

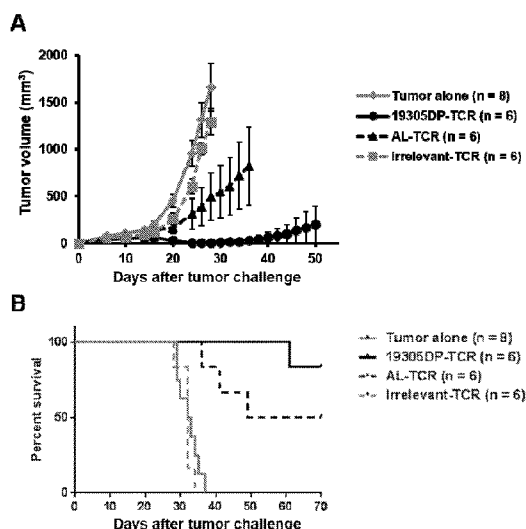


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- (71) **Applicant:** HEALTH RESEARCH, INC. [US/US]; Elm and Carlton Streets, Buffalo, NY 14263 (US).
- (72) **Inventors:** ODUNSI, Adekunle; 14 Emerald Trail, Williamsville, NY 14221 (US). TSUJI, Takemasa; 249 Palmdale Drive, Apt. #5, Williamsville, NY 14221 (US). MATSUZAKI, Junko; 249 Palmdale Drive, Apt. #5, Williamsville, NY 14221 (US).
- (74) **Agents:** WATT, Rachel, S. et al.; Hodgson Russ LLP, The Guaranty Building, 140 Pearl Street, Suite 100, Buffalo, NY 14202-4040 (US).
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[Continued on next page]

(54) **Title:** COMPOSITIONS AND LIBRARIES COMPRISING RECOMBINANT T-CELL RECEPTORS AND METHODS OF USING RECOMBINANT T-CELL RECEPTORS

Figure 8



(57) **Abstract:** Provided are recombinant polynucleotides, including expression vectors encoding an alpha chain and/or a beta chain of a TCR having amino acid sequences from any of novel the AL, KQ, PP, 19305CD8, BB, KB, ST, JD, 19305DP, PB-P, PB-T, and PB 13.2 T Cell Receptors (TCRs) that are embodiments of the invention. Cells comprising the polynucleotides are provided, as are libraries of the recombinant polynucleotides and expression vectors. Methods are provided and involve administering to an individual modified human T cells that express a novel recombinant TCR. The methods are 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 includes cancer cells which express NY-ESO-1 and/or its highly homologous LAGE-1 antigen.



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COMPOSITIONS AND LIBRARIES COMPRISING RECOMBINANT T-CELL RECEPTORS AND METHODS OF USING RECOMBINANT T-CELL RECEPTORS

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 **[0001]** This application claims priority to U.S. provisional patent application no. 62/275,600, filed January 6, 2016, the disclosure of which is incorporated herein by reference.

FIELD

- 10 **[0002]** The present disclosure relates generally to immunotherapy and more specifically to recombinant T cell receptors that can impart direct tumor recognition capability to T cells.

BACKGROUND

- 15 **[0003]** Tumor antigen-specific T cells recognize and kill cancer cells by using unique T cell receptor (TCR) alpha and beta chain complex which is specific against tumor antigen peptide/HLA complex. Extremely diverse TCR alpha/beta sequence alone determines peptide-specificity, HLA restriction, and strength of recognition. Gene-engineering of polyclonally expanded peripheral T cells with tumor antigen-specific TCR generates large numbers of tumor antigen-specific T cells that can be used in adoptive T cell therapy of cancer patients using autologous gene-engineered T cells. Currently, only a few therapeutic
20 TCR gene products has been tested in clinical trials, which significantly restricts applicability of this powerful therapeutic strategy to limited patients by their HLA types as well as antigen expression in cancer cells. There is thus an ongoing and unmet need for improved compositions and methods for use in adoptive immune therapy involving recombinant TCRs. The present disclosure meets this need.

25 **BRIEF SUMMARY**

- 30 **[0004]** The present disclosure provides in one aspect a modified human T cell comprising a recombinant polynucleotide encoding an alpha chain and/or a beta chain of a TCR, wherein the TCR is one of the TCRs referred to herein as AL, KQ, PP, 19305CD8, BB, KB, ST, JD, 19305DP, PB-P, PB-T, or PB13.2, as described further below. In another aspect the disclosure comprises 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 and/or its highly homologous LAGE-1 antigen, the method comprising administering to the individual modified human T cells that express a recombinant TCR of this disclosure.

[0005] In another aspect the disclosure comprises an expression vector encoding a TCR, wherein the TCR comprises an alpha chain and/or a beta chain having the sequence of 19305DP AL, KQ, PP, 19305CD8, BB, KB, ST, JD, PB-P, PB-T, or PB13.2 as further described below.

[0006] In another aspect the disclosure provides a library comprising a plurality of expression vectors, wherein the expression vectors encode at least one alpha chain and/or a beta chain or a combination thereof selected from the group of TCR alpha and beta chains described herein for TCRs 19305DP, AL, KQ, PP, 19305CD8, BB, KB, ST, JD, PB-P, PB-T, or PB13.2 as further described below. In one example, the library can further comprise at least one expression vector encoding the alpha chain, the beta chain, or a combination thereof for the JM, 5B8, SB95 TCRs which are also further described below.

[0007] In another aspect the disclosure provides a method comprising selecting an expression vector from a library of this disclosure, wherein the selection is based at least in part on the HLA type of an individual diagnosed with or suspecting of having a NY-ESO-1/LAGE-1 positive cancer, and distributing the selected expression vector to a party for use in introducing the expression vector into immune cells of the diagnosed individual.

[0008] In another aspect the disclosure provides a method comprising selecting an expression vector from an expression vector library of this disclosure, and introducing the expression vector into immune cells obtained from an individual diagnosed with a NY-ESO-1/LAGE-1 positive cancer, wherein the HLA type of the TCR encoded by the expression vector is matched to the HLA type of the individual, the method optionally comprising introducing the immune cells comprising the expression vector into an individual in need thereof.

[0009] In another aspect the disclosure provides a method comprising testing a sample from an individual to determine whether or not the individual has a NY-ESO-1/LAGE-1 positive cancer, and subsequent to a determination that the individual has the NY-ESO-1/LAGE-1 positive cancer, selecting an expression vector from a library of this disclosure based at least in part on the HLA type of the individual, and introducing the expression vector into immune cells of the individual, the method optionally further comprising introducing the immune cells comprising the expression vector into the individual.

[0010] In another aspect the disclosure provides computer-based methods for selecting and/or retrieving a TCR from a library of this disclosure for use in an immunotherapy. In embodiments the disclosure includes a database comprising nucleotide and/or amino acid sequences of the TCRs, or other indicia of the TCRs. In certain
5 embodiments the disclosure includes a system that comprises a processor programmed to match a TCR of a library of this disclosure with the HLA type of a sample.

BRIEF DESCRIPTION OF THE FIGURES

[0011] Figures 1A-1D provide graphical depictions of vectors of this disclosure.

Figure 1A. For transduction of T cells, murine stem cell virus (MSCV)-derived vectors have
10 been widely used because of strong promoter activity by MSCV long terminal repeats (LTR) and in vivo stability of transgene expression in hematopoietic cells. As 3'-LTR is copied to 5'-LTR during integration to host cells (T cells) and responsible for the transcription of transgenes, 3'-LTR in the plasmids is important in the expression. On the other hand, 5'-LTR is responsible for transcription for virus production. A Schematic representation for classical
15 MSCV-derived vectors (pMIG-II and pMIG-w) is shown in Figure 1A. Both vectors have MSCV-derived LTR at 5' and 3' sites, packaging signal (ψ), multiple cloning sites (MCS). A transgene is cloned in the multiple cloning site (MCS), which is followed by the internal ribosomal entry site (IRES) and the green fluorescent protein gene (GFP) to efficiently detect transduced cells. pMIG-w vector has additional woodchuck hepatitis virus post-
20 transcriptional regulatory element (WRE), which enhances expression of the transgene. Further modifications can be introduced, such as those found in the commercial retroviral vector, pDON-5 (Clontech). pDON-5, which is derived from a murine leukemia virus (MLV) vector, and replaces the 5'-LTR with the CMV/MLV hybrid LTR for enhanced virus production through strong CMV promoter activity in virus packaging cell lines. Additionally,
25 a partial intron from the human elongation factor 1 α gene can be introduced to provide a splice acceptor site (SA), which together with an endogenous splice donor site (SD) induces splicing and enhances transcription.

[0012] Figure 1B. To create a retrovirus vector which can produce high-titer retrovirus that induces high level transgene (TCR) expression in T cells, we amplified a DNA
30 fragment from 5'-LTR to the intron containing a splice acceptor site in the pDON-5 plasmid. The forward primer was designed to append SgrAI restriction enzyme recognition site before 5'-LTR and the reverse primer was designed to append NotI and SalI sites after the intron. PCR-amplified fragment was treated with SgrAI and SalI and inserted into pMIG-II and

pMIG-w plasmids so that 5'-LTR to GFP is replaced.

[0013] Figure 1C. The depicted plasmids only have NotI and SalI recognition sites for cloning. The use of SalI a known 6-mer sequence is not preferred as this short recognition sequence may appear in the transgene. To provide additional restriction enzyme recognition site which is believed to be absent from most transgenes that are pertinent to this disclosure, we amplified a 1.8kb DNA fragment (stuffer) with a forward primer with a NotI restriction site, and a reverse primer with PacI-SalI sites. The amplified fragment was treated with NotI and SalI restriction enzymes and inserted into new plasmids.

[0014] Figure 1D. The TCR expression cassette was amplified with the forward primer with the NotI restriction site and the reverse primer with the PacI restriction enzyme site. The amplified expression cassette was treated with NotI and PacI restriction enzymes and inserted into the new plasmids.

[0015] Figure 2. Expression of 19305DP-TCR on polyclonally activated T cells. Polyclonally activated T cells from peripheral blood mononuclear cells were transduced twice with retroviral vector encoding 19305DP-TCR, whose beta chain variable region was a Vb8 subtype. Expression was measured by staining with anti-TCR Vb8 antibody together with anti-CD4 and anti-CD8 antibodies.

[0016] Figure 3. Cancer cell recognition by 19305DP-TCR-transduced T cells. 19305DP-TCR transduced T cells were co-cultured with NY-ESO-1⁺SK-MEL-37 and NY-ESO1-SK⁺MEL-29 melanoma cells lines for 6 hours in the presence of Golgi Stop and IFN-g production was measured by intracellular IFN-g staining in combination with cell surface CD8 staining.

[0017] Figure 4, panel A. Retrovirus vector used to express TCRs. LTR: long-terminal repeat; ψ : packaging signal; MCS: multiple cloning site; IRES: internal ribosome entry site; eGFP: enhanced green fluorescent protein. Figure 4 panel B. TCR expressing cassette. (I) TCR β and α chain-coding cDNA sequences are connected by a GSG (Gly-Ser-Gly) linker and a P2A ribosomal skipping sequence. (II) TCR β and α chain-coding cDNA sequences are connected by a furin protease recognition site (RAKR (Arg-Ala-Lys-Arg) ((SEQ ID NO:59)), a SGSG (Ser-Gly-Ser-Gly) (SEQ ID NO:60) linker, V5 epitope, and a P2A ribosomal skipping sequence.

[0018] Figure 5. Cell surface expression of CD4 and CD8 molecules on HLA-A*02-restricted T cell clones. 19305DP-TCR CD4/CD8 double positive T cell clone and AL CD8 single positive T cell clone were stained by anti-CD4 and anti-CD8 monoclonal antibodies

and analyzed by flow cytometry.

[0019] Figure 6. Effect of blocking antibodies on recognition of cancer targets.

19305DP-TCR CD4/CD8 double positive T cell clone and JD CD8 single-positive T cell clone were co-cultured with HLA-A*02+NY-ESO-1+ melanoma cell line MZ-MEL-9 in the presence or absence of the indicated antibodies. Anti-HLA class I antibody (aABC) inhibited recognition by both 19305DP and JD. Although two anti-CD8 antibodies significantly inhibited recognition by JD, recognition by 19305DP was not inhibited, indicating CD8-independent recognition of cancer targets. * indicates significant ($p < 0.05$) inhibition.

[0020] Figure 7. Effect of alanine substitution on NY-ESO-1 peptide recognition.

Each amino acid residue in NY-ESO-1(157-170) peptide (DP4 peptide SLLMWITQCFLPVF (SEQ ID NO:61) was substituted with alanine residue. HLA-A*02-positive and NY-ESO-1-negative cancer cell was pulsed or un-pulsed (-) with alanine substituted (1-14) or natural DP4 peptide (DP4). Recognition by 19305DP CD4/CD8 double -positive T cell clone, AL CD8 single -positive T cell clone and JD CD8 single positive T cell clone was investigated by measuring IFN- γ in the supernatant. Alanine substitution of amino acids at positions 2, 3, 4, 5, 6, and 7 (LLMWIT-SEQ ID NO: 62) significantly decreased the recognition by 19305DP, indicating that these residues are important for T cell recognition. In silico analysis showed that there is no known proteins with LLMWIT sequence, except for NY-ESO-1 and LAGE-1.

[0021] Figure 8. In vivo anti-tumor activity of 19305DP-TCR-transduced T cells.

NOD/Scid/IL-2R γ -chain-deficient (NSG) mice were subcutaneously injected with 1×10^6 HLA-A2⁺NY-ESO-1⁺ A375 cell line. Peripheral blood mononuclear cells (PBMCs) were polyclonally activated and transduced with 19305DP-TCR, AL-TCR or irrelevant-TCR by retroviral vectors. On day 11, 2.5×10^5 TCR transduced T cells were intravenously injected. Tumor volume was measured every other days after T-cell injection by digital caliper. 19305-TCR-transduced T cells significantly inhibited tumor growth (Panel A) and prolonged survival (Panel B). Five out of 6 mice treated with 19305DP-TCR-transduced T cells were tumor free at day 40, while 4 out of 6 mice injected with AL-TCR-transduced T cells had tumor at this time point.

[0022] Figure 9. In vitro CTL activity of CD8⁺ and CD4⁺ T cells transduced with

19305DP-TCR. CD4⁺ or CD8⁺ T cells were depleted from PBMC using biotin-conjugated anti-CD4 or anti-CD8 antibody followed by anti-biotin Dynabeads. CD4-depleted and CD8-depleted PBMC were polyclonally activated and transduced with 19305DP-TCR, AL-TCR or irrelevant-TCR by retroviral vectors. (Panel A) The purity of CD8⁺ (CD4-depleted cells) and CD4⁺ (CD8-depleted cells) T cells transduced with TCR were determined by flow cytometry

prior to in vivo experiment. (Panel B) Cytotoxic activity of CD8⁺ or CD4⁺ T cells transduced with indicated TCR against HLA-A2⁺NY-ESO-1⁺ Mel624.38 cell line was tested by Calcein-AM assay after 4 hours incubation. (Panel C) Cancer cell apoptotic cell death after cocultured with CD4⁺ or CD8⁺ T cells transduced with indicated TCR. HLA-A2⁺NY-ESO-1⁺ A375 cell line (2.5x10⁵) was cocultured with 5x10⁵ T cells for 20 hours. Apoptotic cell death was determined by annexin-V and PI staining. Whereas 19305DP-TCR-transduced CD4⁺ T cells showed weak cytotoxic activity compared with CD8⁺ T cells by short-term cytotoxic assay (Panel B), the CD4⁺ T cells induced apoptotic cell death (Panel C). Cancer cell death was not observed when cocultured with AL-TCR-transduced CD4⁺ T cells.

[0023] Figure 10. Anti-tumor activity of CD8⁺ and CD4⁺ T cells transduced with 19305DP-TCR. CD4⁺ or CD8⁺ T cells were depleted from PBMC using biotin-conjugated anti-CD4 or anti-CD8 antibody followed by anti-biotin Dynabeads. CD4-depleted and CD8-depleted PBMC were polyclonally activated and transduced with 19305DP-TCR, AL-TCR or irrelevant-TCR by retroviral vectors. NOD/Scid/IL-2R γ -chain-deficient (NSG) mice were subcutaneously injected with 1x10⁶ HLA-A2⁺NY-ESO-1⁺ A375 cell line. On day 11, 0.6x10⁵ TCR transduced CD8⁺ T cells (25% of whole PBMC) and 1.9x10⁵ TCR transduced CD4⁺ T cells (75% of whole PBMC) were intravenously injected. Tumor volume was measured every other days after T-cell injection by digital caliper. Both 19305DP-TCR and AL-TCR-transduced CD8⁺ T cells controlled tumor growth (left), while only 19305DP-TCR transduced CD4⁺ T cells showed tumor regression (right).

DETAILED DESCRIPTION

[0024] Unless defined otherwise herein, all technical and scientific terms used in this disclosure have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains.

[0025] Every numerical range given throughout this specification includes its upper and lower values, as well as every narrower numerical range that falls within it, as if such narrower numerical ranges were all expressly written herein.

[0026] Each polynucleotide described herein includes its complementary sequence and its reverse complementary sequence, as well as RNA equivalents of DNA sequences wherein each T in a DNA sequence is replaced with U. All polynucleotide sequences encoding the amino acid sequences described herein are included within the scope of this disclosure.

[0027] The present disclosure provides compositions and methods for prophylaxis and/or therapy of a variety of cancers. In general, the cancers are those which express the

well-known NY-ESO-1/LAGE-1 antigen.

[0028] The disclosure includes each of the recombinant TCRs described herein, polynucleotides encoding them, expression vectors comprising the polynucleotides, cells into which the polynucleotides have been introduced, including but not necessarily limited CD4⁺ T cells, CD8⁺ T cells, natural killer T cells, $\gamma\delta$ T cells, and progenitor cells, such as hematopoietic stem cells. As used in this disclosure, a “recombinant TCR” means a TCR that is expressed from a polynucleotide that was introduced into the cell, meaning prior to the initial introduction of the polynucleotide the TCR was not encoded by a chromosomal sequence or other polynucleotide in the cell.

[0029] In embodiments, the cells into which the polynucleotides are introduced are lymphoid progenitor cells, immature thymocytes (double-negative CD4-CD8-) cells, or double-positive thymocytes (CD4+CD8+). In embodiments, the progenitor cells comprise markers, such as CD34, CD117 (c-kit) and CD90 (Thy-1). The disclosure includes methods of making the TCRs, methods of modifying cells so that they express the TCRs, the modified cells, and methods of using the modified cells for anti-cancer approaches. Libraries of distinct TCRs are also included.

[0030] In particular embodiments, the disclosure 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/LAGE-1 antigen. This approach comprises in one aspect administering to the individual modified human cells comprising a recombinant polynucleotide encoding a TCR of this disclosure. The modified human cells, such as modified T cells, are enhanced relative to their unmodified T cell counterparts in their ability to fight cancer. Thus, in embodiments practicing a method of this disclosure results in a therapeutic response which can include but is not necessarily limited to slowing the growth rate of cancer cells and/or tumors, reducing tumor volume and/or reducing an increase in the rate of tumor volume increase, killing cancer cells, extending life span of an individual who has been diagnosed with a cancer as more fully described herein, and other parameters that will be apparent to those skilled in cancer treatment.

[0031] The effect of practicing embodiments of this disclosure can be compared to any suitable reference, such as a positive or negative control. In embodiments an effect can be compared to a reference value obtained, for example, from a control cells expressing a TCR with known effects, or a TCR that is not specific for the particular antigen in question,

or a TCR that is mismatched with respect to HLA type and/or T lymphocyte type, or any other suitable reference value that will be apparent to those skilled in the art when provided the benefit of this disclosure. In embodiments a suitable reference comprises a known value or range of values. In embodiments, the reference comprises a statistical value, such as an area under a curve, or another area or plot on a graph, and/or is obtained from repeated measurements.

[0032] Various and non-limiting embodiments of the disclosure are demonstrated using HLA-I and HLA-II restricted TCRs.

[0033] HLA- I restricted TCRs

[0034] In one aspect the disclosure encompasses novel TCRs that are specific for the NY-ESO-1 antigen as presented in an HLA class I context. Specific examples of the α chain and β chain of HLA-I restricted TCRs and polynucleotide sequences encoding them are described further below as “19305DP”, “AL”, “KQ”, “PP”, “19305CD8”, “BB”, “KB”, “ST”, and “JD”. These TCRs are specific for NY-ESO-1/LAGE-1-derived peptides presented by different HLA class I types, including HLA-A*02; B*27; B*35; Cw*03; and Cw*15.

