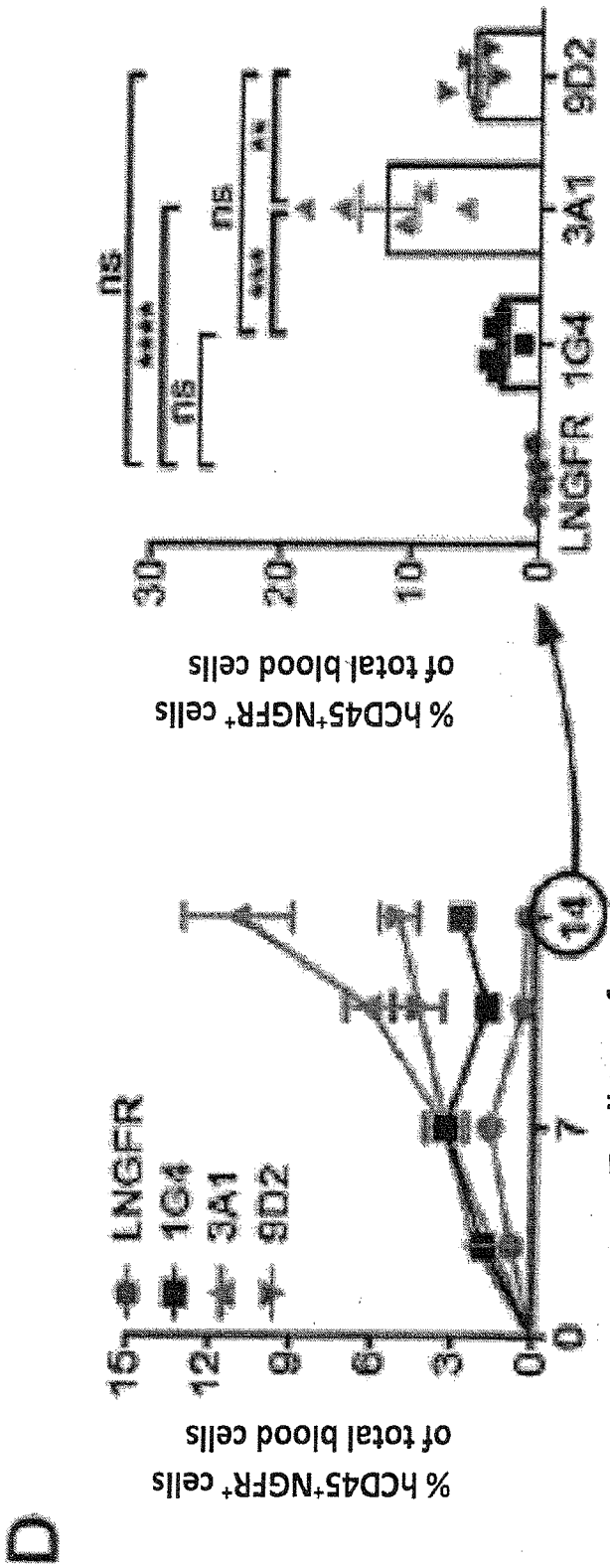
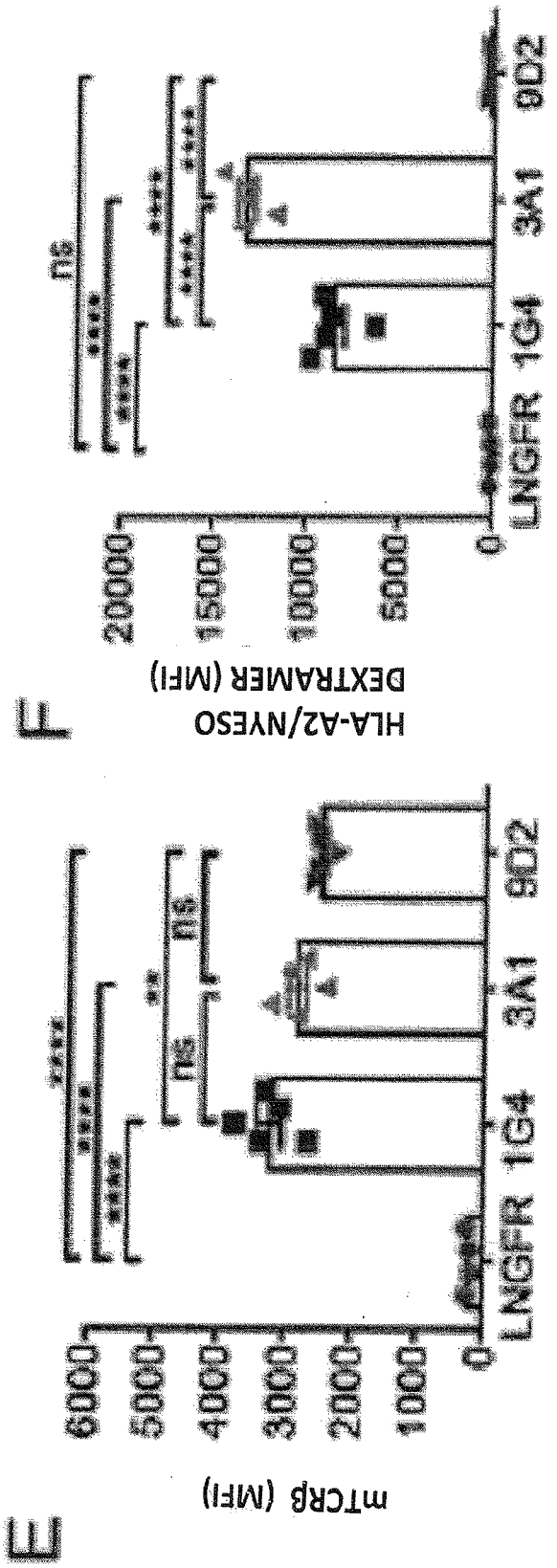

D
% hCD45+NGFR+ cells
of total blood cells
15
12
9
6
3
0
LNGFR
1G4
3A1
9D2
0
7
14
Days post T cell transfer
% hCD45+NGFR+ cells
of total blood cells
30
20
10
0
LNGFR
1G4
3A1
9D2
ns
****
ns
ns
****
**

FIG. 4D


F
HLA-A2/NYESO
DEXTRAMER (MFI)
20000
15000
10000
5000
0
LNGFR 1G4 3A1 9D2
ns
****
E
mTCRβ (MFI)
6000
5000
4000
3000
2000
1000
0
LNGFR 1G4 3A1 9D2
ns
**
***

FIGS. 4E-4F

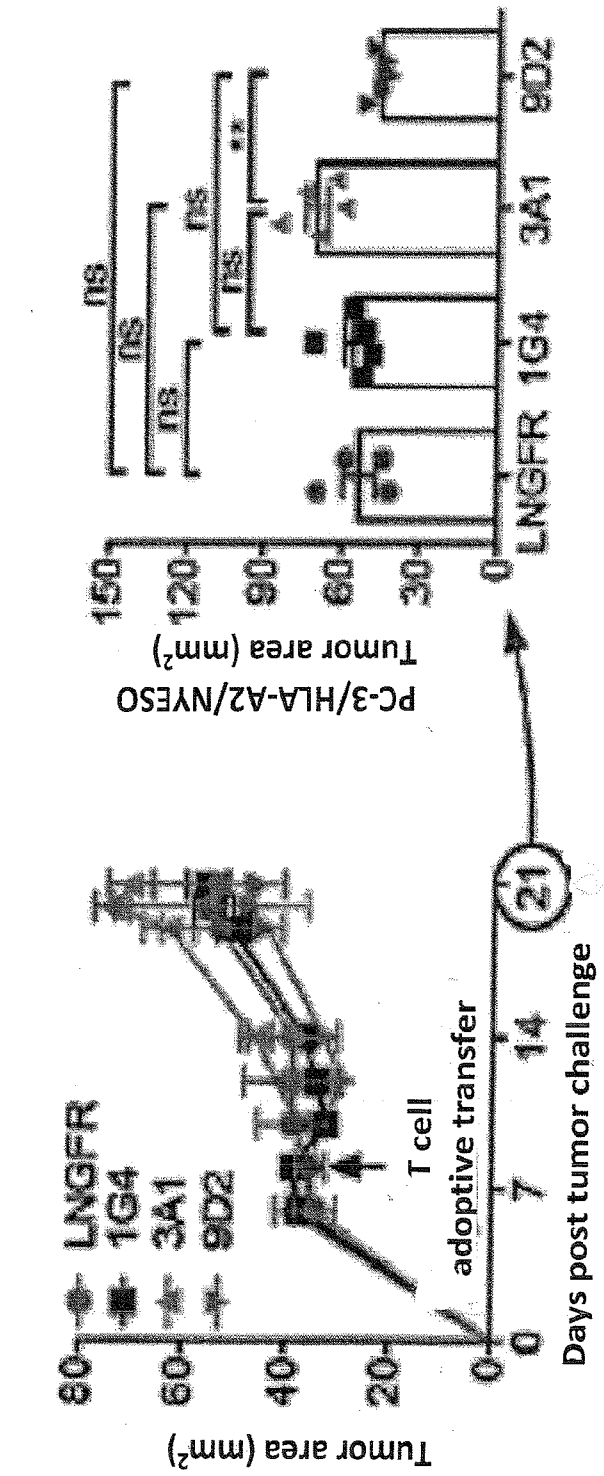

G
Tumor area (mm²)
60
40
20
0
LNGFR
1G4
3A1
9D2
T cell
adoptive transfer
0
7
14
21
Days post tumor challenge
PC-3/HLA-A2/NYESO
Tumor area (mm²)
150
120
90
60
30
0
ns
ns
ns
ns
**
LNGFR
1G4
3A1
9D2

