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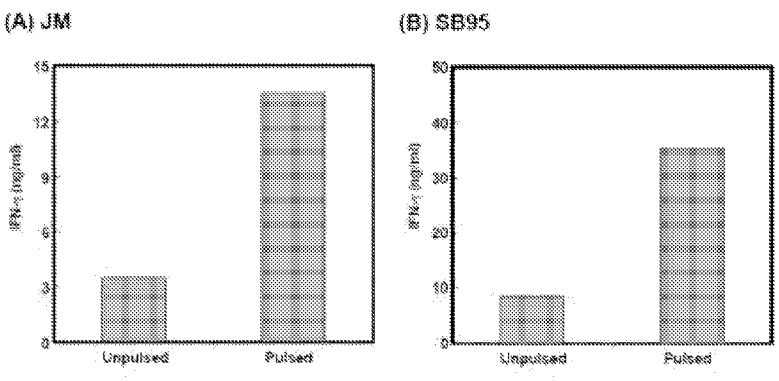
Figure 7



(57) Abstract: Provided are compositions and methods for prophylaxis and/or therapy of a variety of cancers which express a NY-ESO-1 antigen. Included are recombinant T cell receptors (TCRs), polynucleotides encoding them, expression vectors that include the polynucleotides, and cells into which the polynucleotides have been introduced to produce modified cells, including $CD4^+$ T cells, $CD8^+$ T cells, natural killer T cells, γδ T cells, and progenitor cells, such as haematopoietic stem cells. The modified cells are capable of direct recognition of a cancer cell expressing a NY-ESO-1 antigen by human leukocyte antigen (HLA) class II-restricted binding of the TCR to the NY-ESO-1 antigen expressed by the cancer cell without presentation of the antigen by antigen presenting cells. In embodiments, the NY-ESO-1 antigen is displayed by the tumor cells. Also included is a method for prophylaxis and/or therapy of cancer by administering modified cells that express a recombinant TCR. Methods for making expression vectors and/or cells which express a recombinant TCR and identifying TCRs to make the expression vectors are also included.

WO 2014/160030 A2

COMPOSITIONS AND METHODS FOR USE OF RECOMBINANT T CELL RECEPTORS FOR DIRECT RECOGNITION OF TUMOR ANTIGEN

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. application no. 61/778,673, filed March 13, 2013, the disclosure of which is incorporated herein by reference.

FIELD

[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 OF THE INVENTION

[0003] Tumor antigen-specific $CD4^+$ helper T cells play critical roles in the induction and maintenance of anti-tumor immune responses by providing "CD4-help". Activation of $CD4^+$ T cells at the local tumor sites is believed to help overcome multiple immuno-suppression mechanisms and promote tumor eradication by the immune system. However, because of the frequent lack of functional antigen-presenting cells at the local tumor sites, activation of the $CD4^+$ T cells and therefore the provision of CD4-help at the local tumor site is severely limited. There is accordingly an ongoing and unmet need to provide new compositions and methods such that activation of $CD4^+$ T cells and therefore provision of CD4-help can be achieved.

SUMMARY

[0004] 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 the NY-ESO-1 antigen. In embodiments, the disclosure includes recombinant T cell receptors (TCRs), 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, γδ T cells, and progenitor cells, such as haematopoietic stem cells. 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).

[0005] In one aspect the disclosure includes a modified human T cell comprising a recombinant polynucleotide encoding a TCR, wherein the T cell is capable of direct recognition

of a cancer cell expressing a NY-ESO-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 antigen expressed by the cancer cell. In particular embodiments, the TCR encoded by the polynucleotide and expressed by the cell has a TCR alpha chain having the sequence of SEQ ID NO:3 and a TCR beta chain having the sequence of SEQ ID NO:4, or a TCR alpha chain having the sequence of SEQ ID NO:7 and a TCR beta chain having the sequence of SEQ ID NO:8, or a TCR alpha chain having the sequence of SEQ ID NO:11 and a TCR beta chain having the sequence of SEQ ID NO:12. All combination of such alpha and beta chains are included in the disclosure. In an embodiment, the modified cell of claim 1, wherein the sequence encoding the alpha chain and/or the beta chain does not comprise introns. In embodiments, the TCRs of this disclosure include amino acid sequences that are 95%, 96%, 97%, 98%, or 99% amino acid sequence identify across the length of the amino acid sequences disclosed herein.