[0035] HLA-II restricted TCRs

[0036] In another aspect the disclosure encompasses novel TCRs that are specific for the NY-ESO-1 antigen as presented in an HLA class II context. Specific examples of the α chain and β chain of HLA-II restricted TCRs and polynucleotide sequences encoding them are described further below as “PB-P”, “PB-T”, and “PB13.2”. These TCRs are specific for NY-ESO-1/LAGE-1-derived peptides presented by different HLA class II types, including HLA-DRB1*04 and DRB1*07. In certain embodiments these TCRs impart to T cells that express them the capability of direct recognition of tumors and/or cancer cells that express the NY-ESO-1/LAGE-1 antigen.

[0037] In certain aspects, the cells comprising a recombinant TCR of this disclosure that are administered to an individual are allogeneic, syngeneic, or autologous cells. Thus, in one embodiment, the cells are obtained from a first individual, modified, and administered to a second individual who is in need thereof. In another embodiment, the cells are removed from the individual prior to modification, are modified to express the recombinant TCR, and administered back to the same individual. In certain embodiments, the cells that are modified according to this disclosure comprise an immune cell population that is enriched for, comprises or consists of a particular immune cell type. In certain aspects the cells are CD4⁺ T cells, or are CD8⁺ T cells. In one aspect, the cells into which one or more expression

vectors of this disclosure is introduced comprise a mixture of immune cells, such CD4⁺ and CD8⁺ T cells, and/or can comprise peripheral blood mononuclear cells (PBMCs). In one approach, one or more expression vectors which alone or together encode the 19305DP TCR is/are introduced into a mixture of immune cells. In certain embodiments, the T cells are capable of direct recognition of the cancer cells expressing the NY-ESO-1/LAGE-1 antigen. In embodiments the direct recognition comprises HLA class II-restricted binding of the TCR to the NY-ESO-1/LAGE-1 antigen expressed by the cancer cells.

[0038] With respect to 19305DP TCR, the present disclosure demonstrates certain characterizations of its expression and function. For example, Figures 5-7 provide characteristics of parental 19305DP clone. In particular, Figure 5 provides evidence of the CD4/CD8 double positive characteristics. Figure 6 provides a demonstration of CD8-independent recognition which indicates high affinity recognition by the TCR. Figure 7 provides a demonstration of strict NY-ESO-1/LAGE-1 sequence-specificity which supports no cross-reactivity to other human antigens. Figures 8-10 present results demonstrating anti-tumor effects of human T cells transduced by the 19305DP-TCR, in comparison with AL-TCR, which has the same HLA-A*02:01-restriction and NY-ESO-1-specificity but is derived from a CD8 single-positive T-cell clone. Figure 8 provides a demonstration of in vivo growth inhibition of NY-ESO-1 and HLA-A*02:01-expressing human melanoma by human peripheral blood mononuclear cells containing both CD4⁺ and CD8⁺ T cells, that were transduced by 19305DP or AL TCR-expressing retroviral vectors, demonstrating superior anti-tumor effect by 19305DP-TCR-transduced PBMCs. Figure 9 provides a demonstration of in vitro tumor-killing ability of 19305DP and AL TCR-expressing CD4⁺ and CD8⁺ T cells, demonstrating that both CD4⁺ and CD8⁺ T cells efficiently kill NY-ESO-1+HLA-A*02:01+ cancer target when they were transduced by 19305DP-TCR, while only CD8⁺ T cells killed the target when they were transduced by AL-TCR. Figure 10 provides data demonstrating in vivo tumor growth inhibition of 19305DP and AL TCR-expressing CD4⁺ and CD8⁺ T cells, demonstrating that both CD4⁺ and CD8⁺ T cells attack NY-ESO-1+HLA-A*02:01+ cancer targets when they were transduced by 19305DP-TCR, while only CD8⁺ T cells showed tumor growth inhibition when they were transduced by AL-TCR. Thus, in certain embodiments the disclosure relates to the ability to transduce either CD4⁺ T cells, or CD8⁺ T cells, or so-called double positive ("DP") T cells using recombinant TCRs and modified cells of this disclosure, such as cells modified to express the 19305DP TCR. With respect to DP T cells, it is known in the art that they exist at a developmental stage in the thymus. CD4⁺/CD8⁺ double positive T cells are differentiated into the CD4 lineage or the

CD8 lineage by the process known as thymic positive selection, and CD4 and CD8 differentiation paths are believed to be generally mutually exclusive fates. Nevertheless, mature CD4⁺/CD8⁺ DP T cells have been described in the blood and peripheral lymphoid tissues, and have been observed in certain disorders, including cancer - although at low frequency. Without intending to be constrained by any particular theory, it is believed the present disclosure provides the first description of a TCR obtained from a DP T cell, and producing such TCR recombinantly to demonstrate its utility in adoptive immunological approaches, as demonstrated in the Examples and Figures of this disclosure. In certain embodiments, the invention provides mixtures of cells expressing TCRs, or cells expressing more than one TCR described herein, that are specific for distinct cancer antigens, thus providing cell populations that can be considered polyvalent with respect to the TCRs.

[0039] The TCRs provided by the invention are in certain examples capable of recognizing NY-ESO-1;157-170 which is an antigen that consists of the amino acid sequence SLLMWITQCFLPVF (SEQ ID NO:63), or are capable of recognizing NY-ESO-1;95-106, which is an antigen that consists of the amino acid sequence PFATPMEAELAR (SEQ ID NO:64).

[0040] In certain embodiments, the cells provided by the invention are engineered CD8⁺ T cells expressing a TCR of this disclosure that can directly recognize NY-ESO-1/LAGE-1+ cancer cells, and CD4⁺ T cells that are capable of recognizing these NY-ESO-1/LAGE-1 antigens via TCRs which interact with the antigen in association with HLA class II molecules, wherein the HLA class II molecules are displayed by tumor cells.

[0041] The invention includes each and every polynucleotide sequence that encodes one or more TCR polypeptides of the invention and disclosed herein, including DNA and RNA sequences, and including isolated and/or recombinant polynucleotides comprising and/or consisting of such sequences. The invention also includes cells which comprise the recombinant polynucleotides. The cells can be isolated cells, cells grown and/or expanded and/or maintained in culture, and can be prokaryotic or eukaryotic cells. Prokaryotic and eukaryotic cell cultures can be used, for example, to propagate or amplify the TCR expression vectors of the invention. In embodiments, the cells can comprise packaging plasmids, which, for example, provide some or all of the proteins used for transcription and packaging of an RNA copy of the expression construct into recombinant viral particles, such as pseudoviral particles. In embodiments, the expression vectors are transiently or stably introduced into cells. In embodiments, the expression vectors are integrated into the chromosome of cells used for their production. In embodiments, polynucleotides encoding

the TCRs which are introduced into cells by way of an expression vector, such as a viral particle, are integrated into one or more chromosomes of the cells. Such cells can be used for propagation, or they can be cells that are used for therapeutic and/or prophylactic approaches. The eukaryotic cells include CD4⁺ T cells, CD8⁺ T cells, natural killer T cells, $\gamma\delta$ T cells, and their progenitor cells into which a TCR expression construct of the invention has been introduced. The CD4⁺ T cells can be from any source, including but not limited to a human subject who may or may not be the eventual recipient of the CD4⁺ T cells, CD8⁺ T cells, or combinations thereof, once they have been engineered to express a novel TCR according to this disclosure.

[0042] Expression vectors for use with embodiments of this disclosure can be any suitable expression vector. In embodiments, the expression vector comprises a modified viral polynucleotide, such as from an adenovirus, a herpesvirus, or a retrovirus, such as a lentiviral vector. The expression vector is not restricted to recombinant viruses and includes non-viral vectors such as DNA plasmids and in vitro transcribed mRNA.

[0043] With respect to the polypeptides that are encoded by the polynucleotides/ expression vectors described above, in certain aspects the invention provides functional TCRs and expression vectors encoding them, wherein the functional TCR which comprises a TCR α and a TCR β chain, wherein the two chains are present in a physical association with one another (e.g., in a complex) and are non-covalently joined to one another, or wherein the two chains are distinct polypeptides but are covalently joined to one another, such as by a disulfide or other covalent linkage that is not a peptide bond. Other suitable linkages can comprise, for example, substituted or unsubstituted polyalkylene glycol, and combinations of ethylene glycol and propylene glycol in the form of, for example, copolymers. In other embodiments, two polypeptides that constitute the TCR α and a TCR β chain can both be included in a single polypeptide, such as a fusion protein. In certain embodiments, the fusion protein comprises a TCR α chain amino acid sequence and a TCR β chain amino acid sequence that have been translated from the same open reading frame (ORF), or distinct ORFs, or an ORF that contain a signal that results in non-continuous translation. In one embodiment, the ORF comprises a P2A-mediated translation skipping site positioned between the TCR α and TCR β chain. Constructs for making P2A containing proteins (also referred to as 2A Peptide-Linked multicistronic vectors) are known in the art. (See, for example, *Gene Transfer: Delivery and Expression of DNA and RNA, A Laboratory Manual*, (2007), Friedman et al., International Standard Book Number (ISBN) 978-087969765-5.

Briefly, 2A peptide sequences, when included between coding regions, allow for stoichiometric production of discrete protein products within a single vector through a novel cleavage event that occurs in the 2A peptide sequence. 2A peptide sequences are generally short sequence comprising 18–22 amino acids and can comprise distinct amino-terminal sequences. Thus, in one embodiment, a fusion protein of the invention includes a P2A amino acid sequence. In embodiments, a fusion protein of the invention can comprise a linker sequence between the TCR α and TCR β chains. In certain embodiments, the linker sequence can comprise a GSG (Gly-Ser-Gly) linker or an SGSG (Ser-Gly-Ser-Gly) (SEQ ID NO:59) linker. In certain embodiments, the TCR α and TCR β chains are connected to one another by an amino acid sequence that comprises a furin protease recognition site, such as an RAKR (Arg-Ala-Lys-Arg) (SEQ ID NO:60) site.

[0044] In one embodiment, the expression construct that encodes the TCR can also encode additional polynucleotides. The additional polynucleotide can be such that it enables identification of TCR expressing cells, such as by encoding a detectable marker, such as a fluorescent or luminescent protein. The additional polynucleotide can be such that it encodes an element that allows for selective elimination of TCR expressing cells, such as thymidine kinase gene. In embodiments the additional polynucleotides can be such that they facilitate inhibition of expression of endogenously encoded TCRs. In an embodiment, the expression construct that encodes the TCR also encodes a polynucleotide which can facilitate RNAi-mediated down-regulation of one or more endogenous TCRs. For example, see Okamoto S, et al. (2009) Cancer Research, 69:9003-9011, and Okamoto S, et al. (2012). Molecular Therapy-Nucleic Acids, 1, e63. In an embodiment, the expression construct that encodes the TCR can encode an shRNA or an siRNA targeted to an endogenously encoded TCR. In an alternative embodiment, a second, distinct expression construct that encodes the polynucleotide for use in downregulating endogenous TCR production can be used.

[0045] In certain approaches distinct TCR chains can be expressed from an expression construct such that the β chain is oriented N-terminally in relation to the α chain, and thus TCRs of the invention can also comprise this chain orientation, or other orientations. In alternative embodiments, the TCR α and β chain proteins can be expressed from distinct expression vectors introduced into the same cell. In certain embodiments, mRNA encoding TCRs can be used as an alternative to expression vectors.

[0046] With respect to use of the engineered CD4⁺ T cells, CD8⁺ T cells, and combinations thereof, the method generally comprises administering an effective amount

(typically 10^{10} cells by intravenous or intraperitoneal injections) of a composition comprising the CD4⁺ T cells to an individual in need thereof. An individual in need thereof, in various embodiments, is an individual who has or is suspected of having, or is at risk for developing a cancer which is characterized by malignant cells that express NY-ESO-1/LAGE-1. As is well known in the art, NY-ESO-1/LAGE-1 is expressed by a variety of cancer cells and tumor types. In particular and non-limiting examples, such cancers include cancers of the bladder, brain, breast, ovary, non-small cell lung cancer, myeloma, prostate, sarcoma and melanoma. Specific embodiments include but are not limited to liposarcomas and intrahepatic cholangiocarcinoma. The individual may have early-stage or advanced forms of any of these cancers, or may be in remission from any of these cancers. In one embodiment, the individual to whom a composition of the invention is administered is at risk for recurrence for any cancer type that expresses NY-ESO-1. In certain embodiments, the individual has or is suspected of having, or is at risk for developing or recurrence of a tumor comprising cells which express a protein comprising the amino acid sequences defined by NY-ESO-1:157-170 and/or NY-ESO-1:95-106. In embodiments, the disclosure includes recombinant TCRs that are specific for peptide fragments of NY-ESO-1 that are between 15 and 24 amino acid residues long, wherein such peptides are presented in a complex with HLA-II. In embodiments, the disclosure includes recombinant TCRs that are specific for peptides that are in a complex with HLA-I, or HLA-II, wherein the peptides comprise or consist of the amino acid sequences of NY-ESO-1:157-170 and/or NY-ESO-1:95-106.

[0047] The nucleotide and amino acid sequences presented below represent those used to demonstrate the invention. As described above, the invention includes any and all polynucleotide sequences encoding the amino acid sequences of the TCR constructs described herein. Further, variations in amino acid sequences in the TCRs are contemplated, so long as they do not adversely affect the function of the TCR. In various embodiments, a TCR comprising one or more amino acid changes as compared to the sequences presented herein will comprise conservative amino acid substitutions or other substitutions, additions or deletions, so long as the cells expressing the recombinant TCRs of the invention can directly and specifically recognize tumor cells that express NY-ESO-1/LAGE-1, wherein that recognition is dependent on expression of NY-ESO-1/LAGE-1 and presentation of peptides processed from it in an HLA class II restricted manner by the tumor cells. In embodiments, a TCR of the present invention comprises any amino acid sequence that facilitates direct recognition of the tumor antigen on the tumor cells, without participation of an antigen presenting cells. In embodiments, the amino acid sequence of a TCR provided by this

disclosure is at least 95%, 96%, 97%, 98% or 99% similar to an amino acid sequences provided in the sequence listing that is part of this disclosure. In various embodiments, any TCR of the invention can have a K_{off} value for its cognate epitope as defined herein that is essentially the same as the K_{off} for the cognate epitope exhibited by a TCR of a naturally occurring TCR for the same epitope. In embodiments, the TCR amino acid sequences can comprise changes in their constant region. In this regard, it is known in the art that in general, the constant region of a TCR does not substantially contribute to antigen recognition. For example, it is possible to replace a portion of the human constant region of a TCR with a murine sequence and retain function of the TCR. (See, for example, Goff SL et al. (2010) Cancer Immunology, Immunotherapy, 59: 1551-1560). Thus, various modifications to the TCR sequences disclosed herein are contemplated, and can include but are not limited to changes that improve specific chain pairing, or facilitate stronger association with T cell signaling proteins of the CD3 complex, or inhibit formation of dimers between the endogenous and introduced TCRs. In embodiments, the amino acid changes can be present in the CDR region, such as the CDR3 region, including but not necessarily limited to substitutions of one, two, three, or more amino acids in the CDR3 sequence. In embodiments, the amino acid changes have no effect on the function of the TCR. The mature TCR proteins are preceded by the amino acid sequences termed a signal peptide or a leader peptide, which direct newly synthesized TCR proteins to the secretory pathway. A signal peptide is removed from the mature TCR protein before cell surface expression. Therefore, replacement of the signal peptide with other natural or artificial sequence does not alter function of mature TCR. Thus, various modifications to the signal peptide sequences disclosed herein are contemplated, including but not limited to deleting or changing some or all of the amino acids is in the signal peptide, or replacing all or some of the amino acids with other amino acids and/or polypeptide sequences, examples of which will be apparent to those skilled in the art given the benefit of the present disclosure. In certain aspects, the disclosure includes expression vectors and other polynucleotides encoding one or more TCR hypervariable, or complementarity determining regions (CDRs) from the TCR alpha chain, beta chain, or a combination thereof. In certain embodiments only one CDR is encoded, or only two CDRs are encoded, or only three CDRs are encoded, or a combination of only certain CDRs from the TCR alpha and beta chains are encoded, and all such combinations of TCR CDR segments and polynucleotides encoding them from the TCR alpha and beta chains described herein are encompassed by this disclosure. Those skilled in the art will be able to recognize TCR CDR segments of each of the TCR amino acid sequence presented herein.

[0048] Libraries

[0049] The disclosure includes a plurality of expression vectors encoding TCRs, i.e., a library comprising a plurality of distinct expression vectors encoding distinct TCRs, wherein at least one member of the library encodes a novel α -chain, and/or a novel β -chain of a TCR of this disclosure. Thus, at least one member of the library can be selected from expression vectors that encode the α chain and/or β chain of at least one of the HLA-I restricted TCRs referred to herein as AL, KQ, PP, 19305CD8, BB, KB, ST, JD, and 19305DP, and at least member of the library can be selected from expression vectors that described herein PB-P, PB-T, PB13.2. Combinations of distinct expression vectors encoding these HLA-I and HLA-II restricted TCRs are included in the disclosure.

[0050] In one non-limiting example a library of this disclosure comprises an expression vector encoding the TCR described herein as 19305DP, which is an HLA-A*02-restricted TCR and is functional in both CD4+ and CD8+ T cells. This TCR was initially obtained from a unique tumor antigen-specific T cells that were CD4+CD8+ double-positive, and this is believed to be the first description of such a TCR. The AL, KQ, PP, 19305CD8, BB, KB, ST, and JD TCR genes were initially obtained from CD8+ single-positive T cells, and PB-P, PB-T and PB13.2 were from CD4+ single-positive T cells.

[0051] In certain aspects, in addition at least one novel TCR/expression vector described herein, a library provided by this disclosure can further comprise expression vectors encoding HLA class II restricted TCRs selected from the TCRs described below as "JM", "5B8" that are HLA DPB1*04-restricted and DRB1*01-restricted "SB95". These constructs are described in PCT/US14/25673, published as WO/2014/160030, from which the description of the TCRs, expression vectors encoding the TCRs, and methods of making and using the TCRs and expression vectors are incorporated herein by reference. The JM, 5B8 and SB95 TCRs were obtained from NY-ESO-1 positive individuals, and these TCRs confer onto T cells, including CD4+ T cells, the ability to directly recognize NY-ESO-1+ cancer cells, as described further below, including but not necessarily limited to PB-P, PB-T and PB13.2. Thus, these TCRs impart to T cells the capability to directly recognize a cancer cell expressing a NY-ESO-1/LAGE-1 antigen, wherein the direct recognition of the cancer cell comprises human leukocyte antigen HLA class II-restricted binding of the TCR to the NY-ESO-1/LAGE-1 antigen expressed by the cancer cell.

[0052] In various embodiments an expression vector library of this disclosure encodes

a diversity of TCRs. In certain aspects, the expression vectors do not comprise any phage or phagemid DNA, and/or none of the TCR polypeptide(s) comprises any phage or phagemid protein. In embodiments the TCRs do not comprise any phage or phagemid protein and thus are not components of, for example, a TCR phage display library.

5 **[0053]** In certain examples expression vectors in a TCR expression vector library of this disclosure encode a plurality of TCRs such that cells expressing the TCRs can function in a diversity of patients with distinct HLA class I types, HLA class II types, and/or combinations thereof.

10 **[0054]** In certain embodiments HLA class I-restricted TCRs encoded by an expression vector library of this disclosure are capable of functioning in patients with an HLA class I type selected from an allele encompassed by HLA-A, -B, -C, and combinations thereof. In certain embodiments, the library is sufficiently diverse to be suitable for use in cancer therapy in at least 50% of the U.S. Caucasian population at the time of the filing of this application or patent. In certain aspects, a library of this disclosure is suitable for use in
15 cancer therapy on the basis of HLA-class I (A*02/B*35/C*04) restricted TCRs, which without intending to be bound by any particular theory is believed to comprise 67% of the HLA types of the U.S Caucasian population at the time of the filing of this application or patent, and HLA class II (DR*01/DR*04/DR*07/DP*04) restricted TCRs, which also without intending to be bound by any particular theory is believed to comprise 87% of the
20 HLA types of U.S Caucasian population at the time of the filing of this application or patent. In certain embodiments a TCR library of this disclosure comprises TCRs specific for the 10 most frequently occurring HLA types in the U.S Caucasian population at the time of the filing of this application or patent, and thus may be suitable for use in cancer therapy at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more of the human population.
25 In certain embodiments, the disclosure provides a TCR library that is restricted to a plurality of NY-ESO-1 specific TCRs that are restricted by HLA types set forth in Table 1. In embodiments, the library comprises TCRs that are restricted by one or a combination of the underlined HLA types in Table 1. In certain aspects the disclosure includes a library of 2 - 3,000 distinct expression vectors encoding distinct TCRs.