FIG. 4G

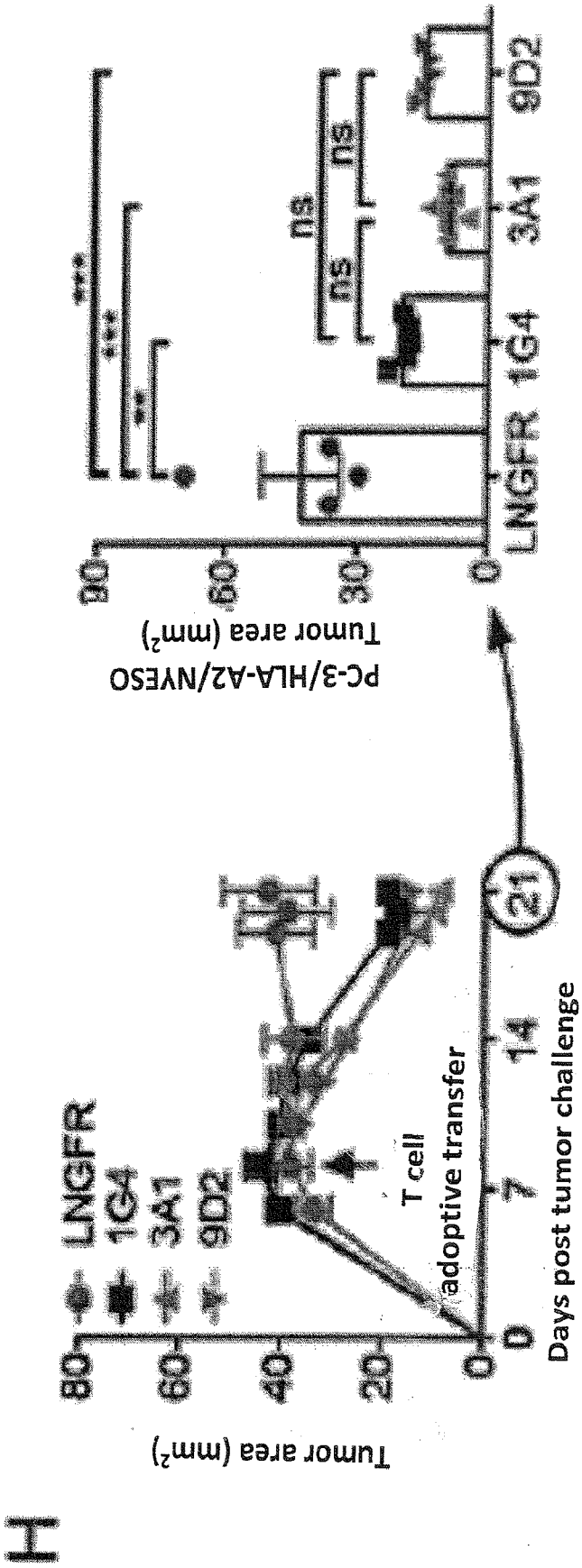

H
Tumor area (mm²)
80
60
40
20
0
LNGFR
1G4
3A1
9D2
T cell
adoptive transfer
0
7
14
21
Days post tumor challenge
PC-3/HLA-A2/NYESO
Tumor area (mm²)
90
60
30
0
LNGFR
1G4
3A1
9D2
***
***
**
ns
ns
ns
***

FIG. 4H

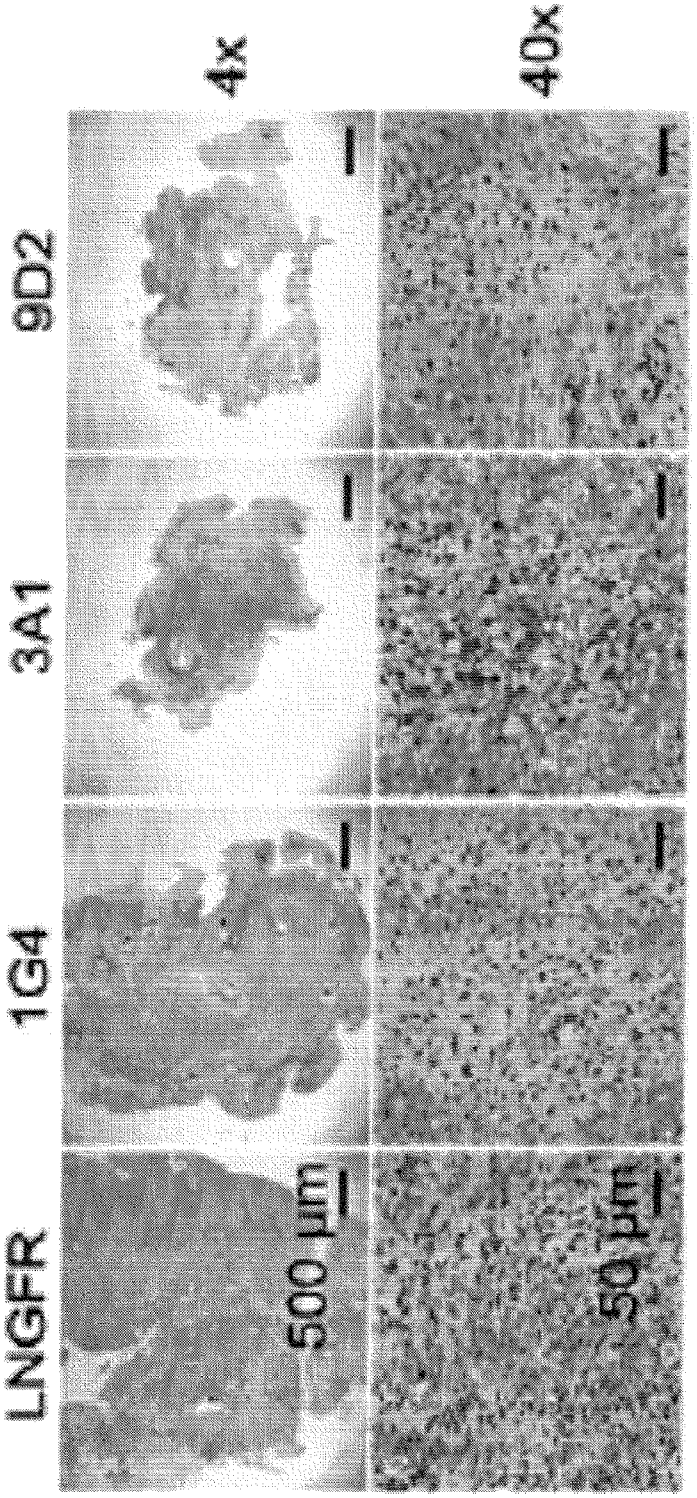


FIG. 4I

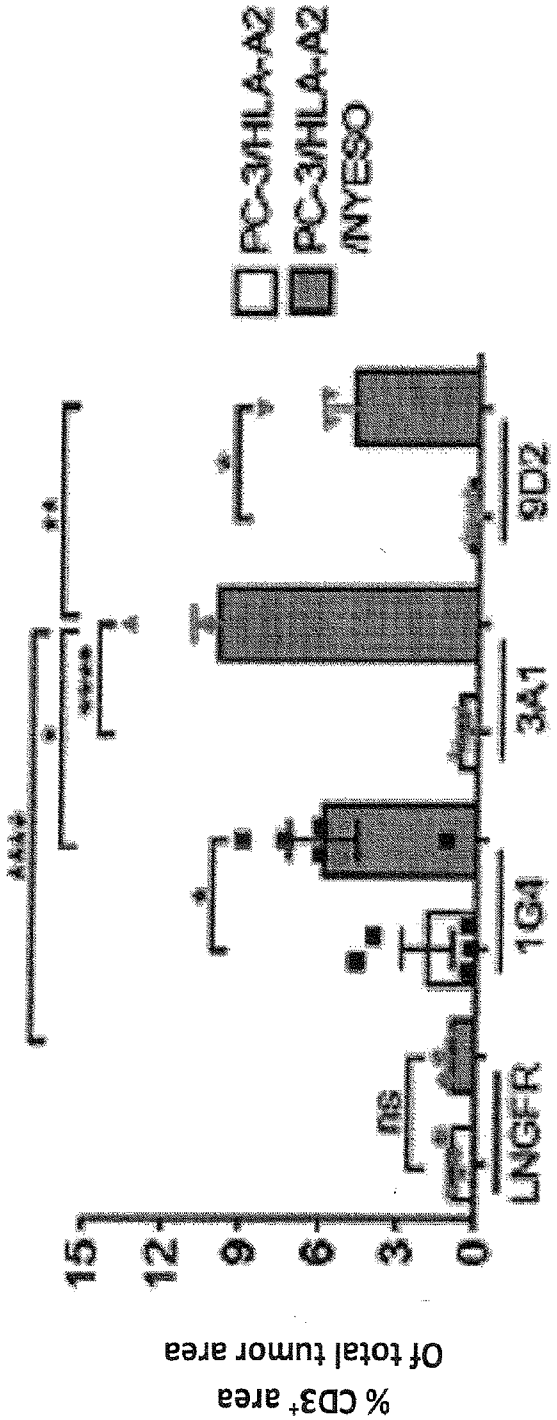

PC-3/HLA-A2
PC-3/HLA-A2 /NYESO
% CD3+ area
Of total tumor area
15
12
9
6
3
0
LNGFR
ns
1G4
3A1
9D2