[0006] In another aspect 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 antigen. This approach comprises administering to the individual modified human T cells comprising a recombinant polynucleotide encoding a TCR, wherein the T cells are capable of direct recognition of the cancer cells expressing the NY-ESO-1 antigen, and wherein the direct recognition of the cancer cells comprises HLA class II-restricted binding of the TCR to the NY-ESO-1 antigen expressed by the cancer cells. In embodiments, the cells comprising the recombinant TCR are human $CD4^+$ T cells. In embodiments, the cells comprising the recombinant TCR that is administered to the 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, modified to express the recombinant TCR, and administered back to the same individual.

[0007] In embodiments, the cancer that expresses the NY-ESO-1 antigen is selected from bladder cancer cells, brain cancer cells, breast cancer cells, gastric cancer cells, esophageal cancer cells, head 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, and combinations thereof.

[0008] In another aspect the disclosure includes one or more expression vectors. The expression vector(s) encode a TCR that is capable of imparting to a cell which expresses it the capability to directly a cancer cell expressing a NY-ESO-1 antigen, wherein the direct

recognition of the cancer cell comprises HLA class II-restricted binding of the TCR to the NY-ESO-1 antigen expressed by the cancer cell.

[0009] In another approach, methods for making expression vectors and/or cells which express a recombinant TCR. The method involves obtaining a plurality of T cells from an individual, identifying T cells that are capable of direct recognition of a cancer cell displaying a NY-ESO-1 antigen in an HLA class II-restricted manner without antigen presenting cells presenting the NY-ESO-1 antigen to the T cells, determining the sequence of the alpha chain of the TCR and the sequence of the beta chain of the TCR, and introducing into an expression vector a polynucleotide sequence encoding the alpha chain of the TCR and the beta chain of the TCR. In an embodiment, this method comprises introducing the expression vector into a cell such that the TCR is expressed.

BRIEF DESCRIPTION OF THE FIGURES

[0010] **Figure 1.** (A) Direct recognition of cancer cells by JM $CD4^+$ T cell clone. Interferon (IFN)-γ and CD107 expression of NY-ESO-$1_{157-170}$ peptide-specific tumor-recognizing $CD4^+$ T cell clone (Clone: JM) (TR-CD4) and non-tumor-recognizing $CD4^+$ T cell clone (NTR-CD4) after coculture with NY-ESO-1-expressing SK-MEL-37 (SK37) and NY-ESO-1-negative SK-MEL-29 (SK29) with or without pulsing with the cognate NY-ESO-$1_{157-170}$ ($ESO_{157-170}$) peptide was investigated by intracellular cytokine staining. (B) Differences in intracellular and extracellular NY-ESO-1 recognition by NY-ESO-1-specific $CD4^+$ and $CD8^+$ T cell clones. NY-ESO-1-negative SK-MEL-29 was unpulsed (Unpulsed) or pulsed with NY-ESO-$1_{157-170}$ peptide (Peptide) or recombinant NY-ESO-1 protein (Protein), or was infected with adenovirus vector which induce intracellular NY-ESO-1 expression. Recognition by TR-CD4, NTR-CD4 and NY-ESO-1-specific $CD8^+$ T cell clone was evaluated by IFN-γ ELISPOT assay.

[0011] **Figure 2.** (A) TR-CD4 (Clone: JM) were co-cultured with SK-MEL-37. Culture supernatant was harvested after 1-4 days of culture. The levels of the indicated cytokines and lytic molecules in the supernatant were measured by ELISA. (B) TR-CD4 and NTR-CD4 was co-cultured with SK-MEL-37 and expression of the early apoptosis marker, Annexin-V, on SK-MEL-37 (SK37) was measured by flow-cytometry.

[0012] **Figure 3.** NY-ESO-1-specific $CD8^+$ T cell clone (ESO-CD8) was co-cultured with SK-MEL-37 at 1:2 ratio in the presence or absence of the indicated ratios of TR-CD4 (Clone: JM). Cytotoxic activity by ESO-CD8 on SK-MEL-37 was evaluated by CFSE-based cytotoxicity assay.

[0013] **Figure 4.** (A) NY-ESO-1-specific $CD8^+$ T cell clone (ESO-CD8) was stimulated

with or without SK-MEL-37 (SK37) in the presence or absence of TR-CD4 (clone: JM). After 4 days, the number of $CD8^+$ T cells were enumerated by trypan blue exclusion assay combined with CD8 staining by flow-cytometry. (B) ESO-CD8 was stimulated with SK37 in the presence or absence of TR-CD4. Before (day 0) and after (day 1 and day 2) stimulation, expression of activation markers (CD25, CD69 and CD122) or central T cell differentiation markers (CD62L, CCR7 and CD127) on ESO-CD8 was measured by flow-cytometry.