30 **[0055]** In certain embodiments, expression vectors in a TCR expression vector library of this disclosure encode a range of TCRs such that cells expressing the TCRs can function in a diversity of patients with an HLA class II type selected from an allele encompassed by HLA- DP, -DM, -DOA, -DOB, -DQ, -DR, and combinations thereof.

[0056] In embodiments, an expression vector library of this disclosure comprises at

least two expression vectors encoding at least two distinct TCRs, at least one of which is selected from 19305DP, AL, KQ, PP, 19305CD8, BB, KB, ST, JD, PB-P, PB-T, and PB13.2. In an embodiment an expression vector library of this disclosure includes an expression vector encoding the alpha chain, the beta chain, or both alpha and beta chains of 19305DP.

5 **[0057]** In certain embodiments, the disclosure comprises a library of distinct expression vectors. In one example, each expression vector can be contained as an isolated DNA preparation, or can be maintained, for example, in a cell culture. Such compositions can be preserved in, for example, separately sealed containers, such as glass or plastic vials, Eppendorf tubes, etc., and can be kept under a reduced temperature, such as a temperature of
10 zero degrees C, or lower. Each separate container or location where a container is kept can comprise indicia of the expression vector(s) in the container. Such indicia can include but are not limited to human or machine perceptible material, such a printed label, a bar code a QR code, and the like, or any other indicia that is useful for identifying the contents / location of the expression vector, and which can be used for retrieving a TCR for use in a method of this
15 disclosure. In certain aspects, the disclosure includes a plurality of distinct containers comprising distinct TCRs, wherein each container is indexed, and wherein the indicia of the containers is maintained in a database. The database can be digitized and can be adapted such that it is integrated with software. In certain aspects, the disclosure provides a computer based method for selecting a TCR from a library of this disclosure, the method comprising using a
20 processor to match an input HLA type of an individual or sample or other HLA information with a TCR in the library that is compatible with said HLA type. In certain implementations, the disclosure can exclude computer based approaches that include signals, carrier waves, or transitory signals.

[0058] In one aspect the disclosure includes a system comprising a library of TCRs,
25 and a database comprising indicia of the nucleotide and/or sequences and/or HLA type of the TCRs, the database in communication with a processor, wherein the processor is programmed to select and/or designate a suitable TCR in the library that is matched with the HLA type of a sample. The system may further comprise an apparatus for retrieval of a container that contains the matched TCR, including but not necessarily a robotized apparatus that can, for
30 example, be directed to the indicia of the suitable TCR, and can select and/or retrieve the indicated TCR from the library. In another aspect the disclosure provides a computer readable medium comprising a database of populated with information about a TCR library of this disclosure. In an embodiment, the disclosure include a computer-readable medium comprising a set of instructions for a computer to select a TCR from a TCR library of this

disclosure, wherein the TCR is matched to the HLA type of a sample.

[0059] In one aspect the disclosure comprises receiving an indication of the HLA type of an individual diagnosed with a NY-ESO-1/LAGE-1 positive cancer, selecting an expression vector from a library of this disclosure based on the HLA type, and distributing the expression vector to a party for use in introducing the expression vector into immune cells of the diagnosed individual. The distributing the expression vector can comprise transporting the expression vector using any suitable approach.

[0060] In one aspect the disclosure comprises selecting an expression vector from a library of this disclosure and introducing the expression vector into immune cells obtained from an individual diagnosed with a NY-ESO-1/LAGE-1 positive cancer, wherein the HLA type of the TCR encoded by the expression vector is matched to the HLA type of the individual. In embodiments the disclosure further comprises introducing the immune cells into an individual in need thereof, which may be the individual who was diagnosed with the NY-ESO-1/LAGE-1 positive cancer, or may be an individual with the same HLA type as the diagnosed individual.

[0061] In one aspect the disclosure comprises testing a sample from an individual to determine whether or not the individual has a NY-ESO-1/LAGE-1 positive cancer, and subsequent to a determination that the individual has the NY-ESO-1/LAGE-1 positive cancer, selecting an expression vector from a library of this disclosure based on the HLA type of the individual, and introducing the expression vector into immune cells of the individual.

[0062] The following examples are intended to illustrate but not limit the invention.

Example 1

[0063] In specific and illustrative embodiments, the polynucleotide sequences encoding the TCRs of the invention, and the amino acid sequences of the TCR α and TCR β chains encoded by the polynucleotides are as follows. Representative and non-limiting examples demonstrating cloning and use of the TCRs is presented in Figures 1-4.

[0064] HLA-A*02-restricted NY-ESO-1₁₅₇₋₁₆₅-specific T-cell clone "AL"

[0065] 1. Nucleotide Sequence

[0066] TCR α chain

[0067] ATGATGAAATCCTTGAGAGTTTTACTAGTGATCCTGTGGCTTCAGTT
GAGCTGGGTTTGGAGCCAACAGAAGGAGGTGGAGCAGAATTCTGGACCCCTCAG

TGTTCCAGAGGGAGCCATTGCCTCTCTCAACTGCACTTACAGTGACCGAGGTTCC
CAGTCCTTCTTCTGGTACAGACAATATTCTGGGAAAAGCCCTGAGTTGATAATGT
CCATATACTCCAATGGTGACAAAGAAGATGGAAGGTTTACAGCACAGCTCAATA
AAGCCAGCCAGTATGTTTCTCTGCTCATCAGAGACTCCCAGCCCAGTGATTCAGC
5 CACCTACCTCTGTGCCGTGGGGGGGACTTACCTCTAGCAACACAGGCAAACCTAATC
TTTGGGCAAGGGACAACCTTTACAAGTAAAACCAGATATCCAGAACCCTGACCCT
GCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCA
CCGATTTTGATTCTCAAACAAATGTGTACAAAGTAAGGATTCTGATGTGTATAT
CACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGC
10 TGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGC
ATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGC
TGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAG
TGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGAC
GCTGCGGCTGTGGTCCAGCTGA (SEQ ID NO:65)

15 [0068] TCR β chain
[0069] ATGGGAATCAGGCTCCTCTGTCTGTGGCCTTTTGTTCCTGGCTGT
AGGCCTCGTAGATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAGGAC
GGGAGAGAAAGTTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATATGTTC
TGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTTCTCATATGATG
20 TTAAAATGAAAGAAAAAGGAGATATTCTGAGGGGTACAGTGTCTCTAGAGAGA
AGAAGGAGCGCTTCTCCCTGATTCTGGAGTCCGCCAGCACCAACCAGACATCTAT
GTACCTCTGTGCCAGCAGTAACCAGATCTATGGCTACACCTTCGGTTCGGGGACC
AGGTTAACCGTTGTAGAGGACCTGAACAAGGTGTTCCACCCGAGGTGCTGTGT
TTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACACTGGTGTGCC
25 TGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGA
AGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCCTCAAGGAGCAGCCCCG
CCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTT
CTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCG
GAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGC
30 GCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCTACCAGCAA
GGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGT
ATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAGGATTT
CTGA (SEQ ID NO:66)

[0070] 2. Amino Acid Sequence of AL (Starting from start codon-coding Methionine to stop codon-coding termination (*))

[0071] TCR α chain

5 [0072] MMKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTY
SDRGSQSFFWYRQYSGKSPELIMSIYSNGDKEDGRFTAQLNKASQYVSLIRDSQPSD
SATYLCAVGGLTSSNTGKLIFGQGTTLQVKPDIQNPDPAVYQLRDSKSSDKSVCLFTD
FDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNNSIPE
DTFFPSPESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRLWSS*
10 (SEQ ID NO:5)

[0073] TCR β chain

[0074] MGIRLLCRVAFCLAVGLVDVKVTQSSRYLVKRTGEKVFLECVQDMD
HENMFWYRQDPGLGLRLIYFSYDVKMKEKGDIEGYSVSREKKERFSLILESASTNQ
TSMYLCASSNQIYGYTFGSGTRLTVVEDLNKVPPEVAVFEPSEAEISHTQKATLVCL
15 ATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQ
NPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLS
ATILYEILLGKATLYAVLVSAVLMLMAMVKRKDF* (SEQ ID NO:6)

[0075] HLA-B*35-restricted NY-ESO-1₉₄₋₁₀₂-specific T-cell clone "KQ"

20 [0076] 1. Nucleotide Sequence

[0077] TCR α chain

[0078] ATGATGGCAGGCATTCGAGCTTTATTTATGTACTTGTGGCTGCAGCT
GGACTGGGTGAGCAGAGGAGAGAGTGTGGGGCTGCATCTTCCTACCCTGAGTGT
CCAGGAGGGTGACAACTCTATTATCAACTGTGCTTATTCAAACAGCGCCTCAGAC
25 TACTTCATTTGGTACAAGCAAGAATCTGGAAAAGGTCCTCAATTCATTATAGACA
TTCGTTCAAATATGGACAAAAGGCAAGGCCAAAGAGTCACCGTTTTATTGAATA
AGACAGTGAAACATCTCTCTCTGCAAATTGCAGCTACTCAACCTGGAGACTCAGC
TGTCTACTTTTGTGCAGAGAATAACCGCCCCACATAATGCAGGCAACATGCTCACC
TTTGGAGGGGGAACAAGGTAAATGGTCAAACCCCATATCCAGAACCCTGACCCT
30 GCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCA
CCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATAT
CACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGC
TGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGC

ATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGC
TGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAG
TGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGAC
GCTGCGGCTGTGGTCCAGCTGA (SEQ ID NO:7)

- 5 [0079] TCR β chain
[0080] ATGGGCCCTGGGCTCCTCTGCTGGGTGCTGCTTTGTCTCCTGGGAGC
AGGCCAGTGGACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGAG
AGGACAGCAAGTGACTCTGAGATGCTCTCCTATCTCTGGGCACAAGAGTGTGTCC
TGGTACCAACAGGTCCTGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAGA
10 AAGAAGAGAGAGGAAGAGGAACTTCCCTGATCGATTCTCAGCTCGCCAGTTCC
CTAACTATAGCTCTGAGCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCCT
GTATCTCTGTGCCAGCAGCTACGACAGGGGGGATAAACTATGGCTACACCTTCGGT
TCGGGGACCAGGTTAACCGTTGTAGAGGACCTGAACAAGGTGTTCCACCCGAG
GTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACA
15 CTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGG
TGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGG
AGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTC
GGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTAC
GGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAG
20 ATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCT
ACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGC
CACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGA
AAGGATTTCTGA (SEQ ID NO:8)

- 25 [0081] 2. Amino Acid Sequence for KQ (Starting from start codon-coding
Methionine to stop codon-coding termination (*))
[0082] TCR α chain
[0083] MMAGIRALFMYLWLQLDWVSRGESVGLHLPTLSVQEGDNSIINCAYS
NSASDYFIWYKQESGKGPQFIIDIRSNMDKRQGQRVTVLLNKTVKHL SLQIAATQPG
30 DSAVYFCAENTAPHNAGNMLTFGGGTRL MVKPHIQNPDP AVYQLRDSKSSDKSVCL
FTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNK SDFACANAFNNS
IIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLK VAGFNLLMTLRLWS
S* (SEQ ID NO:9)

[0084] TCR β chain

[0085] MGPGLLCWVLLCLLGAGPVDAGVTQSPTHLIKTRGQQVTLRCSPISGH
KSVSWYQQVLGQGPQFIFQYYEKEERGRGNFPDRFSARQFPNYSSELNVNALLLGDS
ALYLCASSYDRGINYG YTFGSGTRLTVVEDLNKVPPEVAVFEPSEAEISHTQKATLV
5 CLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATF
WQNPRNHFRQCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQG
VLSATILYEILLGKATLYAVLV SALVLMAMVKRKDF* (SEQ ID NO:10)

[0086] HLA-B*35-restricted NY-ESO-1₉₄₋₁₀₄-specific T-cell clone "PP"

10 [0087] 1. Nucleotide Sequence

[0088] TCR α chain

[0089] ATGGCCTCTGCACCCATCTCGATGCTTGCGATGCTCTTCACATTGAG
TGGGCTGAGAGCTCAGTCAGTGGCTCAGCCGGAAGATCAGGTCAACGTTGCTGA
AGGGAATCCTCTGACTGTGAAATGCACCTATTCAGTCTCTGGAAACCCTTATCTT
15 TTTTGGTATGTTCAATACCCCAACCGAGGCCTCCAGTTCCTTCTGAAATACATCAC
AGGGGATAACCTGGTTAAAGGCAGCTATGGCTTTGAAGCTGAATTTAACAAGAG
CCAAACCTCCTTCCACCTGAAGAAACCATCTGCCCTTGTGAGCGACTCCGCTTTG
TACTTCTGTGCTGTGAGAGATGTTGTGGAGGGGAAATTGCAGTTTGGAGCAGGG
ACCCAGGTTGTGGTCAACCCAGATATCCAGAACCCTGACCCTGCCGTGTACCAGC
20 TGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCT
CAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAAC
GTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGC
AACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCAGAAG
ACACCTTCTTCCCAGCCCAGAAAGTTCTGTGATGTCAAGCTGGTCGAGAAAAG
25 CTTTGAAACAGATACGAACCTAACTTTCAAACCTGTCAGTGATTGGGTTCCGA
ATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGT
CCAGCTGA (SEQ ID NO:11)

[0090] TCR β chain

30 [0091] ATGGGCACCAGCCTCCTCTGCTGGATGGCCCTGTGTCTCCTGGGGG
CAGATCACGCAGATACTGGAGTCTCCCAGGACCCAGACACAAGATCACAAAGA
GGGGACAGAATGTAACTTTCAGGTGTGATCCAATTTCTGAACACAACCGCCTTTA
TTGGTACCGACAGACCCTGGGGCAGGGCCCAGAGTTTCTGACTTACTTCCAGAAT

GAAGCTCAACTAGAAAAATCAAGGCTGCTCAGTGATCGGTTCTCTGCAGAGAGG
 CCTAAGGGATCTTTCTCCACCTTGGAGATCCAGCGCACAGAGCAGGGGGACTCG
 GCCATGTATCTCTGTGCCAGCAGCACCACAAGCTCCTACGAGCAGTACTTCGGGC
 CGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCACCCGAGG
 5 TCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACACT
 GGTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGGGTG
 AATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGA
 GCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCG
 GCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACG
 10 GGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGA
 TCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTA
 CCAGCAAGGGGTCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCC
 ACCTTGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAGAA
 AGGATTCCAGAGGCTAG (SEQ ID NO:12)

15

[0092] 2. Amino Acid Sequence for PP (Starting from start codon-coding Methionine
 to stop codon-coding termination (*))

[0093] TCR α chain

[0094] MASAPISMLAMLFTLSGLRAQSVAQPEDQVNVAEGNPLTVKCTYSVS
 20 GNPYLFWYVQYPNRGLQFLKYITGDNLVKGSYGFEAEFNKSQTSFHLKKPSALVSD
 SALYFCAVRDVVEGKLQFGAGTQVVVTPDIQNPDPVYQLRDSKSSDKSVCLFTDF
 DSQTNVSQSKSDSVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPED
 TFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLK VAGFNLLMTLRLWSS*
 (SEQ ID NO:13)

25 **[0095]** TCR β chain

[0096] MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDP
 HNRLYWYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERP KGSFSTLEIQRTEQG
 DSAMYL CASSTTSSYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLV
 CLATGFYDPDHVELSWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATF
 30 WQNPRNHFRQVQFYGLSENDEWTQDRAKPV TQIVSAEAWGRADCGFTSESYQQG
 VLSATILYEILLGKATLYAVLV SALVLMAMVKRKDSRG* (SEQ ID NO:14)

[0097] HLA-B*27-restricted NY-ESO-1₅₁₋₇₀-specific T-cell clone "19305CD8"

[0098] 1. Nucleotide Sequence**[0099]** TCR α chain

[0100] ATGGAAACTCTCCTGGGAGTGTCTTTGGTGATTCTATGGCTTCAACT
GGCTAGGGTGAACAGTCAACAGGGAGAAGAGGATCCTCAGGCCTTGAGCATCCA
5 GGAGGGTGAATAATGCCACCATGAACTGCAGTTACAAAAGTAGTATAAACAATTT
ACAGTGGTATAGACAAAATTCAGGTAGAGGCCTTGTCACCTAATTTTAATACGT
TCAAATGAAAGAGAGAGAAACACAGTGGAAAGATTAAGAGTCACGCTTGACACTTCC
AAGAAAAGCAGTTCCTTGTTGATCACGGCTTCCCGGGCAGCAGACACTGCTTCTT
ACTTCTGTGCTACGGACGCCGTCGTGGGTGCTGACGGACTCACCTTTGGCAAAGG
10 GACTCATCTAATCATCCAGCCCTATATCCAGAACCCTGACCCTGCCGTGTACCAG
CTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATT
CTCAAACAAATGTGTACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAA
CTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGA
GCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGA
15 AGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAA
AGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTC
GAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTG
GTCCAGCTGA (SEQ ID NO:43)

[0101] TCR β chain

[0102] ATGCTGCTGCTTCTGCTGCTTCTGGGGCCAGGCTCCGGGCTTGGTGC
20 TGTCGTCTCTCAACATCCGAGCTGGGTATCTGTAAGAGTGGAACCTCTGTGAAG
ATCGAGTGCCGTTCCCTGGACTTTCAGGCCACAACCTATGTTTTGGTATCGTCAGTT
CCCGAAACAGAGTCTCATGCTGATGGCAACTTCCAATGAGGGCTCCAAGGCCAC
ATACGAGCAAGGCGTCGAGAAGGACAAGTTTCTCATCAACCATGCAAGCCTGAC
25 CTTGTCCACTCTGACAGTGACCAGTGCCCATCCTGAAGACAGCAGCTTCTACATC
TGCAGTGCTAGAGACCGGGACATAGGACCCTTAGATACGCAGTATTTTGGCCCA
GGCACCCGGCTGACAGTGCTCGAGGACCTGAAAAACGTGTTCCACCCGAGGTC
GCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACACTG
GTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGGGTGA
30 ATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGC
AGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGC
CACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGG
CTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATC

GTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTACC
 AGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCAC
 CTTGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAGAAAG
 GATTCCAGAGGCTAG (SEQ ID NO:44)

5

[0103] 2. Amino Acid Sequence for 19305CD8 (Starting from start codon-coding
 Methionine to stop codon-coding termination (*))

[0104] TCR α chain

[0105] METLLGVSLVILWLQLARVNSQQGEEDPQALSIQEGENATMNCSYKT

10 SINNLQWYRQNSGRGLVHLILIRSNEREKHSGLRVRTLDTSKKSSSLITASRAADTA
 SYFCATDAVVGADGLTFGKGTHLIIQPYIQNPDPVYQLRDSKSSDKSVCLFTDFDSQ
 TNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFF
 PSPESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRLWSS* (SEQ
 ID NO:45)

15 [0106] TCR β chain

[0107] MLLLLLLLGPGLGAVVSQHPSWVICKSGTSVKIECRSLDFQATTMF
 WYRQFPKQSLMLMATSNEGSKATYEQGVKDKFLINHASLTLSTLTVTSAHPEDSSF
 YICSARDRDIGPLDTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCL
 ATGFYDPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQ
 20 NPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLS
 ATILYEILLGKATLYAVLVSAVLMLMAMVKRKDSRG* (SEQ ID NO:46)

[0108] HLA-Cw*15-restricted NY-ESO-1₁₂₇₋₁₃₅-specific T-cell clone "BB"

[0109] 1. Nucleotide Sequence

25 [0110] TCR α chain

[0111] >BBA-2

[0112] ATGATGGCAGGCATTCGAGCTTTATTTATGTACTTGTGGCTGCAGCT
 GGACTGGGTGAGCAGAGGAGAGAGTGTGGGGCTGCATCTTCCTACCCTGAGTGT
 CCAGGAGGGTGACAACTCTATTATCAACTGTGCTTATTCAAACAGCGCCTCAGAC
 30 TACTTCATTTGGTACAAGCAAGAATCTGGAAAAGGTCCTCAATTCATTATAGACA
 TTCGTTCAAATATGGACAAAAGGCAAGGCCAAAGAGTCACCGTTTTATTGAATA
 AGACAGTGAAACATCTCTCTCTGCAAATTGCAGCTACTCAACCTGGAGACTCAGC
 TGTCTACTTTTGTGCAGAGGCGGGAGGAAGCCAAGGAAATCTCATCTTTGGAAA