FIG. 4J

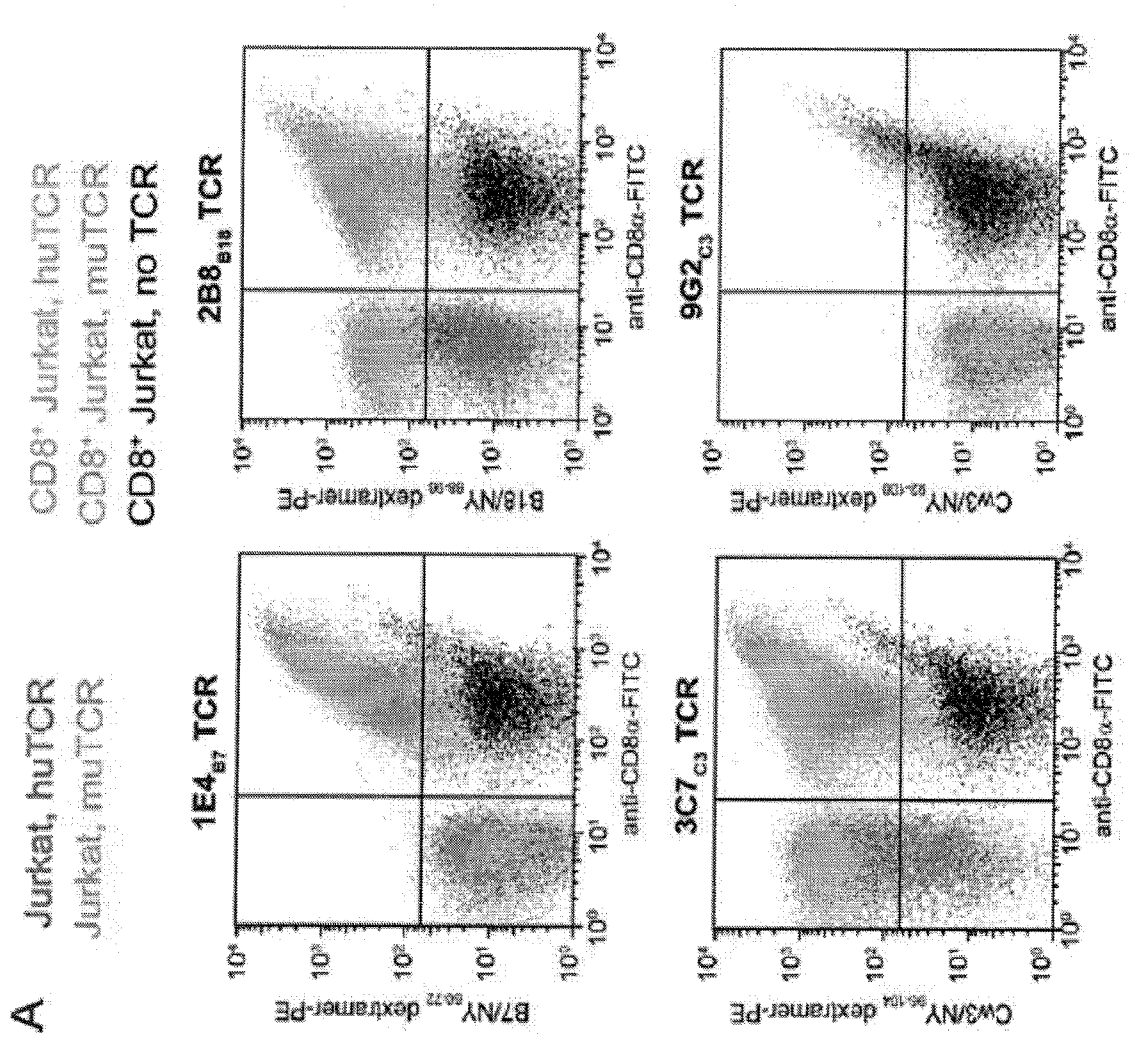

A
Jurkat, huTCR
Jurkat, muTCR
CD8+ Jurkat, huTCR
CD8+ Jurkat, muTCR
CD8+ Jurkat, no TCR
1E4E7 TCR
2B8B18 TCR
3C7C3 TCR
9G2C3 TCR
B7/NY 82-22 dextramer-PE
B18/NY 82-98 dextramer-PE
Cw3/NY 82-104 dextramer-PE
Cw3/NY 82-102 dextramer-PE
anti-CD8α-FITC

FIG. 5A

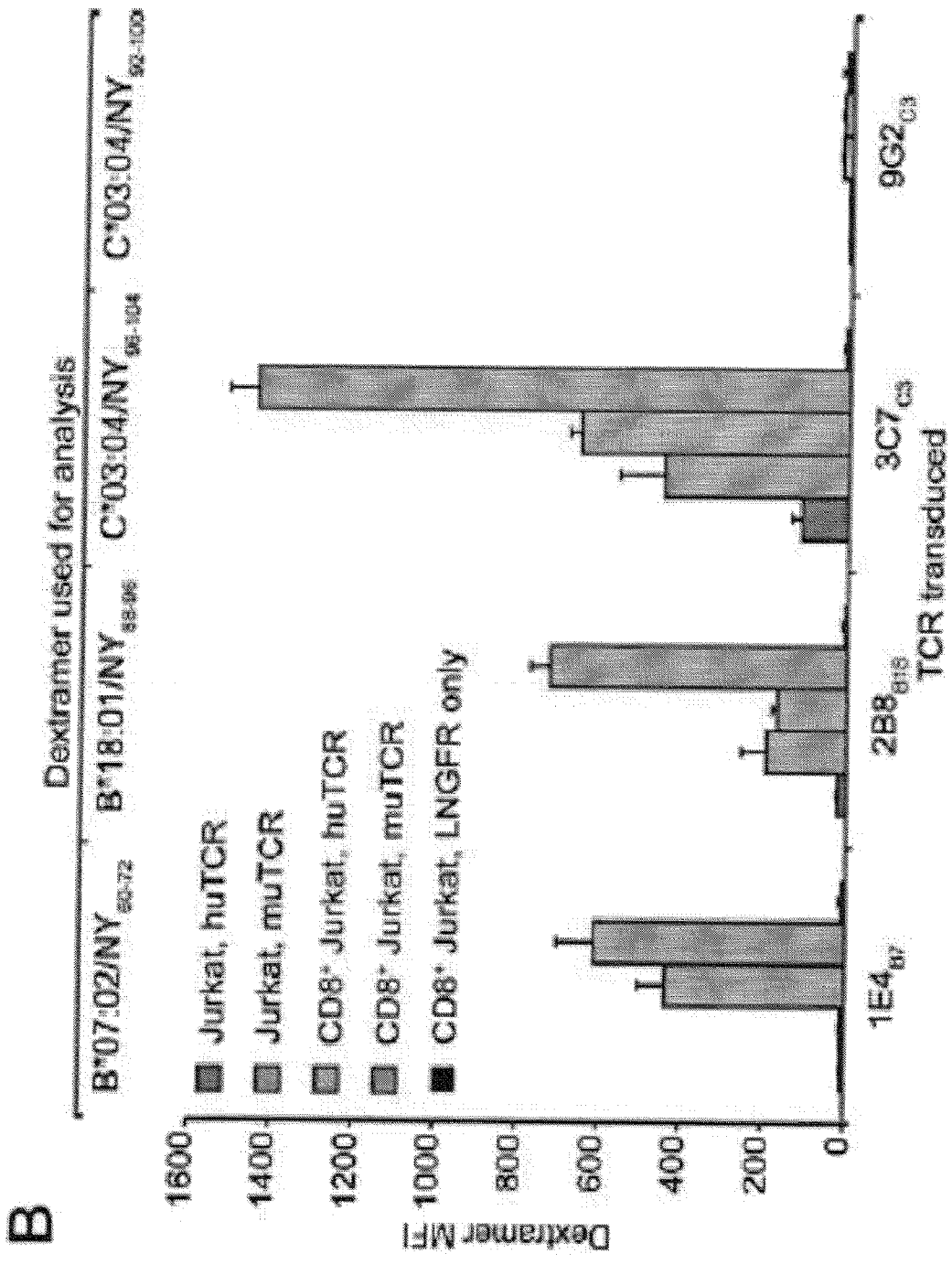

B
Dextramer used for analysis
B*07:02/NY
B*18:01/NY
C*03:04/NY
C*03:04/NY
Jurkat, huTCR
Jurkat, muTCR
CD8+ Jurkat, huTCR
CD8+ Jurkat, muTCR
CD8+ Jurkat, LNGFR only
Dextramer MFI
1600
1400
1200
1000
800
600
400
200
0
1E4
2B8
3C7
9G2
TCR transduced