[0014] **Figure 5.** (A) SK-MEL-37 was inoculated in SCID mice (6 mice / group) with or without tumor-recognizing $CD4^+$ T cell clone (JM: TR-CD4), non-tumor-recognizing $CD4^+$ T cell clone (NTR-CD4), and/or NY-ESO-1-specific $CD8^+$ T cell clone (ESO-CD8). Tumor growth was measured every 2-3 day. (B) Tumor was excised and weighted at day 45 after inoculation.

[0015] **Figure 6.** (A) Retrovirus vector used in the experiments. 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.

[0016] **Figure 7.** Polyclonally activated PBMC were transduced with retroviral vector (A: JM-TCR; B: SB95-TCR). They were cocultured with peptide-pulsed (Pulsed) or unpulsed (Unpulsed) HLA-DRB1*01+DPB1*04+ cells for 20 hours. IFN-γ level in the supernatant was measured by ELISA. NY-ESO-$1_{157-170}$ and NY-ESO-1_{91-110} peptides were used as the cognate peptides for JM-TCR and SB95-TCR, respectively.

DESCRIPTION OF THE INVENTION

[0017] The present disclosure relates to immune cells, including but not necessarily limited to T cells, that have been engineered to be capable of direct recognition of tumor antigen and MHC class II-expressing cancer cells. In embodiments, the immune cells are $CD4^+$ T cells, $CD8^+$ T cells, natural killer T cells, γδ T cells, or their progenitor cells such hematopoietic stem/progenitor cells. In embodiments, the hematopoietic/progenitor cells are characterized by one or more markers selected from CD34, CD117 (c-kit) and CD90 (Thy-1).

[0018] It is well known that $CD4^+$ T cells typically recognize peptide fragments presented on MHC class II (HLA class II in humans) by antigen presenting cells, such as

macrophages and dendritic cells. In addition to antigen-presenting cells, many human cancer cells are also known to express MHC class II constitutively or in an IFN-γ-inducible manner, but the role of MHC class II expression on human cancer cells remains largely unknown.

[0019] We have now discovered that there are two distinct types of tumor antigen-specific $CD4^+$ T cells. One type of tumor antigen-specific $CD4^+$ T cells is referred to herein as tumor-recognizing $CD4^+$ T cells (TR-CD4). This type of $CD4^+$ T cell directly recognizes MHC (HLA in humans) class II-expressing cancer cells in antigen-specific and MHC class II-restricted manner. In contrast, another type of previously known, antigen-specific $CD4^+$ T cells is referred to herein as non-tumor-recognizing $CD4^+$ T cells (NTR-CD4). This type of T cell only recognizes exogenous tumor antigen peptides after processing by antigen-presenting cells. Figs. 1A and 1B depict data demonstrating these distinct functions and reveal direct recognition of cancer cells by TR-CD4.

[0020] Because of their different abilities in direct recognition of cancer cells, these two types of $CD4^+$ T cells (TR-CD4 and NTR-CD4) are believed to play different roles at the local tumor site. Without intending to be constrained by any particular theory, it is believed that TR-CD4 cells provide CD4-help by direct recognition of cancer cells. The present invention takes advantage of this function to provide TCR polypeptides and recombinant polynucleotides encoding them for use in novel prophylactic and/or therapeutic treatment modalities and compositions. By engineering T cells to express the TCRs further described herein, we can endow any $CD4^+$ cell with the capability to directly recognize tumor antigen-expressing cancer cells, without requiring presentation of the antigen by an antigen-presenting cell. Thus, the present invention includes compositions and methods that are useful for creating and using TR-CD4 cells for improved care of cancer patients.

[0021] Previous attempts at making and using recombinant TCRs have been made. For example, U.S. patent no. 8,008,438 (the '438 patent) discloses recombinant TCRs which bind to the peptide sequence SLLMWITQC from the NY-ESO-1 protein (NY-ESO-1:157-165). However, and importantly, the disclosure in the '438 patent pertains to classic $CD8^+$ TCRs, which only recognize the NY-ESO-1:157-165 peptide in the context of the HLA-A*0201 class I restriction element. This constitutes a significant dissimilarity from the present invention because, as described above, the recombinant TCRs of the present invention are class II restricted. Moreover, and as also described above, unlike canonical class II restriction, cells engineered to express a recombinant TCR of the invention surprisingly do not require the assistance of antigen presenting cells to recognize the antigens to which they are specific. Instead, they can recognize the antigens as they exist *in vivo* as a peptide displayed by the tumor