AGGCACTAAACTCTCTGTAAACCAAATATCCAGAACCCTGACCCTGCCGTGTAC
CAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
ATTCTCAAACAAATGTGTACAAAGTAAGGATTCTGATGTGTATATCACAGACAA
AACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTG
5 GAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCA
GAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGA
AAAGCTTTGAAACAGATACGAACCTAAACTTTCAAACCTGTCAGTGATTGGGTT
CCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTG
TGGTCCAGCTGA (SEQ ID NO:15)

10 [0113] TCR β chain

[0114] ATGGGCCCCGGGCTCCTCTGCTGGGCACTGCTTTGTCTCCTGGGAG
CAGGCTTAGTGACGCTGGAGTCACCCAAAGTCCCACACACCTGATCAAAACGA
GAGGACAGCAAGTGACTCTGAGATGCTCTCCTAAGTCTGGGCATGACACTGTGTC
CTGGTACCAACAGGCCCTGGGTCAGGGGCCCCAGTTTATCTTTCAGTATTATGAG
15 GAGGAAGAGAGACAGAGAGGCAACTTCCCTGATCGATTCTCAGGTCACCAGTTC
CCTAACTATAGCTCTGAGCTGAATGTGAACGCCTTGTTGCTGGGGGACTCGGCCC
TCTATCTCTGTGCCAGCAGCTTTTGGGGTCGTTCTCACCTTCAAACCTATGGCTAC
ACCTTCGGTTCGGGGACCAGGTAAACCGTTGTAGAGGACCTGAACAAGGTGTTCC
CACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAA
20 AGGCCACACTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAG
CTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCC
CCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTG
AGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCC
AGTTCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCG
25 TCACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTC
GGTGTCTTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTA
GGGAAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGG
TCAAGAGAAAGGATTTCTGA (SEQ ID NO:16)

30 [0115] 2. Amino Acid Sequence for BB (Starting from start codon-coding Methionine
to stop codon-coding termination (*))

[0116] TCR α chain

[0117] MMAGIRALFMYLWLQLDWVSRGESVGLHLPTLSVQEGDNSIINCAYS

NSASDYFIWYKQESGKGPFII DIRSNMDKRQGQRVTVLLNKTVKHL SLQIAATQPG
 DSAVYFCAEAGGSQGNLIFGKGTKLSVKPNIQNPDP AVYQLRDSKSSDKSVCLFTDF
 DSQTNVSQSKSDSVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPED
 TFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLK VAGFNLLMTLRLWSS*

5 (SEQ ID NO:17)

[0118] TCR β chain

[0119] MGPGLLCWALLCLLGAGLVDAGVTQSPH LIKTRGQQVTLRCSPKSG
 HDTVSWYQQALGQGPQFIFQYEEEEERQ RGNFPDRFSGHQFPNYSSELNVNALLLGD
 SALYLCASSFWGRSHPSNYGYTFGSGTRLTVVEDLNK VFPPEVAVFEPSEAEISHTQK
 10 ATL VCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRV
 SATFWQNPRNHFR CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVS
 YQGGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF* (SEQ ID NO:18)

[0120] HLA-Cw*03-restricted NY-ESO-1₉₂₋₁₀₀-specific T-cell clone "KB"

15 [0121] 1. Nucleotide Sequence

[0122] TCR α chain

[0123] ATGAACATGCTGACTGCCAGCCTGTTGAGGGCAGTCATAGCCTCCA
 TCTGTGTTGTATCCAGCATGGCTCAGAAGGTA ACTCAAGCGCAGACTGAAATTTC
 TGTGGTGGAGAAGGAGGATGTGACCTTGGACTGTGTGTATGAAACCCGTGATAC
 20 TACTTATTACTTATTCTGGTACAAGCAACCACCAAGTGGAGAATTGGTTTTCTTA
 TTCGTCGGA ACTCTTTTGATGAGCAAAATGAAATAAGTGGTCGGTATTCTTGGA
 CTTCCAGAAATCCACCAGTTCCTTCAACTTCACCATCACAGCCTCACAAGTCGTG
 GACTCAGCAGTATACTTCTGTGCTCTGAGTGAGGCAAGCGGGAGAGATGACAAG
 ATCATCTTTGGAAAAGGGACACGACTTCATATTCTCCCAATATCCAGAACCCTG
 25 ACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCT
 ATTCACCGATTTTGATTCTCAAACAAATGTGT CACAAAGTAAGGATTCTGATGTG
 TATATCACAGACAAA ACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAAC
 AGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACA
 ACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGT
 30 CAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAACTTTCAAAACCT
 GTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTC
 ATGACGCTGCGGCTGTGGTCCAGCTGA (SEQ ID NO:19)

[0124] TCR β chain

[0125] ATGCTGCTGCTTCTGCTGCTTCTGGGGCCAGGCTCCGGGCTTGGTGC
 TGTCGTCTCTCAACATCCGAGCTGGGTATCTGTAAGAGTGGAACCTCTGTGAAG
 ATCGAGTGCCGTTCCCTGGACTTTCAGGCCACAACCTATGTTTTGGTATCGTCAGTT
 CCCGAAACAGAGTCTCATGCTGATGGCAACTTCCAATGAGGGGCTCCAAGGCCAC
 5 ATACGAGCAAGGCGTCGAGAAGGACAAGTTTCTCATCAACCATGCAAGCCTGAC
 CTTGTCCACTCTGACAGTGACCAGTGCCCATCCTGAAGACAGCAGCTTCTACATC
 TGCAGTGCTAGAGTCGACTTTGACCGTGACGAGCAGTTCTTCGGGGCCAGGGACAC
 GGCTCACCGTGCTAGAGGACCTGAAAAACGTGTTCCACCCGAGGTCGCTGTGTT
 TGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACACTGGTGTGCCT
 10 GGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAA
 GGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCAGCCCGC
 CCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTC
 TGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGG
 AGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTCAGCG
 15 CCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTACCAGCAAG
 GGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACCTTGTA
 TGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAGAAAGGATTCC
 AGAGGCTAG (SEQ ID NO:20)

20 [0126] 2. Amino Acid Sequence for KB (Starting from start codon-coding
 Methionine to stop codon-coding termination (*))

[0127] TCR α chain

[0128] MNMLTASLLRAVIASICVVSSMAQKVTQAQTEISVVEKEDVTLDVCVY
 ETRDTTYLFWYKQPPSGELVFLIRNSFDEQNEISGRYSWNFQKSTSSFNFTITASQV
 25 VDSAVYFCALSEASGRDDKIIFGKGTRLHILPNIQNPDPAVYQLRDSKSSDKSVCLFT
 DFDSQTNVSQSKSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIP
 EDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLWSS*
 (SEQ ID NO:21)

[0129] TCR β chain

30 [0130] MLLLLLLLGPGLGAVVSQHPSWVICKSGTSVKIECRSLDFQATTMF
 WYRQFPKQSLMLMATSNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSF
 YICSARVDFDRDEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLA
 TGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQN

PRNHFR CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSA
TILYEILLGKATLYAVLV SALVLMAMVKRKDSRG* (SEQ ID NO:22)

[0131] HLA-Cw*03-restricted NY-ESO-1₉₆₋₁₀₄-specific T-cell clone "ST"

5 [0132] 1. Nucleotide Sequence

[0133] TCR α chain

[0134] ATGGAACTCTCCTGGGAGTGTCTTTGGTGATTCTATGGCTTCAACT
GGCTAGGGTGAACAGTCAACAGGGAGAAGAGGATCCTCAGGCCTTGAGCATCCA
GGAGGGTGAAAATGCCACCATGAACTGCAGTTACAAAAC TAGTATAACAATTT
10 ACAGTGGTATAGACAAAATTCAGGTAGAGGCCTTGTCCACCTAATTTTAATACGT
TCAAATGAAAGAGAGAGAAACACAGTGGAAGATTAAGAGTCACGCTTGACACTTCC
AAGAAAAGCAGTTCCTTGTTGATCACGGCTTCCCGGGCAGCAGACACTGCTTCTT
ACTTCTGTGCTACGGACGCAGAGTATAACAATGCCAGACTCATGTTTGGAGATGG
AACTCAGCTGGTGGTGAAGCCCAATATCCAGAACCCTGACCCTGCCGTGTACCAG
15 TTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTC
TCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAAC
TGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAG
CAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAA
GACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAA
20 GCTTTGAAACAGATACGAACCTAACTTTCAAAACCTGTCAGTGATTGGGTTCCG
AATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGG
TCCAGCTGA (SEQ ID NO:23)

[0135] TCR β chain

[0136] ATGGGCACCAGCCTCCTCTGCTGGATGGCCCTGTGTCTCCTGGGGG
25 CAGATCACGCAGATACTGGAGTCTCCAGGACCCCAGACACAAGATCACAAAGA
GGGGACAGAATGTAACTTTCAGGTGTGATCCAATTTCTGAACACAACCGCCTTTA
TTGGTACCGACAGACCCTGGGGCAGGGCCCAGAGTTTCTGACTTACTTCCAGAAT
GAAGCTCAACTAGAAAAATCAAGGCTGCTCAGTGATCGGTTCTCTGCAGAGAGG
CCTAAGGGATCTTTCTCCACCTTGGAGATCCAGCGCACAGAGCAGGGGGACTCG
30 GCCATGTATCTCTGTGCCAGCAGCATGGTAGCTGGGGCCAACGTCCTGACTTTTCG
GGGCCGGCAGCAGGCTGACCGTGCTGGAGGACCTGAAAAACGTGTTCCACCCCG
AGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCA
CACTGGTGTGCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTG

GGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAA
 GGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGT
 CTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTC
 TACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACC
 5 CAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAG
 TCTTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGA
 AGGCCACCTTGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAA
 GAGAAAGGATTCCAGAGGCTAG (SEQ ID NO:24)

10 [0137] 2. Amino Acid Sequence for ST (Starting from start codon-coding Methionine
 to stop codon-coding termination (*))

[0138] TCR α chain

[0139] METLLGVSLVILWLQLARVNSQQGEEDPQALSIQEGENATMNCSYKT
 SINNLQWYRQNSGRGLVHLILIRSNEREKHSGRRLRVTLDTSKKSSSLITASRAADTA
 15 SYFCATDAEYNNARLMFGDGTQLVVKPNIQNPDPVYQLRDSKSSDKSVCLFTDFD
 SQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDT
 FFPSPSSCDVKLVEKSFETDTNLNFQNL SVIGFRILLKLVAGFNLLMTLRLWSS*
 (SEQ ID NO:25)

[0140] TCR β chain

20 [0141] MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPIS
 HNRLYWYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERP KGSFSTLEIQRTEQG
 DSAMYLCASSMVAGANVLTFGAGSRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKAT
 LVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSA
 TFWQNPRNHFRQCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQ
 25 QGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG* (SEQ ID NO:26)

[0142] HLA-A*02-restricted NY-ESO-1₁₅₇₋₁₆₅-specific T-cell clone “**19305DP**”

[0143] 1. Nucleotide Sequence

[0144] TCR α chain

30 [0145] ATGGAAACTCTCCTGGGAGTGTCTTTGGTGATTCTATGGCTTCAACT
 GGCTAGGGTGAACAGTCAACAGGGAGAAGAGGATCCTCAGGCCTTGAGCATCCA
 GGAGGGTGAAAATGCCACCATGAACTGCAGTTACAAAACCTAGTATAACAATTT
 ACAGTGGTATAGACAAAATTCAGGTAGAGGCCTTGTCCACCTAATTTTAATACGT

TCAAATGAAAGAGAGAAACACAGTGGAAGATTAAGAGTCACGCTTGACACTTCC
AAGAAAAGCAGTTCCTTGTTGATCACGGCTTCCCGGGCAGCAGACACTGCTTCTT
ACTTCTGTGCTACGGACGGGGGGGGCACCTCACCTTTGGGAAGGGGACTATGCT
TCTAGTCTCTCCAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGAC
5 TCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAA
ATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACACTGTGCTAGA
CATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATC
TGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTC
TTCCCCAGCCCAGAAAGTTCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAA
10 CAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCTCCT
CCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGCTGA
(SEQ ID NO:3)

[0146] TCR β chain

[0147] ATGGACTCCTGGACCCTCTGCTGTGTGTCCCTTTGCATCCTGGTAGC
15 AAAGCACACAGATGCTGGAGTTATCCAGTCACCCCGGCACGAGGTGACAGAGAT
GGGACAAGAAGTGACTCTGAGATGTAAACCAATTTTCAGGACACGACTACCTTTTC
TGGTACAGACAGACCATGATGCGGGGACTGGAGTTGCTCATTACTTTAACAACA
ACGTTCCGATAGATGATTCAGGGATGCCCCGAGGATCGATTCTCAGCTAAGATGCC
TAATGCATCATTCTCCACTCTGAAGATCCAGCCCTCAGAACCCAGGGACTCAGCT
20 GTGTACTTCTGTGCCAGCAAGTGGGGCGGCCTGAAGCTTTCTTTGGACAAGGCA
CCAGACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCACCCGAGGTCGCTGT
GTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACACTGGTGTG
CCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGG
AAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCC
25 GCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCT
TCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTC
GGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAG
CGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCTACCAGCAA
GGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGT
30 ATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAGGATTT
CTGA (SEQ ID NO:4)

[0148] 2. Amino Acid Sequence for 19305DP (Starting from start codon-coding

Methionine to stop codon-coding termination (*))

[0149] TCR α chain

[0150] METLLGVSLVILWLQLARVNSQQGEEDPQALSIQEGENATMNC SYKT
SINN LQWYRQNSGRGLVHLILIRSNEREKHSGRRLRVTLDTSKKSSSLITASRAADTA
5 SYFCATDGGGTLTFGKG TMLLVSPDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTN
VSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSP
ESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLWSS* (SEQ ID
NO:1)

[0151] TCR β chain

10 [0152] MDSWTLCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEVTLRCKPISGH
DYLFWYRQTMMRGLELLIYFNNNPIDDSGMPEDRFS AKMPNASFSTLKIQPSEPRD
SAVYFCASKWGGTEAFFGQGTRLTVVEDLNKVFPEVAVFEPSEAEISHTQKATLVC
LATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFW
QNPRNHFRQCQVQFYGLSENDEWTQDRAKPV TQIVSAEAWGRADCGFTSVSYQQGV
15 LSATILYEILLGKATLYAVLV SALVLMAMVKRKDF* (SEQ ID NO:2)

[0153] HLA-A*02-restricted NY-ESO-1₁₅₇₋₁₆₅-specific T-cell clone "JD"

[0154] 1. Nucleotide Sequence

[0155] TCR α chain

20 [0156] ATGATGAAATCCTTGAGAGTTTTACTAGTGATCCTGTGGCTTCAGTT
GAGCTGGGTTTGGAGCCAACAGAAGGAGGTGGAGCAGAATTCTGGACCCCTCAG
TGTTCCAGAGGGAGCCATTGCCTCTCTCAACTGCACTTACAGTGACCGAGGTTCC
CAGTCCTTCTTCTGGTACAGACAATATTCTGGGAAAAGCCCTGAGTTGATAATGT
TCATATACTCCAATGGTGACAAAGAAGATGGAAGGTTTACAGCACAGCTCAATA
25 AAGCCAGCCAGTATGTTTCTCTGCTCATCAGAGACTCCCAGCCCAGTGATTCAGC
CACCTACCTCTGTGCCGTGGGTGCTACAAACAAGCTCATCTTTGGAAGTGGCACT
CTGCTTGCTGTCCAGCCAAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGA
GAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCA
AACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAAGTGT
30 GCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAA
CAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATCCAGAAGAC
ACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCT
TTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAAT

CCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCC
AGCTGA (SEQ ID NO:27)

[0157] TCR β chain

5 [0158] ATGGTTTCCAGGCTTCTCAGTTTAGTGTCCTTTGTCTCCTGGGAGC
AAAGCACATAGAAGCTGGAGTTACTCAGTTCCCCAGCCACAGCGTAATAGAGAA
GGGCCAGACTGTGACTCTGAGATGTGACCCAATTTCTGGACATGATAATCTTTAT
TGGTATCGACGTGTTATGGGAAAAGAAATAAAATTTCTGTTACATTTTGTGAAAG
AGTCTAAACAGGATGAGTCCGGTATGCCCAACAATCGATTCTTAGCTGAAAGGA
10 CTGGAGGGACGTATTCTACTCTGAAGGTGCAGCCTGCAGAACTGGAGGATTCTG
GAGTTTATTTCTGTGCCAGCAGCCAAGCGTACGGCACTGAAGCTTTCTTTGGACA
AGGCACCAGACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCCAACCCGAGGT
CGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACACT
GGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTG
15 AATGGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGA
GCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCG
GCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACG
GGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGA
TCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCTA
20 CCAGCAAGGGGTCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCC
ACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAA
AGGATTTCTGA (SEQ ID NO:28)

25 [0159] 2. Amino Acid Sequence for JD (Starting from start codon-coding Methionine
to stop codon-coding termination (*))

[0160] TCR α chain

[0161] MMKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTY
SDRGSQSFFWYRQYSGKSPELIMFIYSNGDKEDGRFTAQLNKASQYVSLIRDSQPSD
SATYLCAVGATNKLIFGTGTLAVQPNIQNPDAVYQLRDSKSSDKSVCLFTDFDSQT
30 NVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFPP
SPESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRLWSS* (SEQ ID
NO:29)

[0162] TCR β chain

[0163] MVSRLLSLVSLCLLGAKHIEAGVTQFP SHSVIEKGQTVTLRCDPISGHD
 NLYWYRRVMGKEIKFLLHFVKESKQDESGMPNNRFLAERTGGTYSTLKVQPAELED
 SGVYFCASSQAYGTEAFFGQGTRLTVVEDLNKVPPEVAVFEPSEAEISHTQKATLVC
 LATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRV SATFW
 5 QNPRNHFRQCQVQFYGLSENDEWTQDRAKPV TQIVSAEAWGRADCGFTSVSYQQGV
 LSATILYEILLGKATLYAVLVSALVLMAMVKRKDF* (SEQ ID NO:30)

[0164] HLA-DRB1*07-restricted NY-ESO-1(139-160)-specific T-cell clone "PB-P"

10 [0165] 1. Nucleotide sequence

[0166] TCR α -chain

[0167] ATGGCCATGCTCCTGGGGGCATCAGTGCTGATTCTGTGGCTTCAGC
 CAGACTGGGTAAACAGTCAACAGAAGAATGATGACCAGCAAGTTAAGCAAAATT
 CACCATCCCTGAGCGTCCAGGAAGGAAGAATTTCTATTCTGAACTGTGACTATAC
 15 TAACAGCATGTTTGATTATTTCTATGGTACAAAAAATACCCTGCTGAAGGTCCT
 ACATTCTGATATCTATAAGTTCCATTAAGGATAAAAAATGAAGATGGAAGATTCA
 CTGTCTTCTTAAACAAAAGTGCCAAGCACCTCTCTCTGCACATTGTGCCCTCCCA
 GCCTGGAGACTCTGCAGTGTACTTCTGTGCAGCAAGCCCCTCTGGCAACACAGGC
 AAATAATCTTTGGGCAAGGGACAACCTTTACAAGTAAAACCAGATATCCAGAAG
 20 CCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCT
 GCCTATTCACCGATTTTGATTCTCAAACAAATGTGTACAAAGTAAGGATTCTGA
 TGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGC
 AACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCA
 ACAACAGCATTATTCAGAAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGA
 25 TGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAACTTTCAAAA
 CCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTG
 CTCATGACGCTGCGGCTGTGGTCCAGCTGA (SEQ ID NO:31)

[0168] TCR β -chain

30 [0169] ATGGGCTCCAGGCTGCTCTGTTGGGTGCTGCTTTGTCTCCTGGGAGC
 AGGCCAGTAAAGGCTGGAGTCACTCAAACCTCCAAGATATCTGATCAAAACGAG
 AGGACAGCAAGTGACACTGAGCTGCTCCCCTATCTCTGGGCATAGGAGTGTATCC

TGGTACCAACAGACCCCAGGACAGGGCCTTCAGTTCCTCTTTGAATACTTCAGTG
 AGACACAGAGAAACAAAGGAACTTCCCTGGTCGATTCTCAGGGCGCCAGTTCT
 CTA ACTCTCGCTCTGAGATGAATGTGAGCACCTTGGAGCTGGGGGACTCGGCCCT
 TTATCTTTGCGCCAGCAGCCCAGACAGGGCCATGAACACTGAAGCTTTCTTTGGA
 5 CAAGGCACCAGACTCACAGTTGTAGAGGACCTGAACAAGGTGTTCCACCCGAG
 GTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACA
 CTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGG
 TGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCCTCAAGG
 AGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTC
 10 GGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTAC
 GGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAG
 ATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCCT
 ACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGC
 CACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGA
 15 AAGGATTTCTGA (SEQ ID NO:32)