FIG. 5B

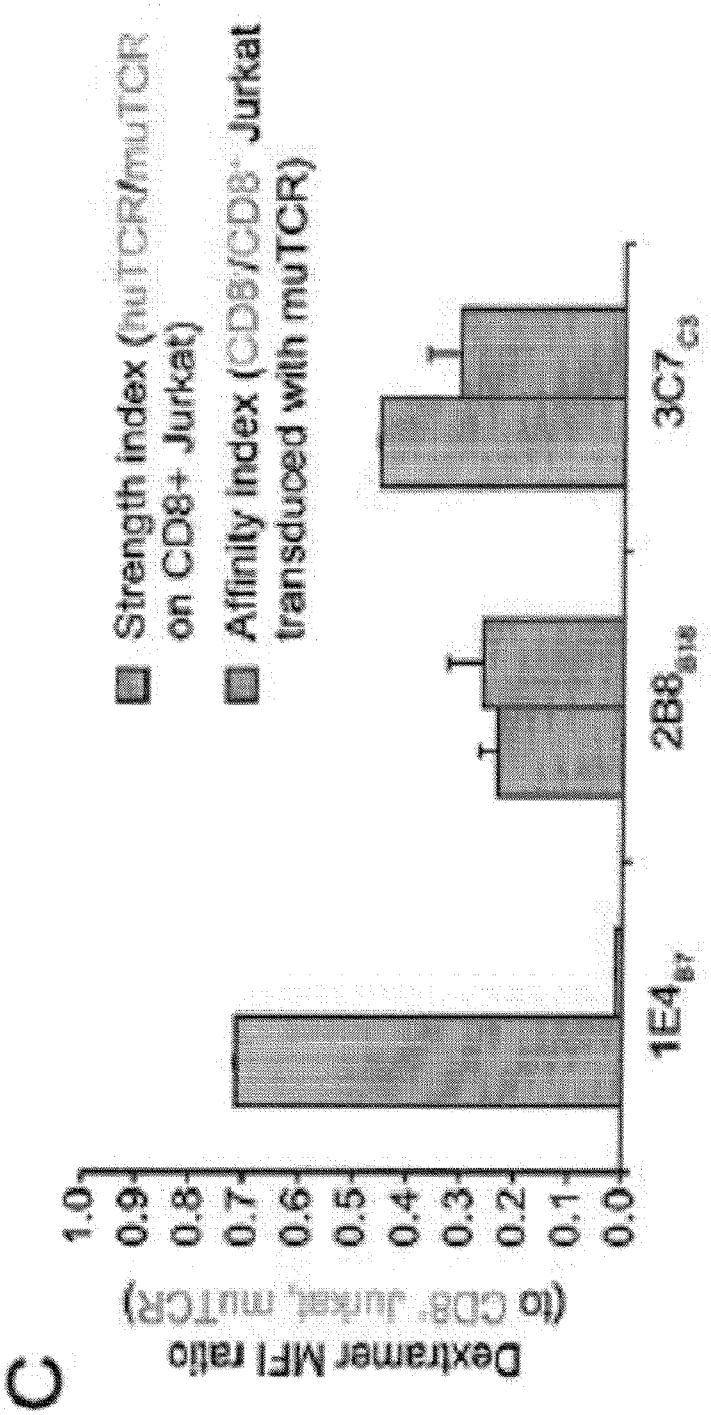
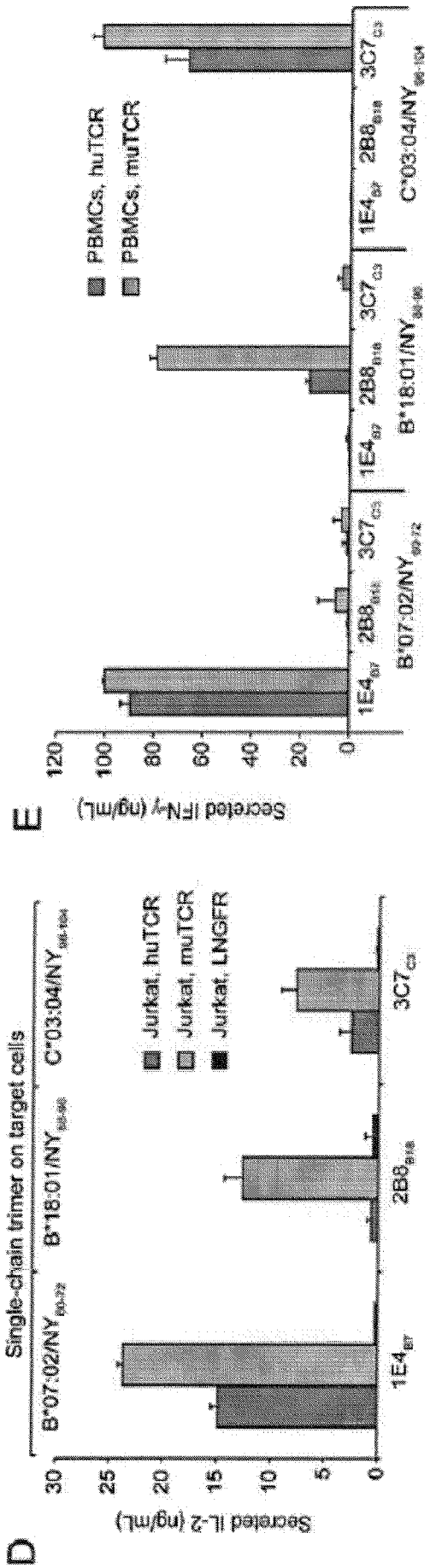
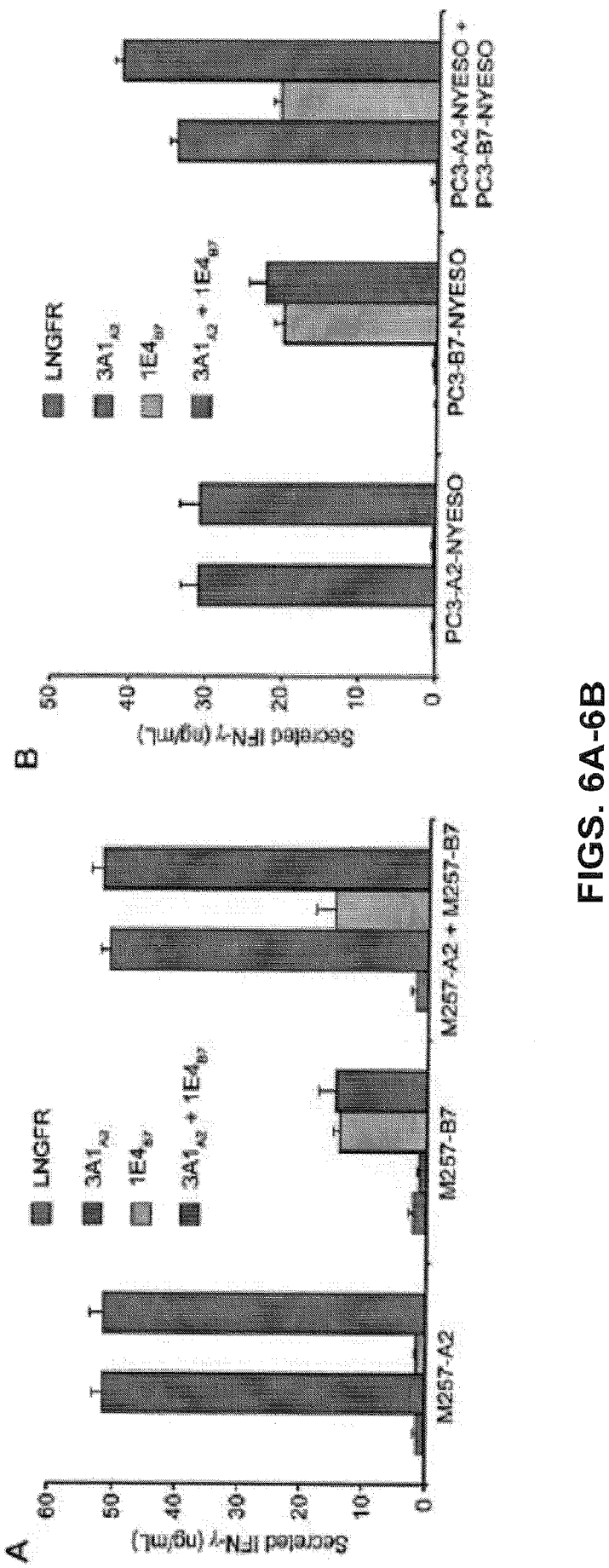