cells. Further, the TCRs of the present invention recognize peptides by those disclosed in the '438 patent. Accordingly, the present invention is a significant and unexpected departure from the prior art. In an embodiment, a TR-CD4 is a CD4+ cell that exhibits cytokine secretion (such as IFN-gamma production) when the TR-CD4 is directly exposed to cells which express an antigen for which the TCR is specific in an HLA-II context. The ability to confer capability for direct recognition of NY-ESO-1-expressing tumors by CD4+ T cells by introducing a TCR from a naturally occurring cell having this capability was unexpected.

[0022] In one embodiment, the invention includes transforming any $CD4^+$ T cell into a TR-CD4 by introducing a polynucleotide encoding a recombinant TCR of the invention into polyclonally expanded $CD4^+$ T cells and allowing expression of the TCR polypeptide coding region(s) of the polynucleotide.

[0023] In various embodiments, the present invention provides isolated and/or recombinant polynucleotides encoding particular TCR polypeptides, cells engineered to express the TCR polypeptides, pharmaceutical formulations comprising cells which express the TCR polypeptides, and methods of using the pharmaceutical formulations to achieve a prophylactic and/or therapeutic effect against cancer in a subject. 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 presenting cell populations that can be considered polyvalent with respect to the TCRs. 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 introduction of the polynucleotide the TCR was not encoded by a chromosomal sequence in the cell.

[0024] The TCRs provided by the invention are capable of recognizing NY-ESO-1;157-170 which is an antigen that consists of the amino acid sequence SLLMWITQCFLPVF, or are capable of recognizing NY-ESO-1;95-106, which is an antigen that consists of the amino acid sequence PFATPMEAELAR. As described above, in certain embodiments, the cells provided by the invention are engineered $CD4^+$ T cells that are capable of recognizing these antigens via TCRs which interact with the antigen in association with HLA class II molecules, wherein the HLA class II molecules and antigen are displayed by tumor cells.

[0025] 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, γδ 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 once they have been engineered to express a TCR according to the invention.

[0026] 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.

[0027] With respect to the polypeptides that are encoded by the polynucleotides described above, in certain aspects the invention provides functional TCRs 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) 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) site.

[0028] 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.

[0029] Figure 6 provides representative configurations of TCR polypeptides of the invention and polynucleotides/expression vectors encoding them. In one embodiment, as outlined in Figure 6, an amino acid sequence that is C-terminal to the TCR β chain protein is removed by furin protease-mediated cleavage, resulting in functional TCR α and β chain proteins. It will be also recognized from Figure 6 that the 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.

[0030] In connection with the present invention, we have also made the following discoveries: in certain instances, intracellular tumor antigen is loaded on HLA class II through recycling of the HLA class II in tumors; direct tumor recognition by tumor-recognizing $CD4^+$ T cells leads to *in vivo* tumor growth inhibition; $CD4^+$ T cells efficiently augment $CD8^+$ T cell cytotoxicity through direct tumor recognition; $CD4^+$ T cells support proliferation, survival, and memory differentiation of cognate antigen-specific $CD8^+$ T cells through direct tumor recognition without antigen presenting cells. It is expected that practicing the present invention in a clinical setting will also result in direct tumor recognition by the engineered tumor-recognizing $CD4^+$ T cells and lead to *in vivo* tumor growth inhibition in human subject, and will also result in the efficient augmentation of $CD8^+$ T cell cytotoxicity by the engineered $CD4^+$ T cells, and that the engineered $CD4^+$ T cells will support proliferation, survival, and memory differentiation of cognate antigen-specific $CD8^+$ T cells in human subjects who receive $CD4^+$ T cells engineered according to the invention.

[0031] With respect to use of the engineered $CD4^+$ T cells of the present invention, 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-I. As is well known in the art, NY-ESO-I 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 cholagiocarcinoma. 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-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.

[0032] The present disclosure includes recombinant TCRs, cells expressing them, and therapeutic/prophylactic methods that involve presentation of NY-ESO-1 antigens in conjunction with any HLA-class II complex that will be recognized by the TCRs. In embodiments, the HLA-II is selected from HLA-DP, HLA-DQ, and HLA-DR. In embodiments, the NY-ESO-1 antigen is recognized by the TCR in conjunction with HLA-DRB1*01 or HLA-DPB1*04.