[0170] 2. Amino Acid Sequence for PB-P (Starting from start codon-coding
 Methionine to stop codon-coding termination (*))

[0171] TCR α -chain

20 **[0172]** MAMLLGASVLILWLQPDWVNSQQKNDDQQVKQNSPSLSVQEGRISIL
 NCDYTNSMFDYFLWYKKYPAEGPTFLISSIKDKNEDGRFTVFLNKS AKHLSLHIVP
 SQPGDSAVYFCAASPSGNTGKLIFGQGTTLQVKPDIQKPDPAVYQLRDSKSSDKSVC
 LFTDFDSQTNVSQSKSDSVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNN
 SIIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLW
 25 SS* (SEQ ID NO:33)

[0173] TCR β -chain

[0174] MGSRLLCWVLLCLLGAGPVKAGVTQTTRYLIKTRGQQVTLSCSPISGH
 RSVSWYQQTPGQGLQFLFEYFSETQRNKG NFPGRFSGRQFSNSRSEMNVSTLELGDS
 ALYLCASSPDRAMNTEAFFGQGTRLTVVEDLNKVFPP EVAVFEPSEAEISHTQKATL
 30 VCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSAT
 FWQNPRNHFR CQVQFYGLSENDEWTQDRAKPV TQIVSAEAWGRADCGFTSVSYQQ
 GVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF* (SEQ ID NO:34)

 [0175] HLA-DRB1*04-restricted NY-ESO-1(111-143)-specific T-cell clone “**PB-T**”

[0176] 1. Nucleotide sequence

[0177] TCR α -chain

5 [0178] ATGGCCTCTGCACCCATCTCGATGCTTGCGATGCTCTTCACATTGAG
 TGGGCTGAGAGCTCAGTCAGTGGCTCAGCCGGAAGATCAGGTCAACGTTGCTGA
 AGGGAATCCTCTGACTGTGAAATGCACCTATTCAGTCTCTGGAAACCCTTATCTT
 TTTTGGTATGTTCAATACCCCAACCGAGGCCTCCAGTTCCTTCTGAAATACATCAC
 AGGGGATAACCTGGTTAAAGGCAGCTATGGCTTTGAAGCTGAATTTAACAAGAG
 10 CCAAACCTCCTTCCACCTGAAGAAACCATCTGCCCTTGTGAGCGACTCCGCTTTG
 TACTTCTGTGCTGTGAGAGACAGGGAGAGAGATGACAAGATCATCTTTGGAAAA
 GGGACACGACTTCATATTCTCCCAATATCCAGAAGCCTGACCCTGCCGTGTACC
 AGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGA
 TTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAA
 15 ACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGG
 AGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAG
 AAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAA
 AAGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTC
 CGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGT
 20 GGTCCAGCTGA (SEQ ID NO:35)

[0179] TCR β -chain

[0180] ATGGATACCTGGCTCGTATGCTGGGCAATTTTGTAGTCTCTTGAAAG
 CAGGACTCACAGAACCTGAAGTCACCCAGACTCCCAGCCATCAGGTCACACAGA
 TGGGACAGGAAGTGATCTTGCGCTGTGTCCCCATCTCTAATCACTTATACTTCTAT
 25 TGGTACAGACAAATCTTGGGGCAGAAAGTCGAGTTTCTGGTTTCCTTTTATAATA
 ATGAAATCTCAGAGAAGTCTGAAATATTCGATGATCAATTCTCAGTTGAAAGGCC
 TGATGGATCAAATTTCACTCTGAAGATCCGGTCCACAAAGCTGGAGGACTCAGCC
 ATGTACTTCTGTGCCAGCAGAGCGGAGATCACAGATACGCAGTATTTTGGCCCAG
 GCACCCGGCTGACAGTGCTCGAGGACCTGAAAAACGTGTTCCACCCGAGGTCTG
 30 CTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACACTGG
 TGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGGGTGAA
 TGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCA
 GCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCTGAGGGTCTCGGCC

ACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGC
 TCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCG
 TCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTACCA
 GCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACC
 5 TTGTATGCCGTGCTGGTCAAGTGCCTCGTGCTGATGGCCATGGTCAAGAGAAAGG
 ATTCCAGAGGCTAG (SEQ ID NO:36)

[0181] 2. Amino Acid Sequence for PB-T (Starting from start codon-coding
 Methionine to stop codon-coding termination (*))

10 [0182] TCR α -chain

[0183] MASAPISMLAMLFTLSGLRAQSVAQPEDQVNVAEGNPLTVKCTYSVS
 GNPYLFWYVQYPNRGLQFLLKYITGDNLVKGSYGFEAEFNKSQTSFHLKKPSALVSD
 SALYFCAVRDRERDDKIIFGKGTRLHILPNIQKPDPAVYQLRDSKSSDKSVCLFTDFDS
 QTNVSQSKDSVDYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNNSIIPEDTF
 15 FPSPESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRLWSS* (SEQ
 ID NO:37)

[0184] TCR β -chain

[0185] MDTWLVCWAIFSLKAGLTEPEVTQTPSHQVTQMGQEVILRCVPISNH
 20 LYFYWYRQILGQKVEFLVSFYNNSEISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSA
 MYFCASRAEITDTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLA
 TGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQN
 PRNHFRQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSA
 TILYEILLGKATLYAVLVSAVLMMAMVKRKDSRG* (SEQ ID NO:38)

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[0186] HLA-DRB1*07-restricted NY-ESO-1(139-160)-specific T-cell clone
 "PB13.2"

[0187] 1. Nucleotide sequence

[0188] TCR α -chain

30 [0189] ATGGCCATGCTCCTGGGGGCATCAGTGCTGATTCTGTGGCTTCAGC
 CAGACTGGGTAAACAGTCAACAGAAGAATGATGACCAGCAAGTTAAGCAAAATT
 CACCATCCCTGAGCGTCCAGGAAGGAAGAATTTCTATTCTGAACTGTGACTATAC

TAACAGCATGTTTGATTATTTTCCTATGGTACAAAAAATACCCTGCTGAAGGTCCT
ACATTCCTGATATCTATAAGTTCCATTAAGGATAAAAAATGAAGATGGAAGATTCA
CTGTCTTCTTAAACAAAAGTGCCAAGCACCTCTCTCTGCACATTGTGCCCTCCCA
GCCTGGAGACTCTGCAGTGTACTTCTGTGCAGCAAGCGCGATAGGAGGTGCTGA
5 CGGACTCACCTTTGGCAAAGGGACTCATCTAATCATCCAGCCCTATATCCAGAAC
CCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCT
GCCTATTCACCGATTTTGATTCTCAAACAAATGTGTACAAAGTAAGGATTCTGA
TGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGC
AACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCA
10 ACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGA
TGTCAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAACTTTCAAAA
CCTGTGAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTG
CTCATGACGCTGCGGCTGTGGTCCAGCTGA (SEQ ID NO:39)

15 [0190] TCR β -chain
[0191] ATGAGCCTCGGGCTCCTGTGCTGTGGGGCCTTTTCTCTCCTGTGGGC
AGGTCCAGTGAATGCTGGTGTCACTCAGACCCCAAAATTCCGGGTCCTGAAGAC
AGGACAGAGCATGACACTGCTGTGTGCCAGGATATGAACCATGAATACATGTA
CTGGTATCGACAAGACCCAGGCATGGGGCTGAGGCTGATTCATTACTCAGTTGGT
20 GAGGGTACAACCTGCCAAAGGAGAGGTCCCTGATGGCTACAATGTCTCCAGATTA
AAAAAACAGAATTTCTGCTGGGGTTGGAGTCGGCTGCTCCCTCCCAAACATCTG
TGTAATTCTGTGCCAGCAGTATTCGGGGAAAAAGGTACAATGAGCAGTTCTTCGG
GCCAGGGACACGGCTCACCGTGCTAGAGGACCTGAAAAACGTGTTCCCACCCGA
GGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCAC
25 ACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGG
GTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAG
GAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCT
CGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTA
CGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCA
30 GATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCT
TACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGG
CCACCTTGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAG
AAAGGATTCCAGAGGCTAG (SEQ ID NO:40)

[0192] 2. Amino Acid Sequence for PB13.2 (Starting from start codon-coding Methionine to stop codon-coding termination (*))

[0193] TCR α -chain

5 [0194] MAMLLGASVLILWLQPDWVNSQQKNDDQQVKQNSPSLSVQEGRISIL
 NCDYTNMFDYFLWYKKYPAEGPTFLISSIKDKNEDGRFTVFLNKS AKHLSLHIVP
 SQPGDSAVYFCAASAIGGADGLTFGKGTHLIIQPYIQNPDP AVYQLRDSKSSDKSVCL
 FTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNS
 IIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLWS
 10 S* (SEQ ID NO:41)

[0195] TCR β -chain

[0196] MSLGLLCCGAFSLLWAGPVNAGVTQTPKFRVLKTGQSM TLLCAQDM
 NHEYMYWYRQDPGMGLRLIHYSVGEGTTAKGEVPDGYNV SRLKKQNFLLGLESAA
 15 PSQTSVYFCASSIRGKRYNEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKA
 TLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVS
 ATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESY
 QQGVLSATILYEILLGKATLYAVLV SALVLMAMVKRKDSRG* (SEQ ID NO:42)

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[0197] In connection with the foregoing sequences of this Example, this Example provides a non-limiting demonstration of generating a NY-ESO-1-specific TCR-expressing retroviral vector (Figure 1). These figures illustrate the following:

25 [0198] Figure 1A. For transduction of T cells, murine stem cell virus (MSCV)-derived vectors have been widely used because of strong promoter activity by MSCV long terminal repeats (LTR) and in vivo stability of transgene expression in hematopoietic cells. As 3'-LTR is copied to 5'-LTR during integration to host cells such as T cells and responsible for the transcription of transgenes, 3'-LTR in the plasmids is important in the
 30 expression. On the other hand, 5'-LTR is responsible for the transcription for virus production. Schematic representation for classical MSCV-derived vectors (pMIG-II and pMIG-w) is shown in Figure 1. Both vectors have MSCV-derived LTR at 5' and 3' sites,

packaging signal (ψ), multiple cloning sites (MCS). Transgene is cloned in the MCS, which is followed by the internal ribosomal entry site (IRES) and the green fluorescent protein gene (GFP) to efficiently detect transduced cells. pMIG-w vector has additional woodchuck hepatitis virus post-transcriptional regulatory element (WRE), which enhances expression of the transgene. In recent retrovirus vectors, further modifications are introduced as found in the commercial retroviral vector, pDON-5 (Clontech). pDON-5, which is derived from a murine leukemia virus (MLV) vector, replaces the 5'-LTR with the CMV/MLV hybrid LTR for enhanced virus production through strong CMV promoter activity in virus packaging cell lines. Furthermore, the partial intron from the human elongation factor 1 alpha gene is introduced to provide a splice acceptor site (SA), which together with an endogenous splice donor site (SD) induces splicing and enhances expression.

[0199] Figure 1B. To create a retrovirus vector which can produce high-titer retrovirus that induces high level transgene (TCR) expression in T cells, we amplified a DNA fragment from 5'-LTR to the intron containing a splice acceptor site in the pDON-5 plasmid. The forward primer was designed to append SgrAI restriction enzyme recognition site before 5'-LTR and the reverse primer was designed to append NotI and SalI sites after the intron. PCR-amplified fragment was treated with SgrAI and SalI and inserted into pMIG-II and pMIG-w plasmids so that 5'-LTR to GFP is replaced.

[0200] Figure 1C. The plasmids depicted in Figure 1C only have NotI and SalI recognition sites for cloning. The use of SalI which recognizes a specific 6-mer nucleotide sequence is not preferable because it may appear frequently enough to be present in the transgene, thus resulting in its cleavage. To provide additional restriction enzyme recognition sites which are believed to be unlikely to appear in most TCR transgenes, we amplified a 1.8kb DNA fragment (stuffer) with the forward primer with a NotI restriction site, and the reverse primer with PacI-SalI sites. The amplified fragment was treated with NotI and SalI restriction enzymes and inserted into the new plasmids.

[0201] Figure 1D. The depicted TCR expression cassette was amplified with the forward primer with the NotI restriction site and the reverse primer with the PacI restriction enzyme site. The amplified expression cassette was treated with NotI and PacI restriction enzymes and inserted into the new plasmids.

[0202] Transduction of 19305DP-TCR gene into polyclonally activated T cells (Figure 2). T cells from healthy individuals were preactivated for 2 days using phytohemagglutinin (PHA, obtained from Remel) in the presence of low-dose IL-2, IL-7, and

IL-12. Retroviral particles were coated on non-tissue culture plate that were pre-coated with Retronectin (obtained from Clontech) and blocked with bovine serum albumin (BSA from Sigma). Activated T cells were infected by retrovirus by culturing on retrovirus-coated plate. Viral infection was repeated 24 hours later. Total number of infection was 2 times. Infected cells were expanded in the presence of IL-2 and IL-7. IL-12 was included for 5 days from the activation of T cells with PHA. After infection, more than 95% TCR gene-transduced T cells expressed transduced TCR by staining with TCR V β subtype-specific antibody.

[0203] Anti-tumor function of 19305DP-TCR-transduced T cells (Figures 3 and 8-10). TCR gene-transduced T cells were co-cultured with HLA-A*02+NY-ESO-1+ melanoma cell line, SK-MEL-37 or HLA-A*02+NY-ESO-1- melanoma cell line, SK-MEL-29 for 6 hours in the presence of Golgi Stop (BD Biosciences). Cells were stained by fluorochrome-conjugated anti-CD4 and anti-CD8 antibodies and fixed and permeabilized using BD Cytotfix/Cytoperm Kit (BD Biosciences). Intracellular IFN- γ was stained by fluorochrome-conjugated anti-IFN- γ antibody. Both TCR gene-transduced CD4⁺ and CD8⁺ T cells produced IFN- γ only when they were co-cultured with NY-ESO-1+ SK-MEL-37 but not SK-MEL-29. Untransduced CD4⁺ and CD8⁺ T cells did not produce IFN- γ against SK-MEL-37 or SK-MEL-29 (Figure 3). In Figure 8, immuno-deficient NOD/SCID/IL-2R γ -chain-deficient (NSG) mice were inoculated with 1 million SK-MEL-37. After the tumor growth was confirmed on day 9, 10 million 19305DP-TCR gene-transduced T cells were injected. Control tumor-bearing mice were injected with irrelevant TCR-transduced T cells. Mice treated with 19305DP-TCR-transduced T cells showed significant delay in tumor growth and most mice eventually reject tumor xenograft. Tumor was continuously grew in control mice received irrelevant TCR-transduced T cells.

Example 2

[0204] This Example provides a description of additional TCR sequences that can be included with any one of the TCR sequences described in Example 1 in libraries of this disclosure. These TCRs impart the capability to CD4⁺ T cells to directly recognize NY-ESO-1/LAGE-1 positive cancer cells.

[0205] “JM” HLA-DPB1*0401/0402-restricted NY-ESO-1₁₅₇₋₁₇₀-specific tumor-recognizing CD4⁺ T cell clone

[0206] (a) cDNA nucleotide sequences of TCR α and β chains

[0207] TCR α chain

[0208] ATGAAGTTGGTGACAAGCATTACTGTACTCCTATCTTTGGGTATTA
TGGGTGATGCTAAGACCACACAGCCAAATTCAATGGAGAGTAACGAAGAAGAGC
CTGTTCACTTGCCTTGTAACCACTCCACAATCAGTGGAAGTATTACATACATTG
GTATCGACAGCTTCCCTCCCAGGGTCCAGAGTACGTGATTTCATGGTCTTACAAGC
5 AATGTGAACAACAGAATGGCCTCTCTGGCAATCGCTGAAGACAGAAAGTCCAGT
ACCTTGATCCTGCACCGTGCTACCTTGAGAGATGCTGCTGTGTACTACTGCATCC
CTAATAACAATGACATGCGCTTTGGAGCAGGGACCAGACTGACAGTAAAACCAA
ATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGA
CAAGTCTGTCTGCCTATTACCGATTTTGATTCTCAAACAAATGTGTCACAAAGT
10 AAGGATTCTGATGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGG
ACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGC
AAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCAGCCCAGAA
AGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTA
AACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCG
15 GGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGCTGA (SEQ ID NO:47)

[0209] TCR β chain

[0210] ATGGGCTCCAGGCTGCTCTGTTGGGTGCTGCTTTGTCTCCTGGGAG
CAGGCCCAGTAAAGGCTGGAGTCACTCAAACCTCCAAGATATCTGATCAAAACGA
GAGGACAGCAAGTGACACTGAGCTGCTCCCCTATCTCTGGGCATAGGAGTGTATC
20 CTGGTACCAACAGACCCCAGGACAGGGCCTTCAGTTCCTCTTTGAATACTTCAGT
GAGACACAGAGAAACAAAGGAACTTCCCTGGTCGATTCTCAGGGCGCCAGTTC
TCTAACTCTCGCTCTGAGATGAATGTGAGCACCTTGGAGCTGGGGGACTCGGCCC
TTTATCTTTGCGCCAGCAGCTTCCCCAGGGAACCTAACTATGGCTACACCTTCGG
TTCGGGGACCAGGTAAACCGTTGTAGAGGACCTGAACAAGGTGTTCCACCCGA
25 GGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCAC
ACTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGG
GTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAG
GAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCT
CGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTA
30 CGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCA
GATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTCC
TACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGG
CCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAG

AAAGGATTTCTGA (SEQ ID NO:48)

[0211] (b) amino acid sequences of TCR α and β chains for JM (TCR variable regions are in italic, CDR3 regions are in bold)

5 [0212] TCR α chain

[0213] *MKLVT*SITVLLSLGIMGDAKTTQPNMESNEEEPVHLPCNHSTISGTDYIHW
*YRQLPSQGPEYVIHGLTSNVNNRMASLAIAEDRKSS*TLILHRATLRDAAVYYC**IPNNNDMR**
FGAGTRLTVKPNIQNPDAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDK
 TVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPSSCDVKLVEKSFE
 10 TDTNLFQNL SVIGFRILLK VAGFNLLMTLRLWSS (SEQ ID NO:49)

[0214] TCR β chain

[0215] *MGSRLLCWVLLCLLGAGPVKAGVTQTPRYLIKTRGQQVTLSCSPISGHR*SV
SWYQQTPGQGLQFLFEYFSETQRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCA
 15 **SSFPREPNYGYTFGSGTRLTV**VEDLNKVFPPPEVAVFEPSEAEISHTQKATLVCLATGFF
 PDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNH
 FRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILY
 EILLGKATLYAVLV SALVLMAMVKRKDF (SEQ ID NO:50)

20

[0216] “5B8” HLA-DPB1*0401/0402-restricted NY-ESO-1₁₅₇₋₁₇₀-specific tumor-recognizing CD4⁺ T cell clone

[0217] (a) cDNA nucleotide sequences of TCR α and β chains

[0218] TCR α chain

25 [0219] ATGGCCCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGG
 AGGCAGAGACTGTGACCCTGAGTTGCACATATGACACCAGTGAGAATAATTATT
 ATTTGTTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTTATTCGCCA
 AGAAGCTTATAAGCAACAGAATGCAACGGAGAATCGTTTCTCTGTGAACTTCCA
 GAAAGCAGCCAAATCCTTCAGTCTCAAGATCTCAGACTCACAGCTGGGGGACAC
 30 TGCGATGTATTTCTGTGCTTTCTCGAGAGGGAGTGGAGGTAGCAACTATAAACTG
 ACATTTGGAAAAGGAACTCTCTTAACCGTGAATCCAAATATCCAGAACCCCTGACC
 CTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATT

CACCGATTTTGAATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTAT
 ATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGT
 GCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACA
 GCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAA
 5 GCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTC
 AGTGATTGGGTTCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATG
 ACGCTGCGGCTGTGGTCCAGCTGA (SEQ ID NO:51)