C
Dextramer MFI ratio
(to CD8+ Jurkat, muTCR)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1E4
2B8
3C7
Affinity index (CD8/CD8- Jurkat transduced with muTCR)
Strength index (muTCR/muTCR on CD8+ Jurkat)

FIG. 5C


D
Secreted IL-2 (ng/mL)
30
25
20
15
10
5
0
Single-chain trimer on target cells
B*07:02/NY
B*18:01/NY
C*03:04/NY
Jurkat, huTCR
Jurkat, muTCR
Jurkat, LNGFR
1E4
2B8
3C7
E
Secreted IFNγ (ng/mL)
120
100
80
60
40
20
0
PBMCs, huTCR
PBMCs, muTCR
B*07:02/NY
B*18:01/NY
C*03:04/NY

FIGS. 5D-5E


A
Secreted IFN-γ (ng/mL)
0
10
20
30
40
50
60
M257-A2
M257-B7
M257-A2 + M257-B7
LNGFR
3A1A2
1E4B7
3A1A2 + 1E4B7
B
Secreted IFN-γ (ng/mL)
0
10
20
30
40
50
PC3-A2-NYESO
PC3-B7-NYESO
PC3-A2-NYESO + PC3-B7-NYESO
LNGFR
3A1A2
1E4B7
3A1A2 + 1E4B7

FIGS. 6A-6B

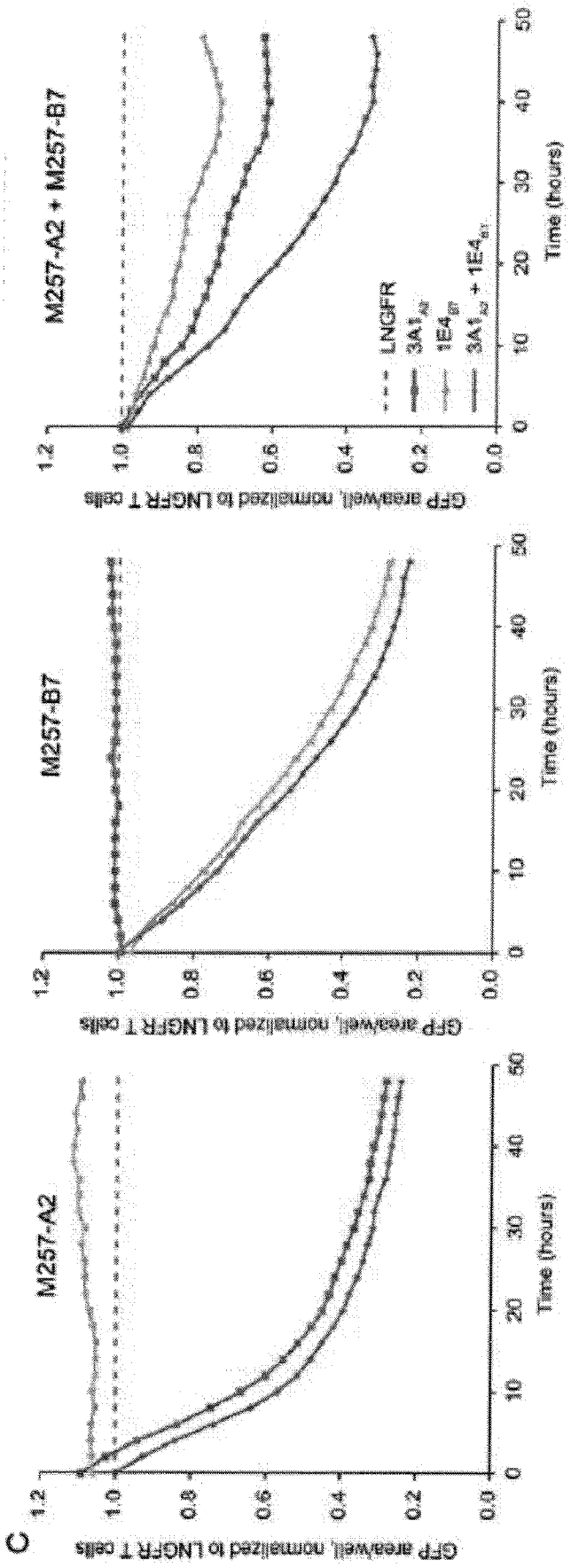

C
M257-A2
M257-B7
M257-A2 + M257-B7
GFP area/well, normalized to LNGFR T cells
Time (hours)
LNGFR
3A1A2
1E4B7
3A1A2 + 1E4B7

FIG. 6C

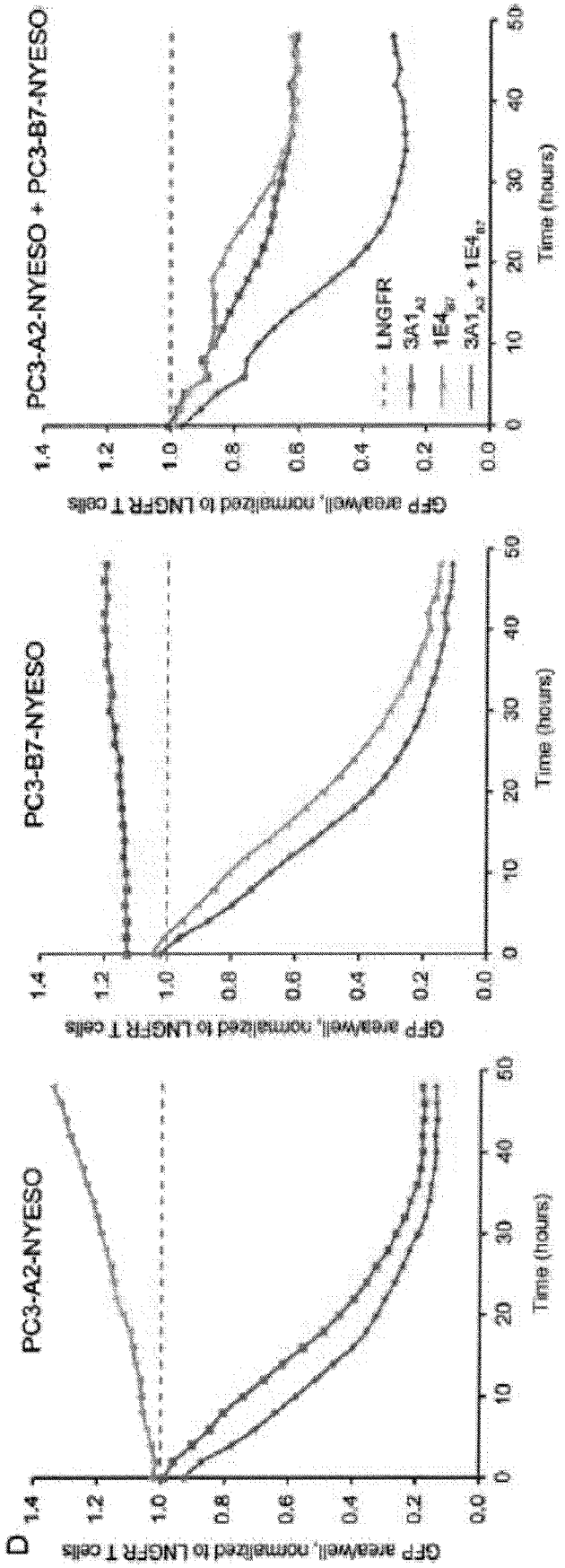

D
PC3-A2-NYESO
PC3-B7-NYESO
PC3-A2-NYESO + PC3-B7-NYESO
GFP area/well, normalized to LNGFR T cells
Time (hours)
LNGFR
3A1A2
1E4B7
3A1A2 + 1E4B7