[0033] We demonstrate in this invention that TR-CD4 we created produce multiple molecules through direct recognition of cancer cells, which induced apoptosis in cancer cells (Fig. 2A and 2B). Importantly, TR-CD4 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 (Fig. 3). Furthermore, $CD8^+$ T cells co-stimulated with TR-CD4 by cancer cells actively proliferated and upregulated central memory T cell markers (Fig. 4A and 4B).

[0034] TR-CD4 showed significant in vivo anti-tumor activity to inhibit the growth of human cancer cells in immuno-deficient mice (Fig. 5). In addition, TR-CD4 and tumor antigen-specific $CD8^+$ T cells co-operatively inhibited *in vivo* tumor growth (Fig. 5). Thus, the data presented herein strongly suggest that the recruitment of TR-CD4 at the local tumor site potentiate the anti-tumor immune responses, and accordingly will likely make an effective and heretofore unavailable therapeutic approach for widespread use in the clinic.

[0035] The following description provides illustrative examples of materials and methods used to make and use various embodiments of the invention.

[0036] To develop a method to efficiently generate a large number of TR-CD4 by gene-engineering with tumor-recognizing T cell receptor (TCR) gene, full length TCR gene from three TR-CD4 clones were cloned and sequenced by using 5'-RACE-PCR technique. The following TCRs were created:

1. HLA-DRB1*01-restricted NY-ESO-1:96-106-specific TR-CD4 (referred to herein as Clone: "SB95")
2. HLA-DPB1*04-restricted NY-ESO-1:157-170-specific TR-CD4 (referred to herein as Clone: "5B8")
3. HLA-DPB1*04-restricted NY-ESO-1:157-170-specific TR-CD4 (referred to herein as Clone: "JM")

[0037] TCR genes from SB95 and JM were inserted into retroviral expression vectors (such as MSCV-derived pMIG-II or pMIG-w vectors). A 5B8 TCR-expressing vector is made in the same manner.

[0038] Retroviral transduction of these TCR genes efficiently transferred reactivity against cognate peptides to polyclonally expanded T cells from peripheral blood mononuclear cells (PBMC) from healthy individuals. The nucleotide and amino acid sequences presented below represent those used to demonstrate the invention. 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, wherein that recognition is dependent on expression of NY-ESO-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 TR-CD4 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.

[0039] 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, wherein translation initiation and stop codons in the polynucleotide sequences are bold:

"JM" HLA-DPB1*0401/0402-restricted NY-ESO-$1_{157-170}$-specific tumor-recognizing $CD4^+$ T cell clone

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

TCR α chain
ATGAAGTTGGTGACAAGCATTACTGTACTCCTATCTTTGGGTATTATGGGTGATGCTAAGAC
CACACAGCCAAATTCAATGGAGAGTAACGAAGAAGAGCCTGTTCACTTGCCTTGTAACCAC
TCCACAATCAGTGGAACTGATTACATACATTGGTATCGACAGCTTCCCTCCCAGGGTCCAGA
GTACGTGATTCATGGTCTTACAAGCAATGTGAACAACAGAATGGCCTCTCTGGCAATCGCTG
AAGACAGAAAGTCCAGTACCTTGATCCTGCACCGTGCTACCTTGAGAGATGCTGCTGTGTAC
TACTGCATCCCTAATAACAATGACATGCGCTTTGGAGCAGGGACCAGACTGACAGTAAAAC
CAAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAA
GTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTG
ATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAG
TGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTA
TTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAA
AGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCT
CCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGC**TGA** (SEQ ID NO:1)

TCR β chain
ATGGGCTCCAGGCTGCTCTGTTGGGTGCTGCTTTGTCTCCTGGGAGCAGGCCCAGTAAAGGC
TGGAGTCACTCAAACTCCAAGATATCTGATCAAAACGAGAGGACAGCAAGTGACACTGAGC
TGCTCCCCTATCTCTGGGCATAGGAGTGTATCCTGGTACCAACAGACCCCAGGACAGGGCCT
TCAGTTCCTCTTTGAATACTTCAGTGAGACACAGAGAAACAAAGGAAACTTCCCTGGTCGAT
TCTCAGGGCGCCAGTTCTCTAACTCTCGCTCTGAGATGAATGTGAGCACCTTGGAGCTGGGG
GACTCGGCCCTTTATCTTTGCGCCAGCAGCTTCCCCAGGGAACCTAACTATGGCTACACCTT
CGGTTCGGGGACCAGGTTAACCGTTGTAGAGGACCTGAACAAGGTGTTCCCACCCGAGGTC
GCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGTGTGCC
TGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGT
GCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTCC
AGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACC
ACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAG
GGCCAAACCCGTCACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTT
ACCTCGGTGTCCTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGG
GAAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAA
AGGATTTC**TGA** (SEQ ID NO:2)