[0220] TCR β chain

[0221] **ATGGGCACCAGGCTCCTCTTCTGGGTGGCCTTCTGTCTCCTGGGGG**
 10 CAGATCACACAGGAGCTGGAGTCTCCAGTCCCCAGTAACAAGGTCACAGAGA
 AGGGAAAGGATGTAGAGCTCAGGTGTGATCCAATTTCAAGGTCATACTGCCCTTTA
 CTGGTACCGACAGAGCCTGGGGCAGGGCCTGGAGTTTTTAATTTACTTCCAAGGC
 AACAGTGCACCAGACAAATCAGGGCTGCCAGTGATCGCTTCTCTGCAGAGAGG
 ACTGGGGGATCCGTCTCCACTCTGACGATCCAGCGCACACAGCAGGAGGACTCG
 15 GCCGTGTATCTCTGTGCCAGCAGCTTAGTCCCCGACAGTGCCTACGAGCAGTACT
 TCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCAC
 CCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGG
 CCACACTGGTATGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTG
 GTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCCT
 20 CAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAG
 GGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAG
 TTCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTC
 ACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCC
 GAGTCTTACCAGCAAGGGGTCTGTCTGCCACCATCCTCTATGAGATCTTGCTAG
 25 GGAAGGCCACCTTGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGT
 CAAGAGAAAGGATTCCAGAGGCTAG (SEQ ID NO:52)

[0222] (b) amino acid sequences of TCR α and β chains for 5B8 (TCR variable regions are in italic, CDR3 regions are in bold)

[0223] TCR α chain

30 [0224] *MAQTVTQSQPEMSVQEAETVTLSCITYDTSENNYYLFWYKQPPSRQMILVIR*
*QEAYKQQNATENRFSVNFQKAASFSLKISDSQLGDTAMYFC****AFSRGSGGSNYKLTFGK***
GTLLTVNPMIQNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDSVYITDKTVL
DMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDT

NLNFQNLSVIGFRILLKLVAGFNLLMTLRLWSS SEQ ID NO:53)

[0225] TCR β chain

[0226] *MGTRLLFWVAFCLLGADHTGAGVSQSPSNKVTEKGKDVELRCDPISGHTA
LYWYRQSLGQGLEFLIYFQGN SAPDKSGLPSDRFSAERTGGSVSTLTIQRTQQEDSAVYLC
5 ASSLV PDSAYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGF
YPDHVELSWWWNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRN
HFRQCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATIL
YEILLGKATLYAVLV SALVLMAMVKRKDSRG SEQ ID NO:54)*

10 [0227] “SB95” HLA-DRB1*0101-restricted NY-ESO-1₉₅₋₁₀₆-specific tumor-
recognizing CD4⁺ T cell clone

[0228] (a) cDNA nucleotide sequences of TCR α and β chains

[0229] TCR alpha

[0230] ATGCTCCTGCTGCTCGTCCCAGTGCTCGAGGTGATTTTTACCCTGGG
15 AGGAACCAGAGCCCAGTCGGTGACCCAGCTTGGCAGCCACGTCTCTGTCTCTGAG
GGAGCCCCTGGTTCTGCTGAGGTGCAACTACTCATCGTCTGTTCCACCATATCTCTT
CTGGTATGTGCAATACCCCAACCAAGGACTCCAGCTTCTCCTGAAGCACACAACA
GGGGCCACCCTGGTTAAAGGCATCAACGGTTTTGAGGCTGAATTTAAGAAGAGT
GAAACCTCCTTCCACCTGACGAAACCCTCAGCCCATATGAGCGACGCGGCTGAGT
20 ACTTCTGTGCTGTGAGTGATTCTAGGGCTGCAGGCAACAAGCTAACTTTTGGAGG
AGGAACCAGGGTGCTAGTTAAACCAAATATCCAGAACCCTGACCCTGCCGTGTA
CCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTT
GATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACA
AAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCT
25 GGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTC
AGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAG
AAAAGCTTTGAAACAGATACGAACCTAACTTTCAAAACCTGTCAGTGATTGGGT
TCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCT
GTGGTCCAGCTGA SEQ ID NO:55)

30 [0231] TCR beta

[0232] ATGGGAATCAGGCTCCTCTGTCTGTGGCCTTTTGTTCCTGGCTGT
AGGCCTCGTAGATGTGAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGAC
GGGAGAGAAAGTTTTTCTGGAATGTGTCCAGGATATGGACCATGAAAATATGTTC

TGGTATCGACAAGACCCAGGTCTGGGGCTACGGCTGATCTATTTCTCATATGATG
 TTTAAATGAAAGAAAAAGGAGATATTCCTGAGGGGTACAGTGTCTCTAGAGAGA
 AGAAGGAGCGCTTCTCCCTGATTCTGGAGTCCGCCAGCACCAACCAGACATCTAT
 GTACCTCTGTGCCAGCAGATTCCCCGGGACAGCCTATAATTCACCCCTCCACTTT
 5 GGGGAATGGGACCAGGCTCACTGTGACAGAGGACCTGAACAAGGTGTTCCACCC
 GAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCC
 AACTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGT
 GGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCA
 AGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGG
 10 TCTCGGCCACCTTCTGGCAGAACCCCGCAACCACTTCCGCTGTCAAGTCCAGTT
 CTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCAC
 CCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGT
 GTCCTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGG
 AAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCA
 15 AGAGAAAGGATTTCTGA (SEQ ID NO:56)

[0233] (b) amino acid sequence of TCR α and β chains for SB95 (TCR variable regions are in italic, CDR3 regions are in bold)

[0234] TCR α chain

[0235] *MLLLVPVLEVIFTLGGTRAQSVTQLGSHVSVSEGA****L****VLLRCNYSSSVPPYLF*
 20 *WYVQYPNQGLQLLLKHHTGATLVKGINGFEAEFKKSETSFHLTKPSAHMSDAAEYFCAV****S***
*DSRAAGNKLT****TF****GGGTRVLVKPMIQNPDP****AVYQLRDSKSSDKSVCLFTDFDSQTNVSQS***
KDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSC
*DVKLVEKSFETDTNLFQNL****SVIGFRILLK****VAGFNLLMTLRLWSS* (SEQ ID NO:57)

[0236] TCR β chain

25 [0237] *MGIRLLCRVAF****C****FLAVGLVDVKVTQSSRYLVKRTGEKVFLECVQDMDHEN*
*MFWYRQDPGLGLRLIYFSYDV****K****MKEKGDIPEGYSVSREKKERFSLILESASTNQTSMYLCA*
*SRFPGTAYNSPLH****FGNGTRLTV****TEDLNKVFPEVAVFEPSEAEISHTQKATLVCLATGF*
*FPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRV****SATFWQNPRN***
HFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATIL
 30 *YEILLGKATLYAVLV****SALVLMAMVKRKDF*** (SEQ ID NO:58)

[0238] Figure 4 provides a non-limiting example of an expression vector that can be used to express any of the TCRs of this Example. For Figure 4: (A) Retrovirus vector used to express TCRs. LTR: long-terminal repeat; ψ : packaging signal; MCS: multiple cloning site;

IRES: internal ribosome entry site; eGFP: enhanced green fluorescent protein. (B) TCR expressing cassette. (I) TCR β and α chain-coding cDNA sequences are connected by a GSG (Gly-Ser-Gly) linker and a P2A ribosomal skipping sequence. (II) TCR β and α chain-coding cDNA sequences are connected by a furin protease recognition site (RAKR (Arg-Ala-Lys-Arg)), a SGSG (Ser-Gly-Ser-Gly) linker, V5 epitope, and a P2A ribosomal skipping sequence.

[0239] As described in PCT PCT/US14/25673, the TCRs of this Example are capable of promoting direct recognition of cancer cells and inducing apoptosis of them. Further, CD4⁺ T cells expressing the TCRs of this Example were found to efficiently enhance the cytotoxic activity of tumor antigen-specific CD8⁺ T cells via direct recognition of cancer cells in the absence of antigen-presenting cells. Additionally, CD8⁺ T cells co-stimulated with CD4⁺ T cells expressing recombinant TCRs of this Example actively proliferated and upregulated central memory T cell markers, and CD4⁺ T cells expressing these TCRs showed significant *in vivo* anti-tumor activity to inhibit the growth of human cancer cells in immuno-deficient mice, and these CD4⁺ T cells with tumor antigen-specific CD8⁺ T cells co-operatively inhibited *in vivo* tumor growth.

[0240] HLA allele frequencies for different ethnic populations in the United States. (Shown for 10 most frequent types in European Americans in Table 1). Data for HLA-A,B,C,DR, and DQ were obtained and modified from The National Marrow Donor Program®(NMDP)/Be The Match® website: bioinformatics.bethematchclinical.org/. Data for HLA-DP were obtained from The Allele Frequency Net Database: www.allelefreqencies.net/contact.asp.

[0241] In an embodiment, a library of this disclosure contains a plurality of NY-ESO-1-specific TCRs restricted by the underlined/bold HLA types in Table 1 below.

Table 1

HLA-A

		EUR_freq	EUR_rank	AFA_freq	AFA_rank	API_freq	API_rank	HIS_freq	HIS_rank
	<u>0201</u>	0.29604	1	0.12458	1	0.09458	3	0.19403	1
	0101	0.17181	2	0.04742	8	0.05082	5	0.06702	4
	0301	0.14347	3	0.08132	3	0.02597	11	0.07907	3
	2402	0.08686	4	0.02205	15	0.18238	1	0.12324	2
	1101	0.05642	5	0.01581	18	0.17899	2	0.04618	7
	2902	0.03279	6	0.03640	12	0.00141	30	0.04167	8
	3201	0.03133	7	0.01414	21	0.01299	18	0.02711	13
	2601	0.02948	8	0.01414	20	0.03896	8	0.02887	11
	6801	0.02503	9	0.03681	11	0.01863	13	0.04694	6

	3101	0.02351	10		0.01040	22		0.03247	9		0.04794	5
	HLA-B											
		EUR_freq	EUR_rank		AFA_freq	AFA_rank		API_freq	API_rank		HIS_freq	HIS_rank
5	0702	0.13987	1		0.07303	2		0.02632	15		0.05453	4
	0801	0.12525	2		0.03838	9		0.01641	21		0.04452	6
	4402	0.09011	3		0.02116	17		0.00764	32		0.03327	9
	1501	0.06654	4		0.00975	23		0.03480	11		0.02876	10
	3501	0.05713	5		0.06494	3		0.04273	5		0.06353	1
10	4001	0.05643	6		0.01328	21		0.07980	1		0.01351	26
	4403	0.04963	7		0.05373	6		0.04244	6		0.06078	2
	1801	0.04620	8		0.03568	10		0.01160	25		0.03952	8
	5101	0.04544	9		0.02178	16		0.06282	2		0.05778	3
	5701	0.03832	10		0.00477	35		0.02066	18		0.01176	29
15	C	EUR_freq	EUR_rank		AFA_freq	AFA_rank		API_freq	API_rank		HIS_freq	HIS_rank
	0701	0.16658	1		0.12401	2		0.03894	12		0.10355	3
	0702	0.15006	2		0.06968	7		0.14560	1		0.11281	2
	0401	0.10534	3		0.18457	1		0.08070	4		0.16508	1
20	0602	0.09301	4		0.08855	4		0.06518	6		0.05878	5
	0501	0.09136	5		0.03526	10		0.00847	18		0.04652	9
	0304	0.08215	6		0.05330	8		0.08352	3		0.06978	4
	0303	0.05457	7		0.01182	16		0.05023	8		0.03402	12
	1203	0.04892	8		0.01783	12		0.02737	13		0.04127	11
25	0802	0.03875	9		0.03733	9		0.00395	22		0.04927	8
	0202	0.03729	10		0.08461	5		0.00395	21		0.04227	10
	DRB1	EUR_freq	EUR_rank		AFA_freq	AFA_rank		API_freq	API_rank		HIS_freq	HIS_rank
	1501	0.14441	1		0.02931	14		0.07919	4		0.06678	4
30	0701	0.13767	2		0.09771	2		0.08201	2		0.10455	1
	0301	0.12916	3		0.07069	4		0.05373	7		0.07329	2
	0101	0.09149	4		0.02599	15		0.02743	14		0.03877	9
	0401	0.09111	5		0.02287	16		0.00905	22		0.01451	22
	1301	0.06283	6		0.05551	7		0.02376	16		0.04202	7
35	1101	0.05654	7		0.08711	3		0.05119	9		0.04202	8
	1302	0.04015	8		0.06445	6		0.03620	10		0.03877	10
	0404	0.03634	9		0.00686	22		0.00905	23		0.05453	6
	1104	0.03189	10		0.00561	23		0.00650	24		0.02551	13
	DQB1	EUR_freq	EUR_rank		AFA_freq	AFA_rank		API_freq	API_rank		HIS_freq	HIS_rank
40	0201	0.23030	1		0.22314	1		0.10915	4		0.18525	3
	0301	0.18450	2		0.17374	3		0.19410	1		0.20046	1
	0602	0.14250	3		0.19801	2		0.04401	10		0.08065	6
	0501	0.12281	4		0.15381	4		0.07394	6		0.11014	4
45	0302	0.09504	5		0.03683	6		0.07614	5		0.18894	2
	0603	0.06512	6		0.02340	9		0.02289	12		0.04055	7
	0303	0.04460	7		0.01646	11		0.12192	2		0.02074	9
	0604	0.03240	8		0.01950	10		0.01364	14		0.02765	8
	0402	0.02529	9		0.06846	5		0.02333	11		0.09078	5

0503 0.02497 10 0.01473 12 0.06206 8 0.01336 12

HLA-DP

		Caucasian		African American	Mexican American
5	0101	0.062	4	0.272	0.031
	0201	0.131	2	0.13	0.093
	0202	0.007	9	NA	0.027
	0301	0.09	8	0.057	0.044
	0401	0.425	1	0.104	0.204
10	0402	0.121	3	0.111	0.398
	0501	0.015	6	0.005	0.035
	0601	0.021	5	0.003	0.009
	0901	0.004	10	0.005	0.013
15	1001	0.013	7	NA	0.027

[0242] While the disclosure has been particularly shown and described with reference to specific embodiments, it should be understood by those having skill in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the present disclosure as disclosed herein.

What is claimed is:

1. A recombinant polynucleotide encoding an alpha chain and/or a beta chain of a T cell receptor (TCR) selected from TCRs 19305DP, AL, KQ, PP, BB, KB, ST, JD, PB-P, PB-T,
5 PB13.2, and 19305CD8, wherein the alpha chain and/or the beta chain comprises at least one of:

i) for 19305DP, an alpha chain comprising the sequence of SEQ ID NO:1 and/or a beta chain comprising the sequence of SEQ ID NO:2;

10

ii) for AL, an alpha chain comprising the sequence of SEQ ID NO:5 and/or a beta chain comprising the sequence of SEQ ID NO:6;

15 iii) for KQ, an alpha chain comprising the sequence of SEQ ID NO:9 and/or a beta chain comprising the sequence of SEQ ID NO:10;

iv) for PP, an alpha chain comprising the sequence of SEQ ID NO:13 and/or a beta chain comprising the sequence of SEQ ID NO:14;

20 v) for BB, an alpha chain comprising the sequence of SEQ ID NO:17 and/or a beta chain comprising the sequence of SEQ ID NO:18;

25 vi) for KB, an alpha chain comprising the sequence of SEQ ID NO:21 and/or a beta chain comprising the sequence of SEQ ID NO:22,

vii) for ST, an alpha chain comprising the sequence of SEQ ID NO:25 and/or a beta chain comprising the sequence of SEQ ID NO:26;

30 viii) for JD, an alpha chain comprising the sequence of SEQ ID NO:29 and/or a beta chain comprising the sequence of SEQ ID NO:30,

ix) for PB-P, an alpha chain comprising the sequence of SEQ ID NO:33 and/or a beta chain comprising the sequence of SEQ ID NO:34;

x) for PB-T, an alpha chain comprising the sequence of SEQ ID NO:37 and/or a beta chain comprising the sequence of SEQ ID NO:38,

5 xi) for PB13.2, an alpha chain comprising the sequence of SEQ ID NO:41 and/or a beta chain comprising the sequence of SEQ ID NO:42, and

xii) for 19305CD8, an alpha chain comprising the sequence of SEQ ID NO:45 and/or a beta chain comprising the sequence of SEQ ID NO:46.

10

2. The recombinant polynucleotide of claim 1 encoding the 19305DP alpha chain comprising the sequence of SEQ ID NO:1 and/or the 19305DP beta chain comprising the sequence of SEQ ID NO:2.

15 3. The recombinant polynucleotide of claim 2 encoding the 19305DP alpha chain comprising the sequence of SEQ ID NO:1 and the 19305DP beta chain comprising the sequence of SEQ ID NO:2.

4. The recombinant polynucleotide of any one of claims 1-3, wherein the recombinant
20 polynucleotide is present in an expression vector.

5. The recombinant polynucleotide of claim 4, wherein the expression vector is present in a cell.

25 6. The recombinant polynucleotide of claim 5, wherein the expression vector is present in a cell that is a CD4⁺ T cell or a CD8⁺ T cell.

7. The recombinant polynucleotide of claim 6, wherein the expression vector encodes the 19305DP alpha chain comprising the sequence of SEQ ID NO:1 and/or the 19305DP beta
30 chain comprising the sequence of SEQ ID NO:2.

8. The recombinant polynucleotide of claim 6, wherein the expression vector encodes the 19305DP alpha chain comprising the sequence of SEQ ID NO:1 and the 19305DP beta chain comprising the sequence of SEQ ID NO:2.

9. 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/LAGE-1 antigen, the method comprising

5 administering to the individual modified human T cells comprising an expression vector of claim 4.

10. The method of claim 9, wherein the cancer cells are selected from bladder cancer cells, brain cancer cells, breast cancer cells, gastric cancer cells, esophageal cancer cells, head
10 and neck cancer cells, hepatobiliary cancer cells, kidney cancer cells, ovary cancer cells, non-small cell lung cancer cells, myeloma, prostate cancer cells, sarcoma cells, testicular cancer cells, melanoma cells, or combinations thereof.

11. The method of claim 9 or claim 10, comprising removing T cells from the individual prior to the administering, and modifying the T cells by introducing into the T cells the
15 expression vector.

12. The method of claim 11, wherein the expression vector encodes the 19305DP alpha chain comprising the sequence of SEQ ID NO:1 and/or the 19305DP beta chain comprising the sequence of SEQ ID NO:2.

20 13. The method of claim 11, wherein the expression vector encodes the 19305DP alpha chain comprising the sequence of SEQ ID NO:1 and the 19305DP beta chain comprising the sequence of SEQ ID NO:2.

14. The method of claim 11, wherein the modified T cells in which the expression vector
25 is present comprise CD4⁺ T cells, CD8⁺ T cells, or a combination thereof.

15. A library comprising a plurality of recombinant polynucleotides encoding the alpha chains and/or beta chain of T cell receptors (TCRs) of claim 1.

30 16. The library of claim 15, wherein the recombinant polynucleotides are present in expression vectors.

17. The library of claim 16, wherein the recombinant polynucleotide encodes the

19305DP alpha chain comprising the sequence of SEQ ID NO:1 and/or the 19305DP beta chain comprising the sequence of SEQ ID NO:2.

18. The library of claim 17, wherein the recombinant polynucleotide encodes the

5 19305DP alpha chain comprising the sequence of SEQ ID NO:1 and the 19305DP beta chain comprising the sequence of SEQ ID NO:2.

19. The library of claim 17, further comprising at least one additional recombinant polynucleotide encoding at least one TCR alpha chain and/or at least one TCR beta chain

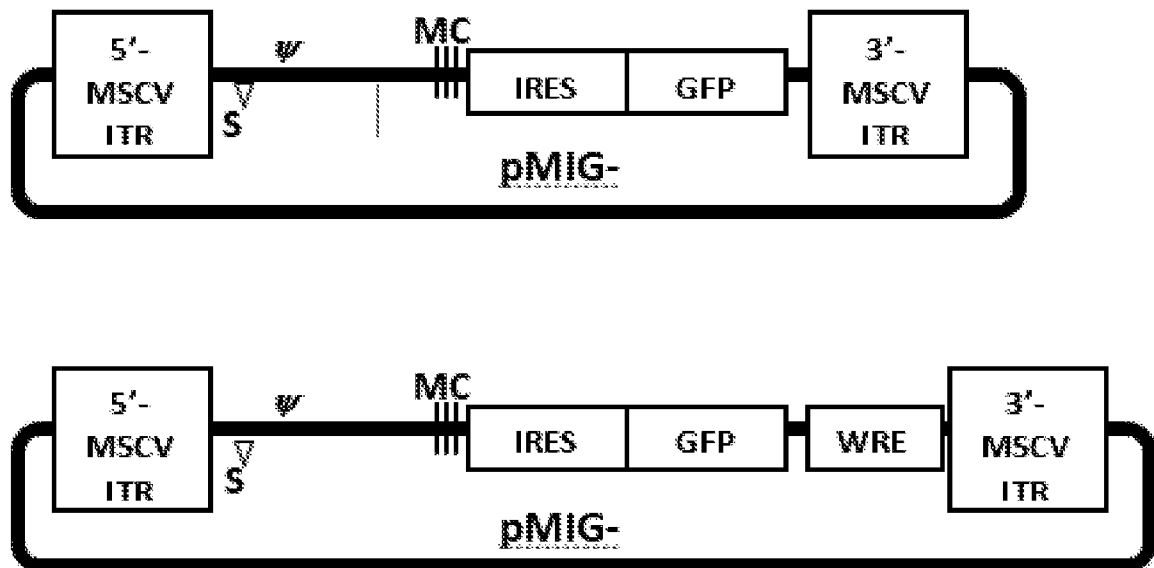
10 from AL, KQ, PP, BB, KB, ST, JD, PB-P, PB-T, PB13.2, 19305CD8, JM, 5B8, and SB95 TCRs.