FIG. 6D

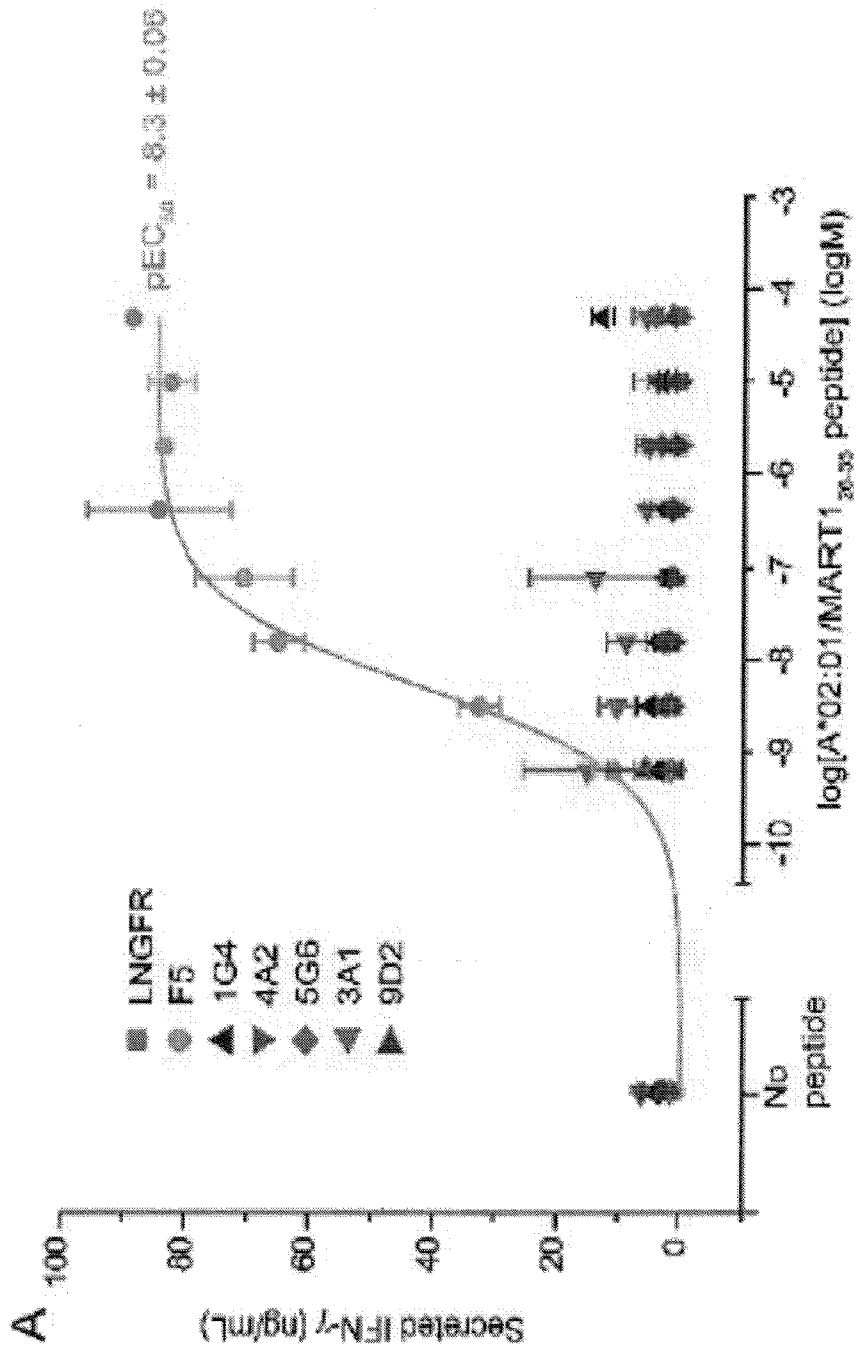

A
Secreted IFN-γ (ng/mL)
100
80
60
40
20
0
No peptide
-10
-9
-8
-7
-6
-5
-4
-3
log[A*02:01/MART1 $_{26-35}$ peptide] (logM)
pEC$_{50}$ = 8.3 ± 0.05
LNGFR
F5
1G4
4A2
5G6
3A1
9D2

FIG. 7A

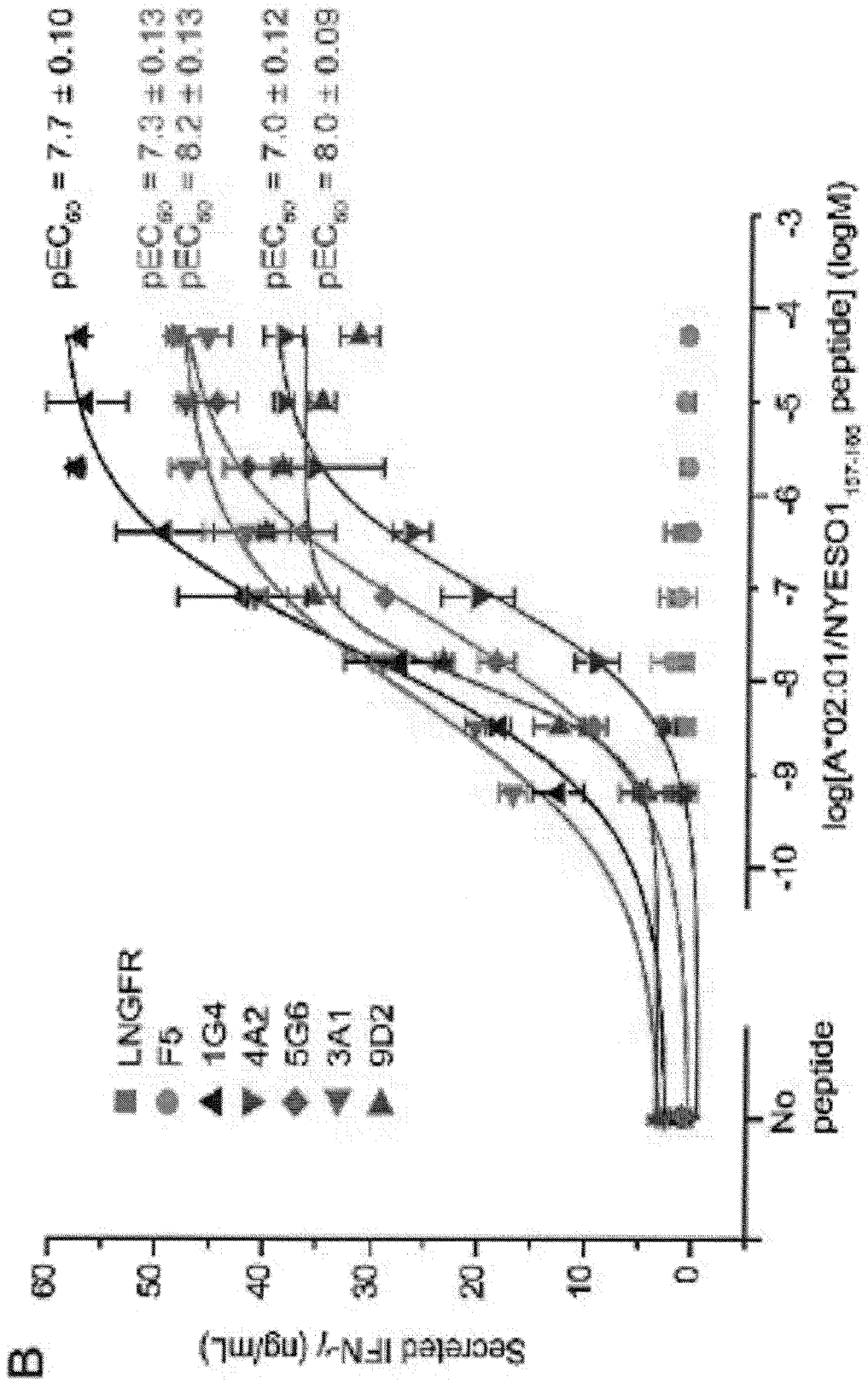

B
Secreted IFN-γ (ng/mL)
60
50
40
30
20
10
0
No peptide
-10
-9
-8
-7
-6
-5
-4
-3
log[A*02:01/NYESO1157-165 peptide] (logM)
LNGFR
F5
1G4
4A2
5G6
3A1
9D2
pEC50 = 7.7 ± 0.10
pEC50 = 7.3 ± 0.13
pEC50 = 8.2 ± 0.13
pEC50 = 7.0 ± 0.12
pEC50 = 8.0 ± 0.09