20. A method comprising testing a sample from an individual to determine whether or not the individual has a NY-ESO-1/LAGE-1 positive cancer, and subsequent to a determination

15 that the individual has the NY-ESO-1/LAGE-1 positive cancer, selecting an expression vector from a library of any one of claims 15-19 based at least in part on the HLA type of the individual, and optionally introducing the expression vector into cells obtained from the individual.

Figure 1A. Plasmids used to create TCR-expressing backbone vectors

(A) Classical retroviral



(B) A recent retroviral

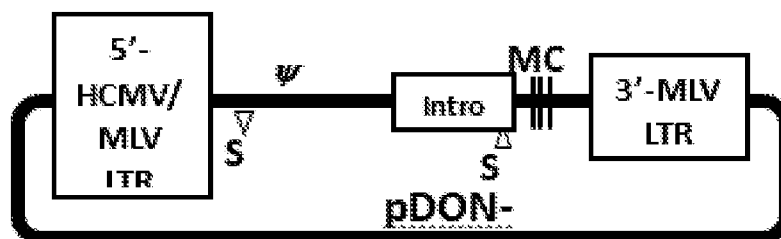
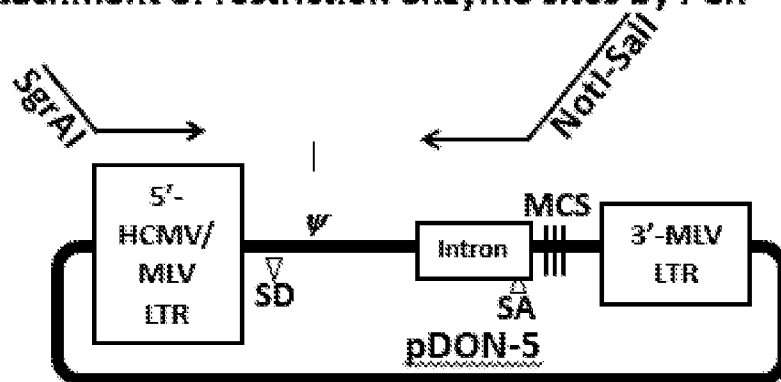


Figure 1B. Cloning of 5'-LTR to Splice Acceptor fragment into pMIG vectors

(A) Attachment of restriction enzyme sites by PCR



(B) Cloning of the PCR fragment into pMIG vectors

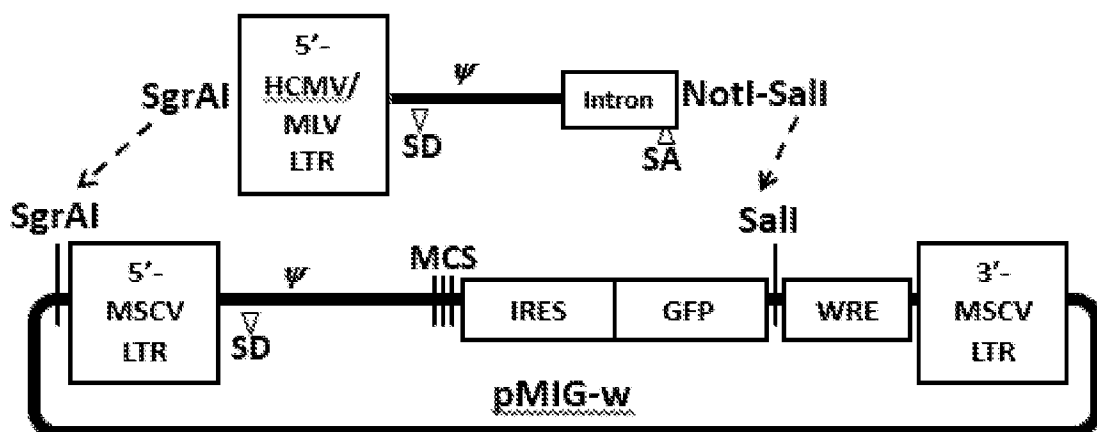
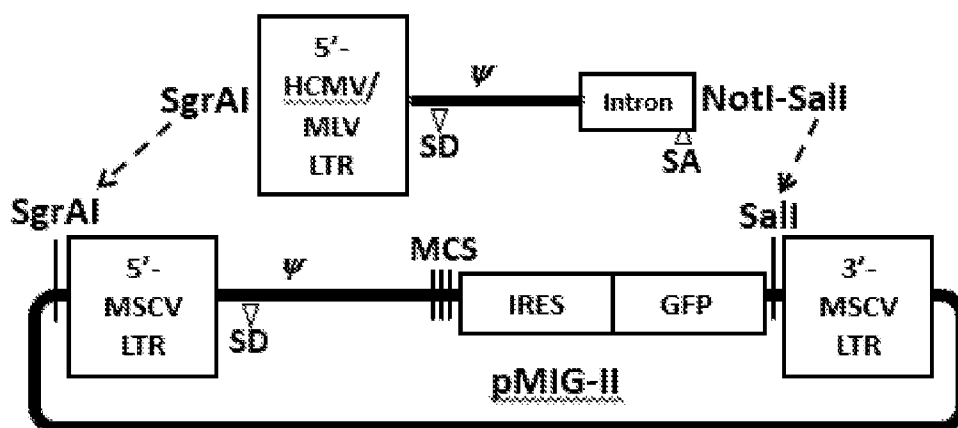
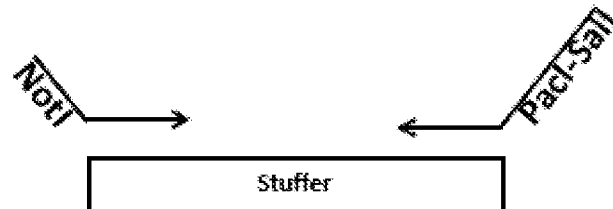


Figure 1C. Introduction of a stuffer fragment and the PacI restriction enzyme site

(A) Attachment of restriction enzyme sites by PCR



(B) Cloning of the stuffer fragment

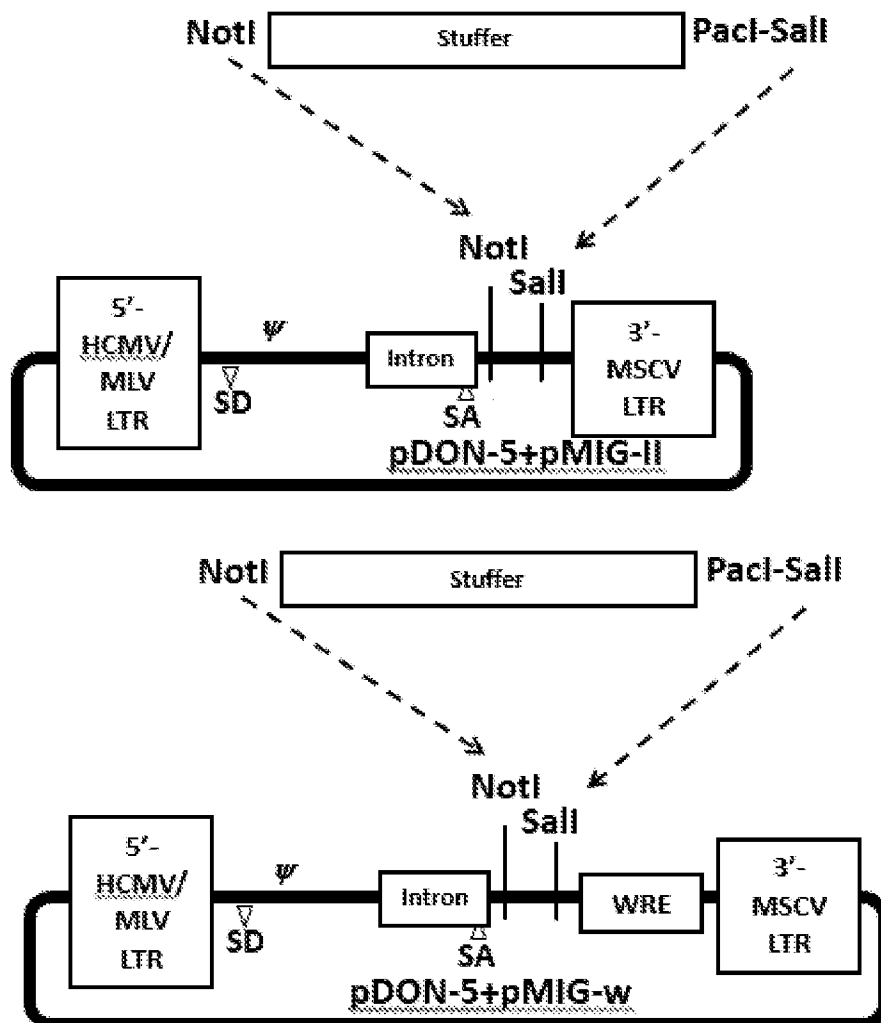


Figure 1D. Cloning of TCR expressing cassettes into vectors

(A) Attachment of restriction enzyme sites by PCR



(B) Cloning of the TCR expressing

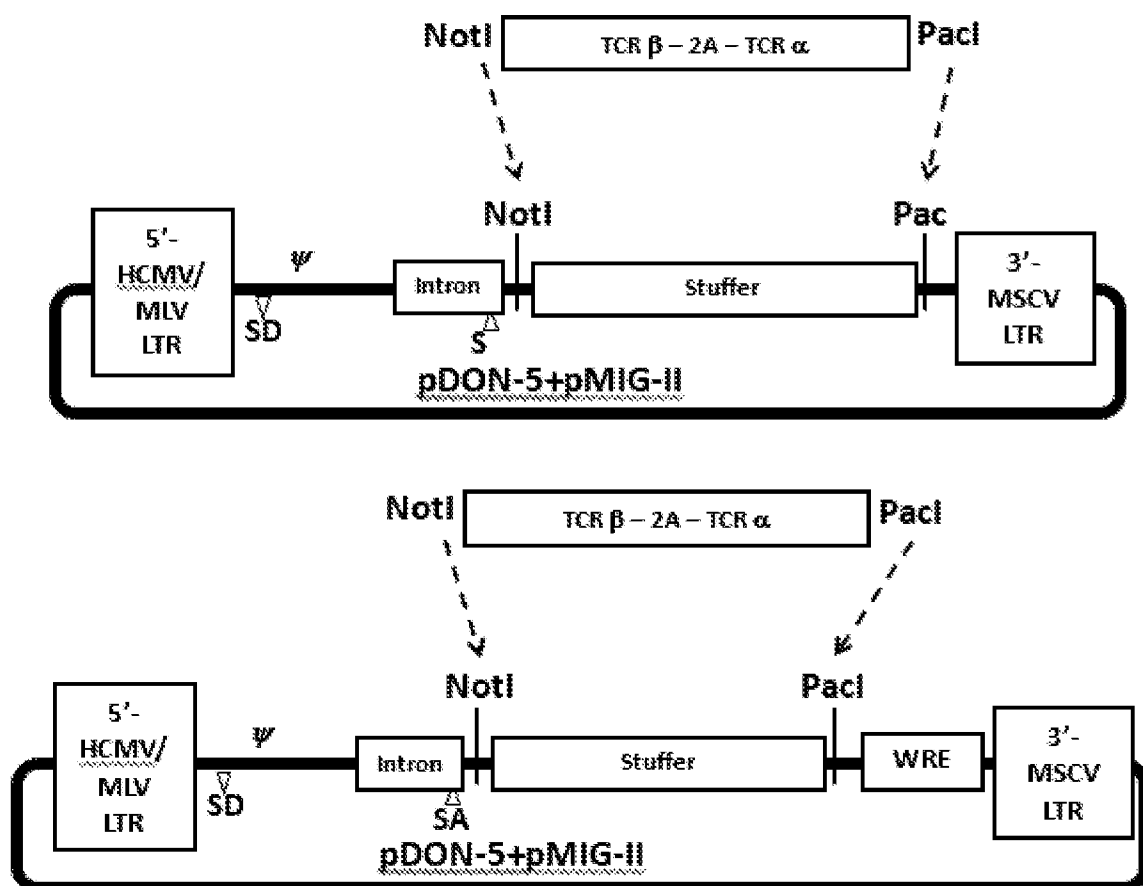
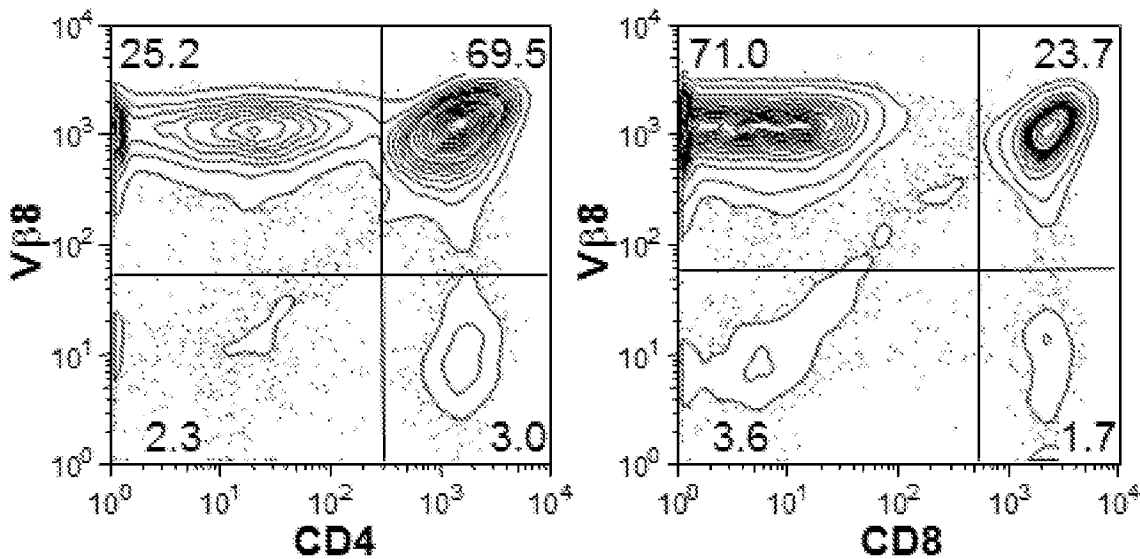


Figure 2

19305DP-TCR transduced PBMC



Untransduced PBMC

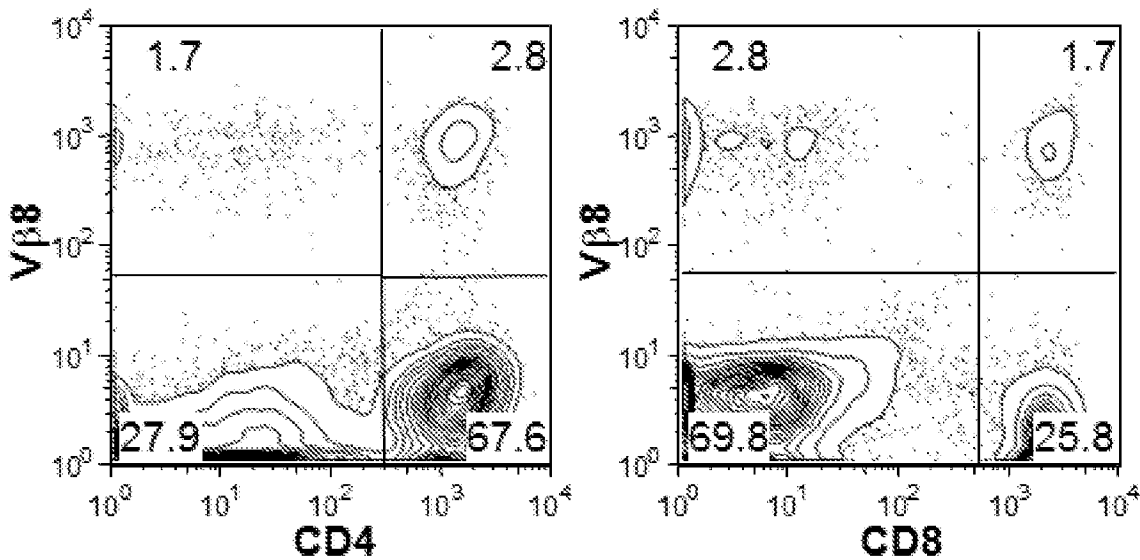
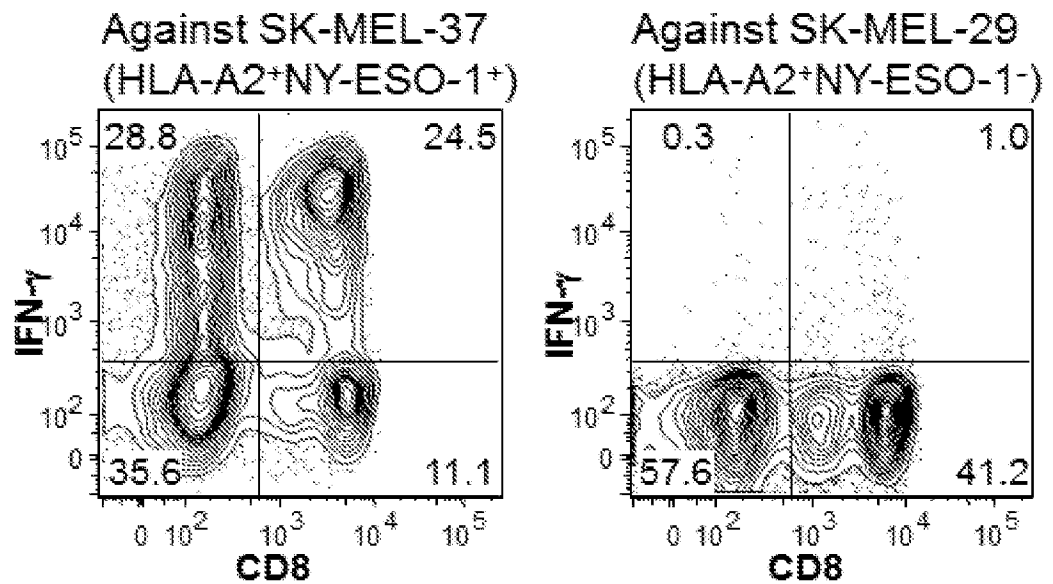


Figure 3

19305DP-TCR transduced PBMC



Untransduced PBMC

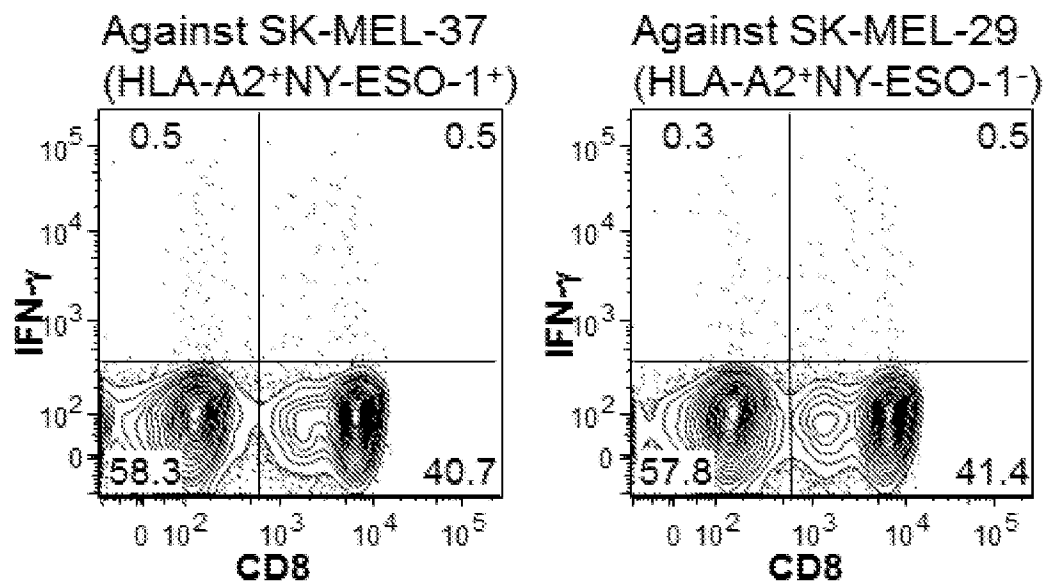


Figure 4

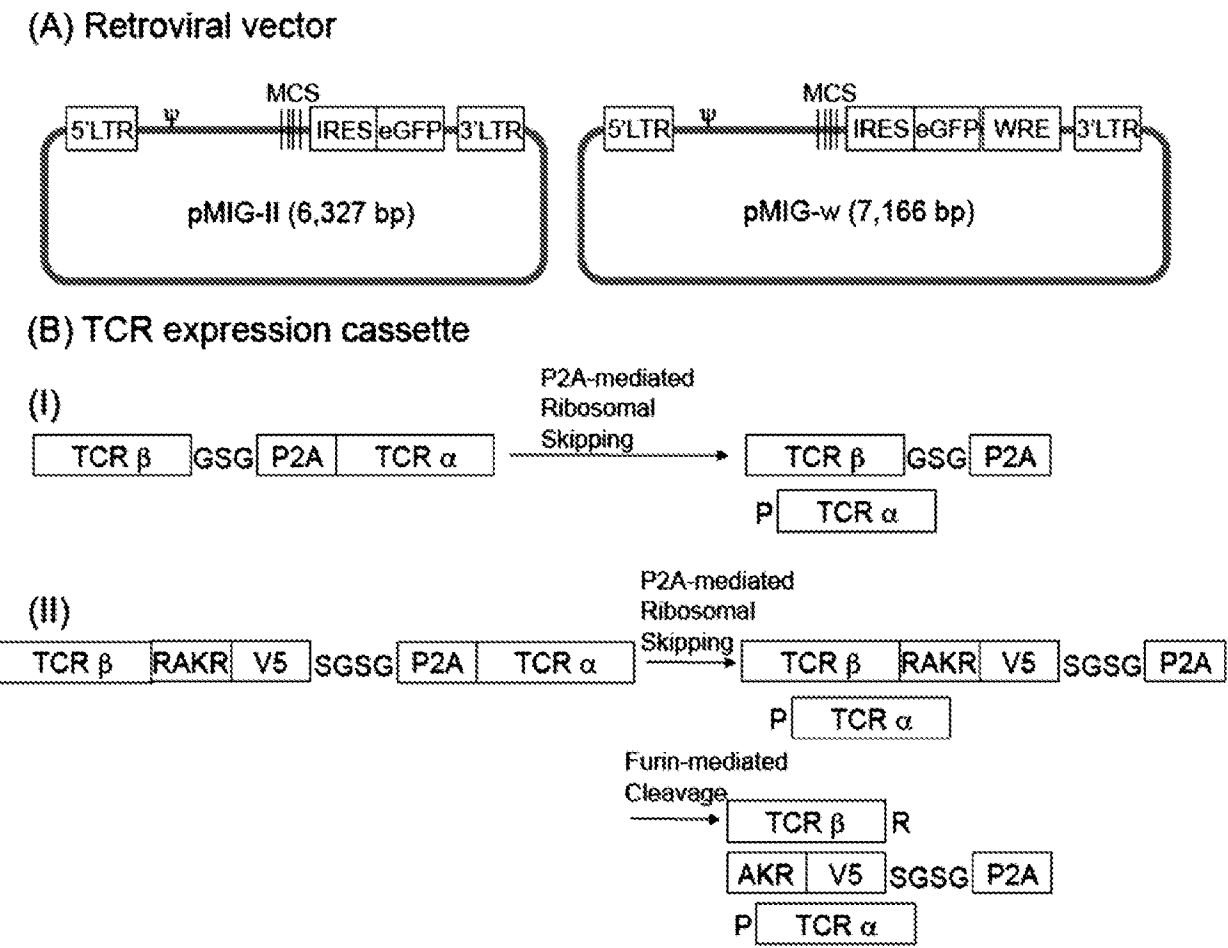


Figure 5

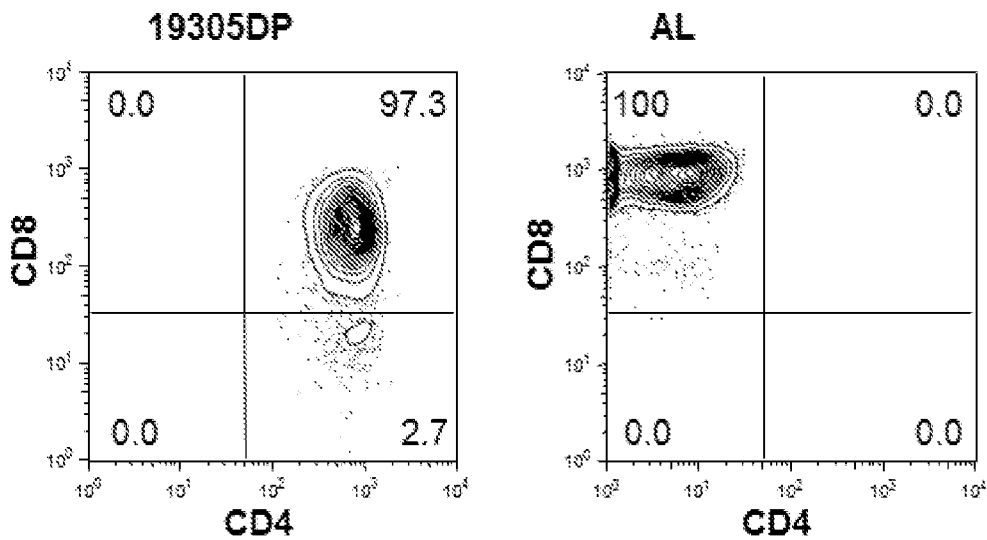


Figure 6

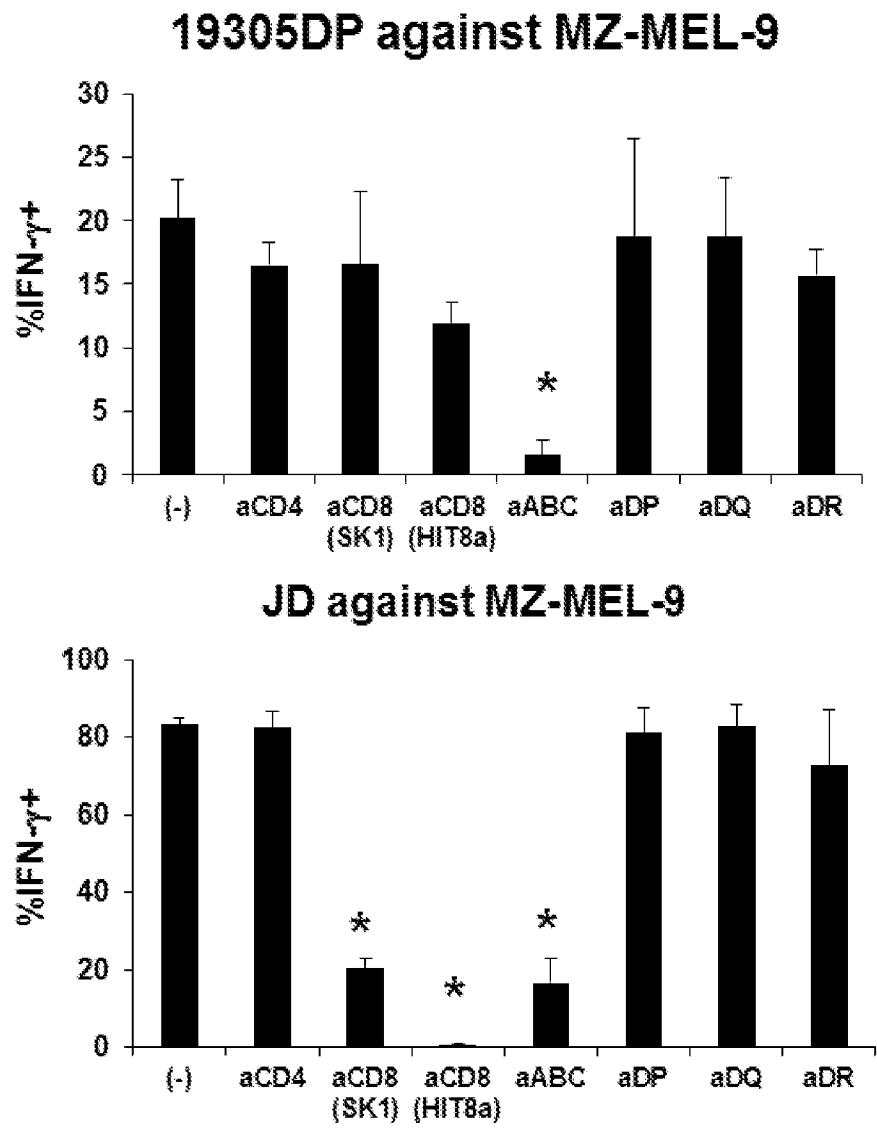


Figure 7

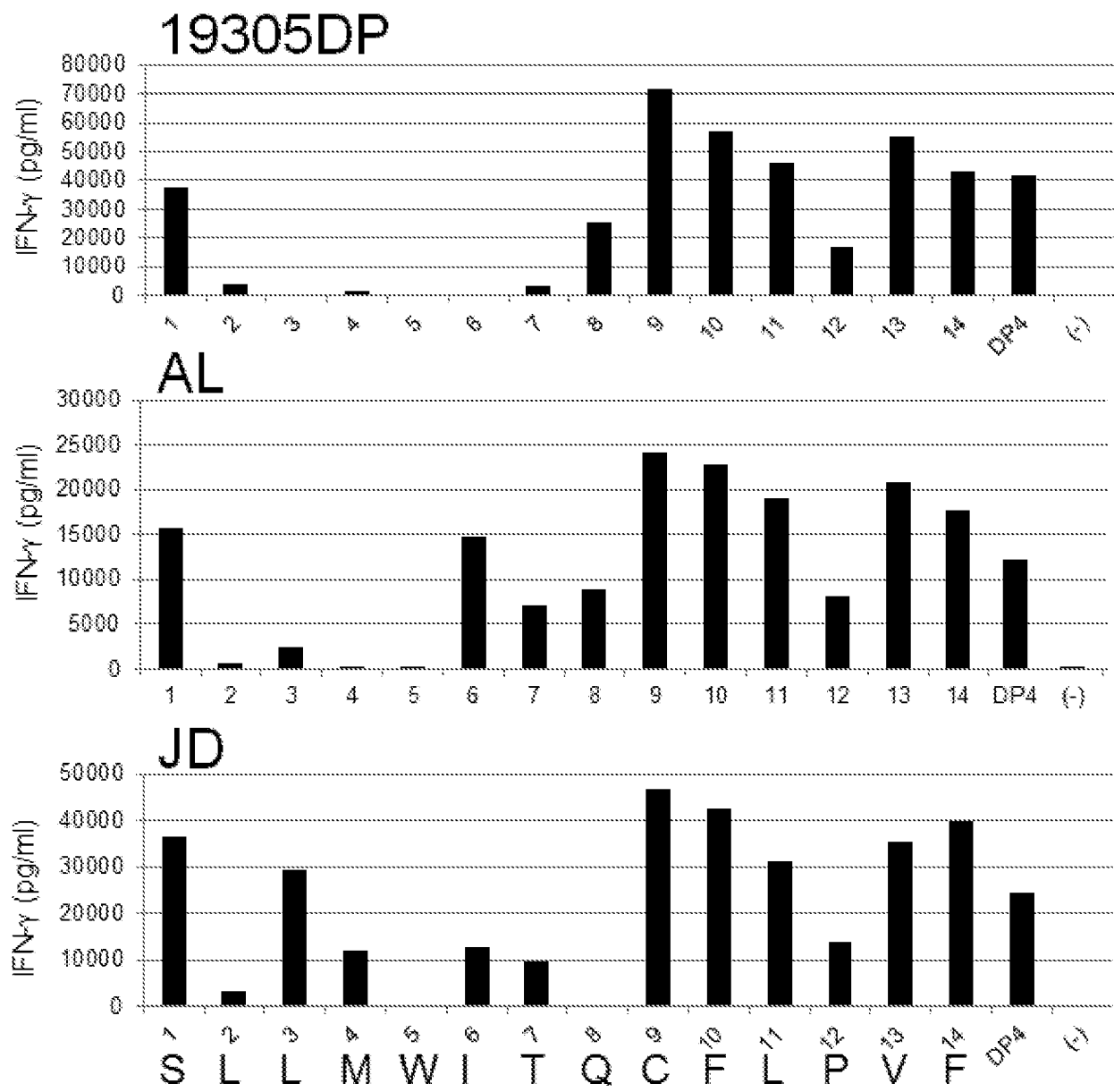


Figure 8

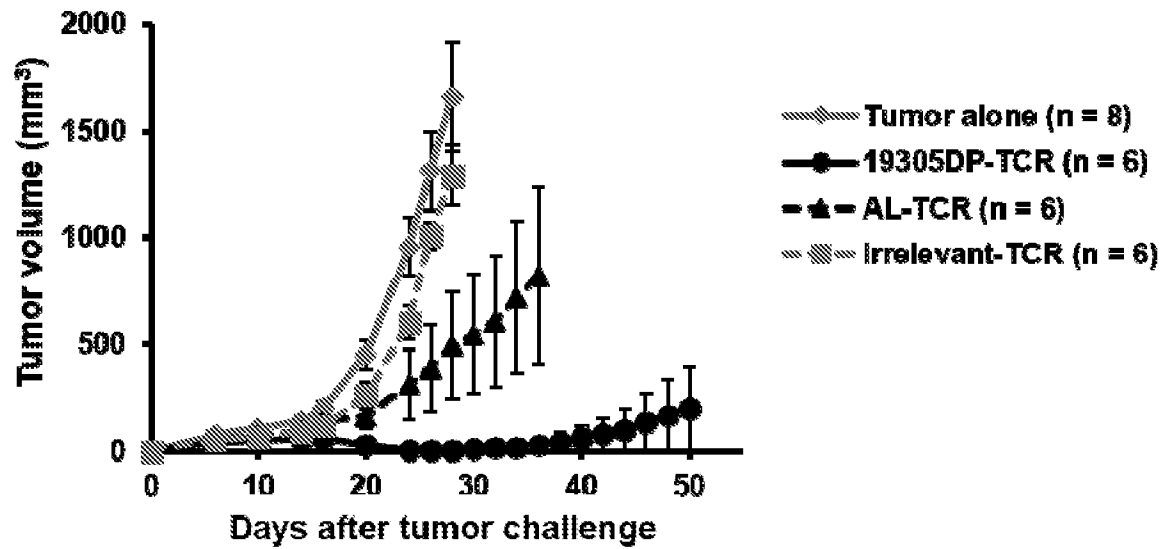
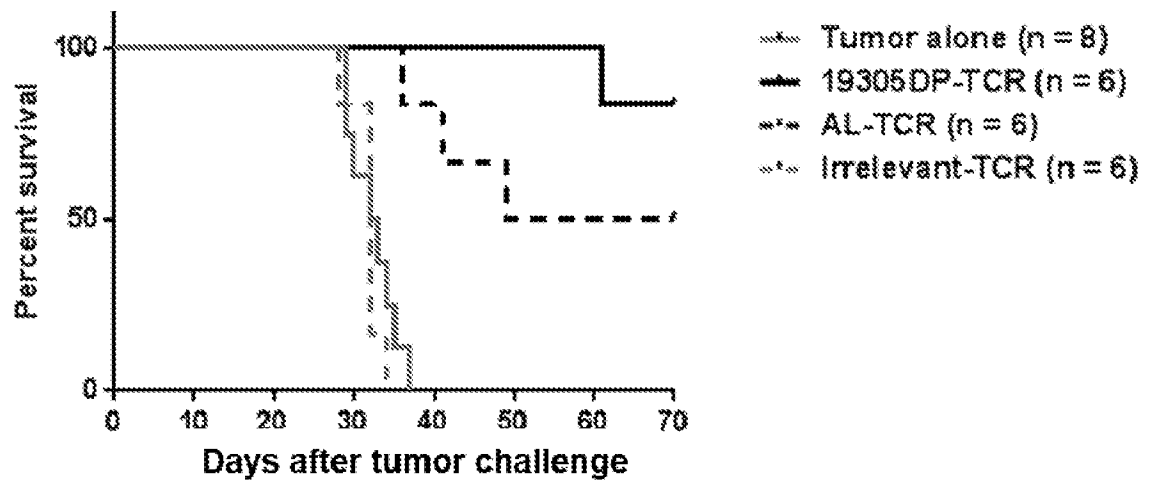
A**B**

Figure 9

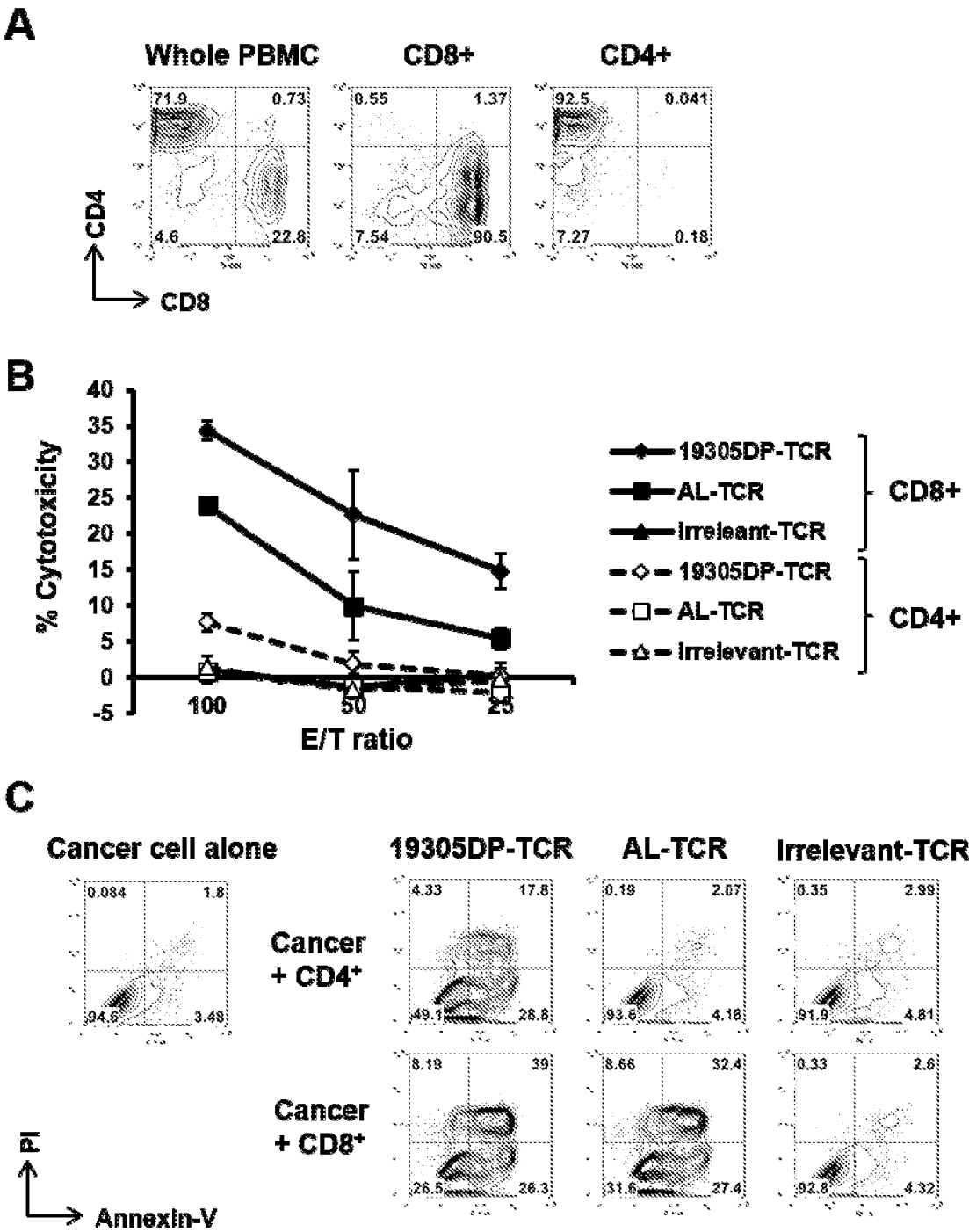


Figure 10