FIG. 7B

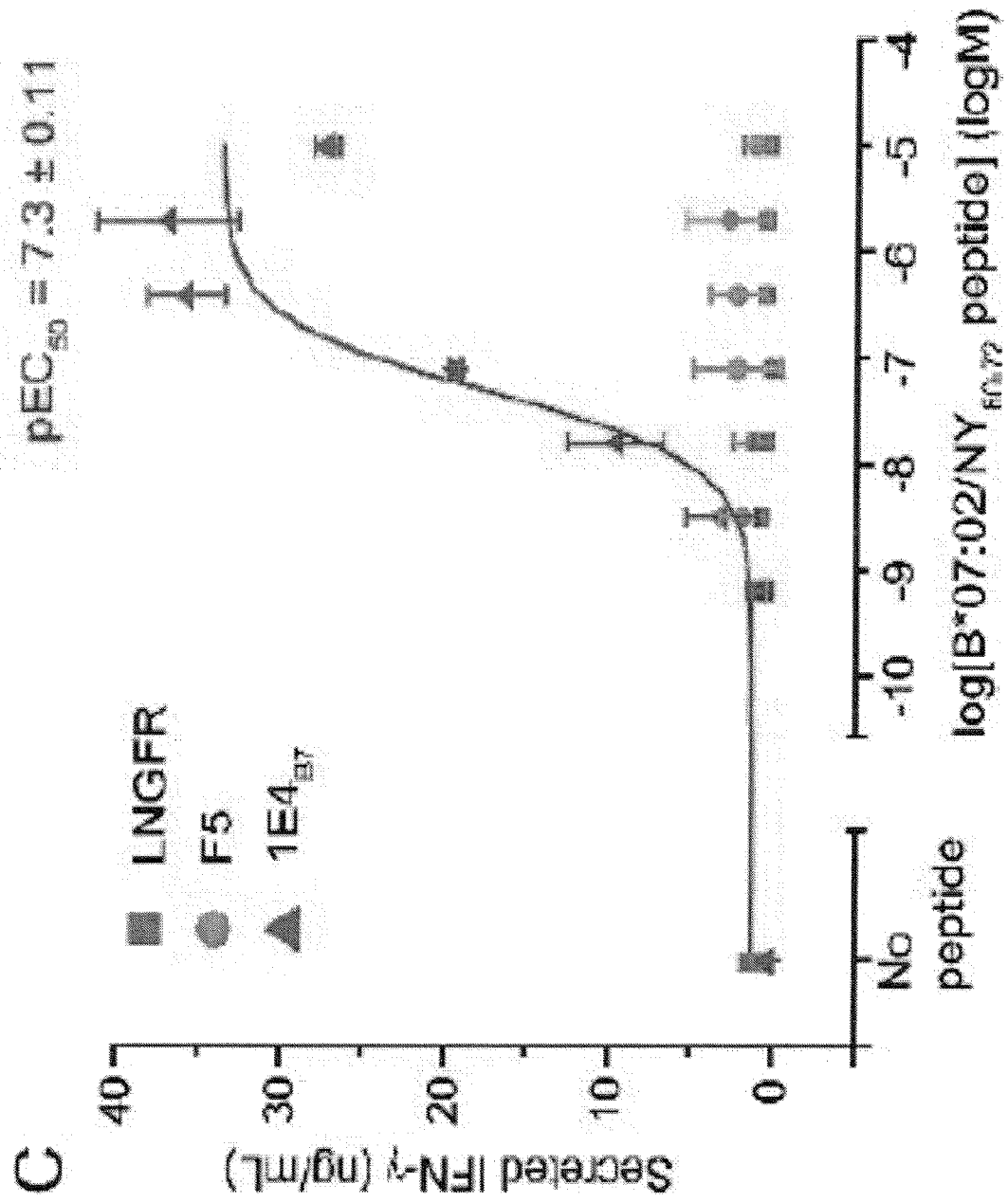

C
Secreted IFN-γ (ng/mL)
40
30
20
10
0
No peptide
-10
-9
-8
-7
-6
-5
-4
log[B*07:02/NY$_{R1\text{-}72}$ peptide] (logM)
LNGFR
F5
1E4$_{E17}$
pEC$_{50}$ = 7.3 ± 0.11

FIG. 7C

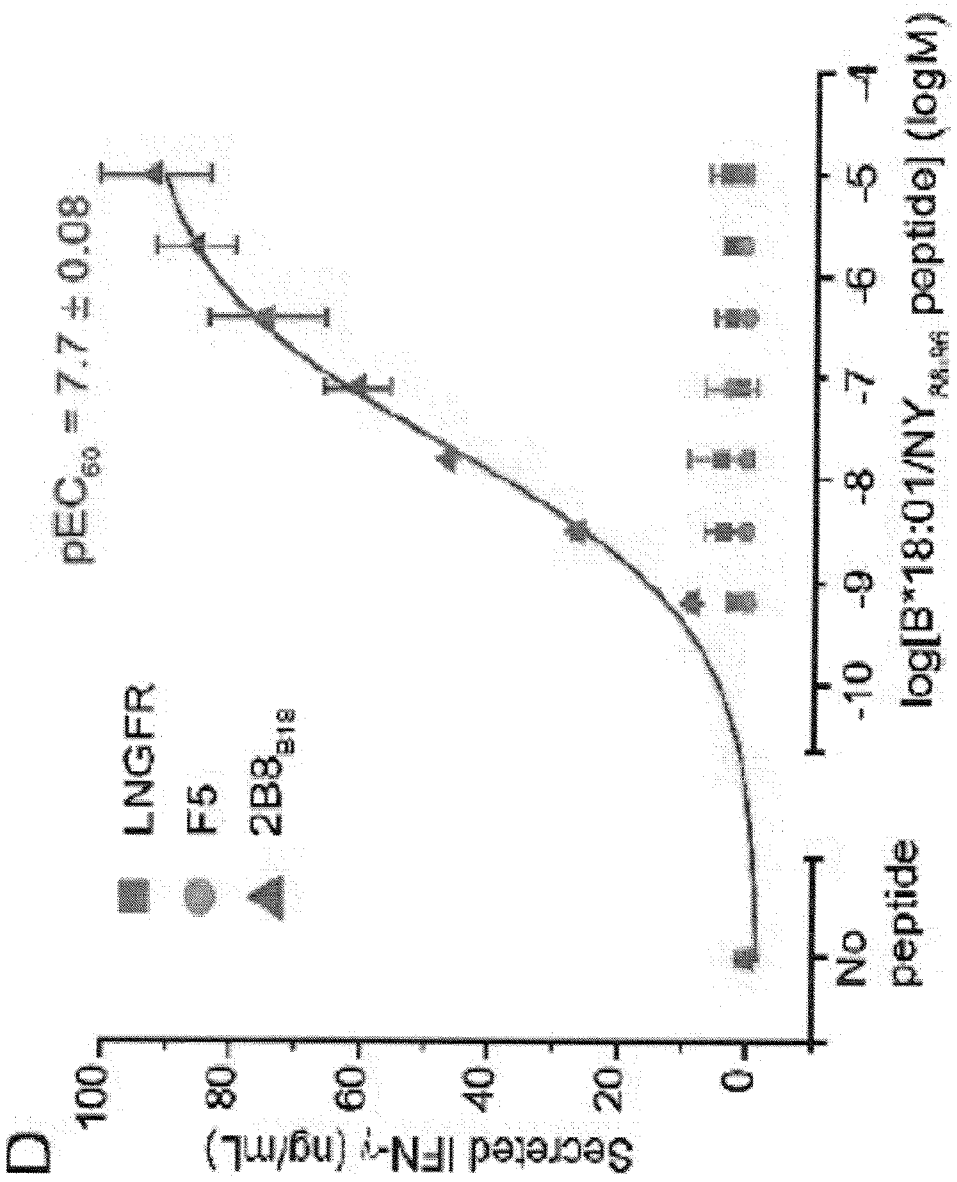

D
Secreted IFN-γ (ng/mL)
100
80
60
40
20
0
pEC50 = 7.7 ± 0.08
LNGFR
F5
2B8B18
No peptide
-10
-9
-8
-7
-6
-5
-4
log[B*18:01/NYR8-9G peptide] (logM)

FIG. 7D

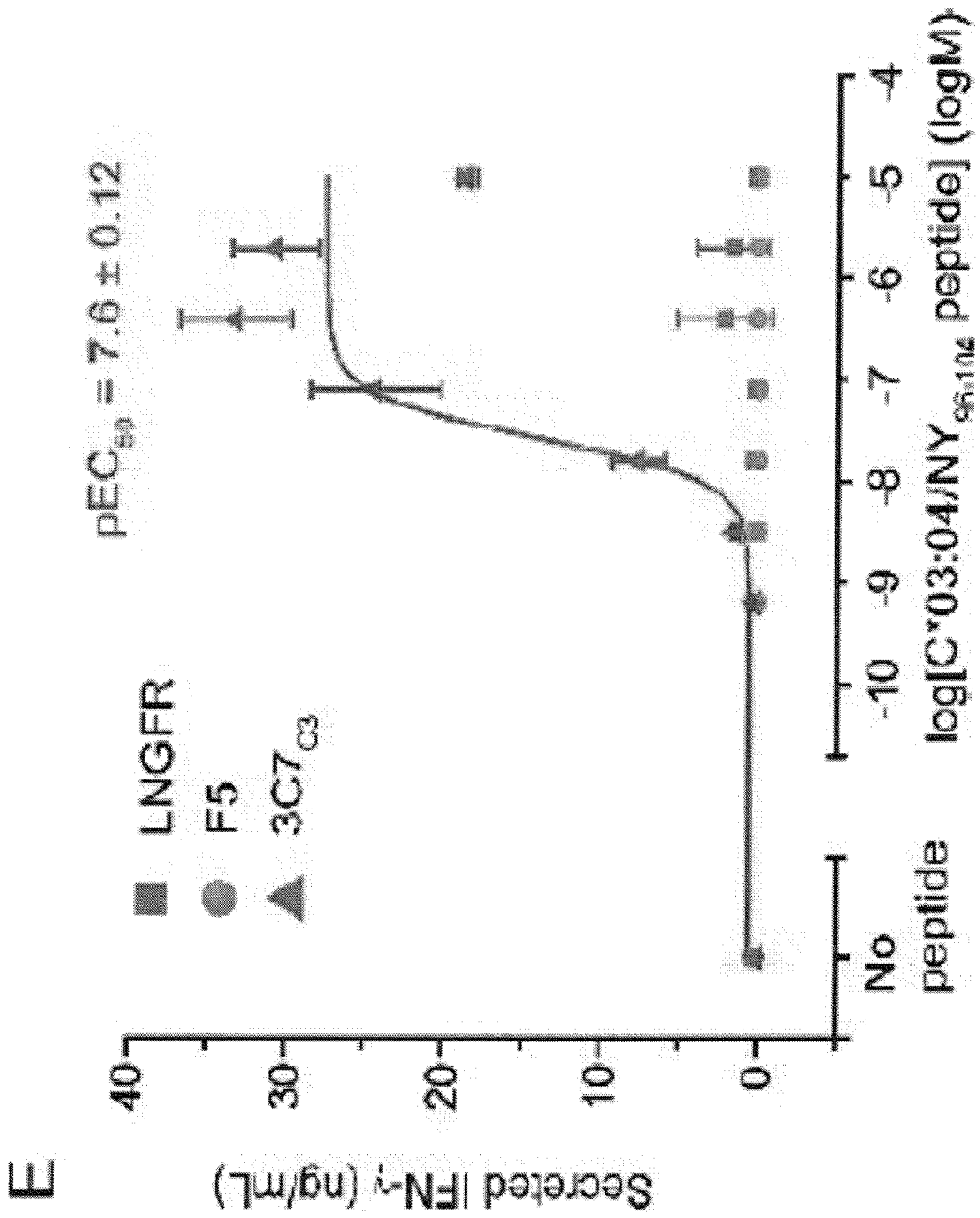

E
Secreted IFN-γ (ng/mL)
40
30
20
10
0
No peptide
-10 -9 -8 -7 -6 -5 -4
log[C*03:04/NY$_{9h1/\alpha}$ peptide] (logM)
LNGFR
F5
3C7$_{C3}$
pEC$_{50}$ = 7.6 ± 0.12

FIG. 7E


A
PC3/A2/NYESO1
PC3/A2
PC3/NYESO1
Secreted IFN-γ (ng/mL)
PBMCs, huTCR
PBMCs, muTCR
LNGFR
F5
1G4
4A2A2
5G6A2
3A1A2
9D2A2

FIG. 8A

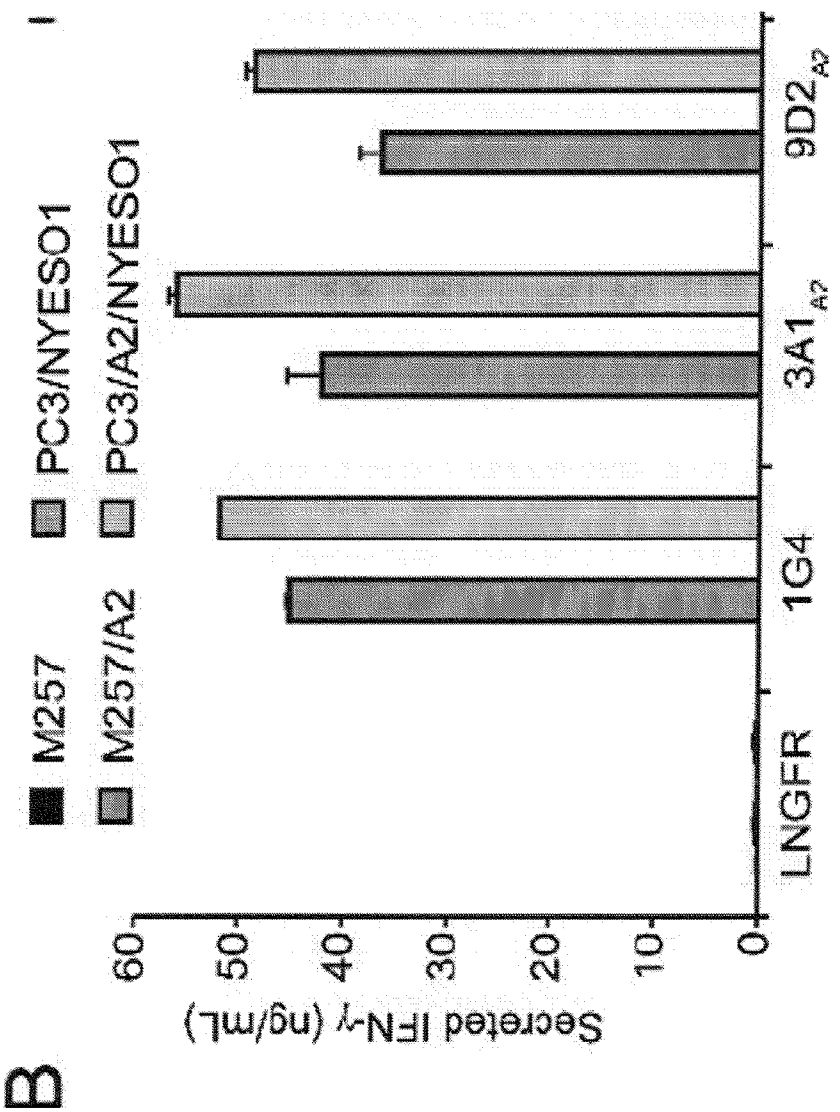

B
Secreted IFN-γ (ng/mL)
0
10
20
30
40
50
60
M257
M257/A2
PC3/NYESO1
PC3/A2/NYESO1
LNGFR
1G4
3A1 A2
9D2 A2

FIG. 8B

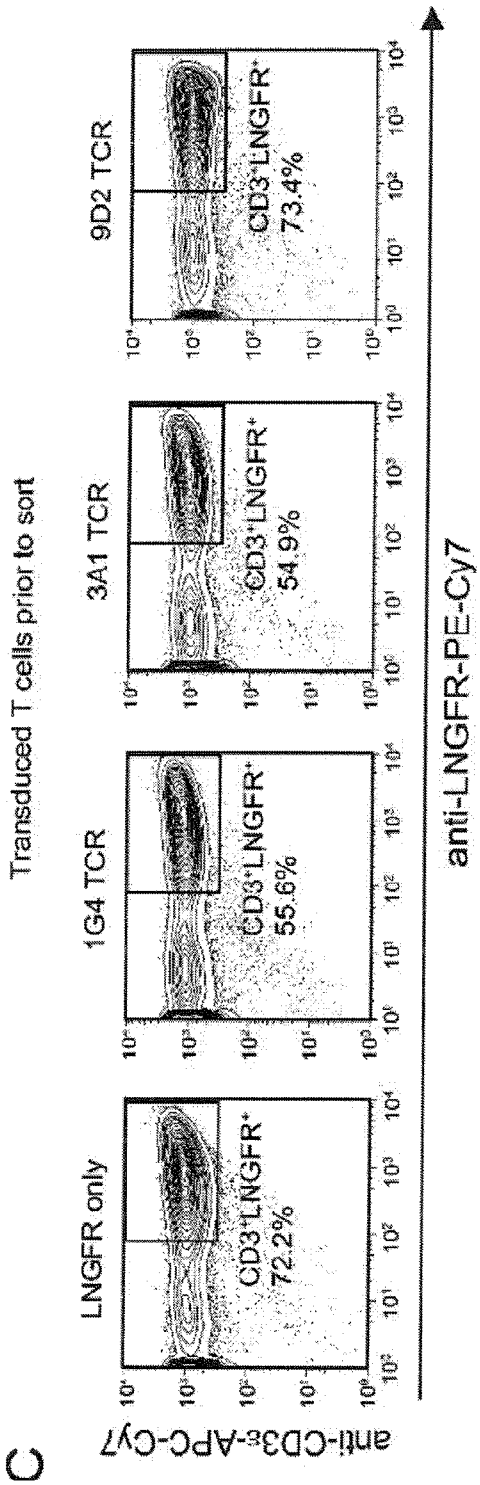

C
Transduced T cells prior to sort
LNGFR only
1G4 TCR
3A1 TCR
9D2 TCR
CD3+LNGFR+ 72.2%
CD3+LNGFR+ 55.6%
CD3+LNGFR+ 54.9%
CD3+LNGFR+ 73.4%
anti-CD3ε-APC-Cy7
anti-LNGFR-PE-Cy7

FIG. 8C