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

TCR α chain
MKLVTSITVLLSLGIMGDAKTTQPNSMESNEEEPVHLPCNHSTISGTDYI 50
HWYRQLPSQGPEYVIHGLTSNVNNRMASLAIAEDRKSSTLILHRATLRDA 100

*AVYYCIPNNNDMRF*GAGTRLTVKPNIQNPDPAVYQLRDSKSSDKSVCLFT 150
DFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANA 200
FNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLK 250
VAGFNLLMTLRLWSS (SEQ ID NO:3)

TCR β chain
MGSRLLCWVLLCLLGAGPVKAGVTQTPRYLIKTRGQQVTLSCSPISGHRS 50
VSWYQQTPGQGLQFLFEYFSETQRNKGNFPGRFSGRQFSNSRSEMNVSTL 100
*ELGDSALYLCASSFPREPNYGYTFGSGTRLTVVE*DLNKVFPPEVAVFEPS 150
EAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQP 200
ALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPV 250
TQIVSAEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALV 300
LMAMVKRKDF (SEQ ID NO:4)

"5B8" HLA-DPB1*0401/0402-restricted NY-ESO-$1_{157-170}$-specific tumor-recognizing $CD4^+$ T cell clone

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

TCR α chain
ATGGCCCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCAGAGACTGTGA
CCCTGAGTTGCACATATGACACCAGTGAGAATAATTATTATTTGTTCTGGTACAAGCAGCCT
CCCAGCAGGCAGATGATTCTCGTTATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACGG
AGAATCGTTTCTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTCAGAC
TCACAGCTGGGGGACACTGCGATGTATTTCTGTGCTTTCTCGAGAGGGAGTGGAGGTAGCA
ACTATAAACTGACATTTGGAAAAGGAACTCTCTTAACCGTGAATCCAAATATCCAGAACCCT
GACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCAC
CGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACA
AAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAA
CAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCT
TCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATAC
GAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCG
GGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGC**TGA** (SEQ ID NO:5)

TCR β chain
ATGGGCACCAGGCTCCTCTTCTGGGTGGCCTTCTGTCTCCTGGGGGCAGATCACACAGGAGC
TGGAGTCTCCCAGTCCCCCAGTAACAAGGTCACAGAGAAGGGAAAGGATGTAGAGCTCAGG
TGTGATCCAATTTCAGGTCATACTGCCCTTTACTGGTACCGACAGAGCCTGGGGCAGGGCCT
GGAGTTTTTAATTTACTTCCAAGGCAACAGTGCACCAGACAAATCAGGGCTGCCCAGTGATC
GCTTCTCTGCAGAGAGGACTGGGGGATCCGTCTCCACTCTGACGATCCAGCGCACACAGCA
GGAGGACTCGGCCGTGTATCTCTGTGCCAGCAGCTTAGTCCCCGACAGTGCCTACGAGCAGT
ACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCACCCGA
GGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGTA
TGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAAGG
AGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATGA
CTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCA
ACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGAT
AGGGCCAAACCTGTCACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCT
TCACCTCCGAGTCTTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTA
GGGAAGGCCACCTTGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAG
AAAGGATTCCAGAGGC**TAG** (SEQ ID NO:6)

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

TCR α chain
MAQTVTQSQPEMSVQEAETVTLSCTYDTSENNYYLFWYKQPPSRQMILVI 50
*RQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDTAMYF**CAFSRGSG*** **100**
***GSNYKLT**FGKGTLLTVNPN*IQNPDPAVYQLRDSKSSDKSVCLFTDFDSQT 150
NVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSII 200
PEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNL 250
LMTLRLWSS (SEQ ID NO:7)

TCR β chain
MGTRLLFWVAFCLLGADHTGAGVSQSPSNKVTEKGKDVELRCDPISGHTA 50
LYWYRQSLGQGLEFLIYFQGNSAPDKSGLPSDRFSAERTGGSVSTLTIQR 100
*TQQEDSAVYL**CASSLVPDSAYEQYF**GPGTRLTVTE*DLKNVFPPEVAVFEP 150
SEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQ 200
PALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKP 250
VTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSAL 300
VLMAMVKRKDSRG (SEQ ID NO:8)

"SB95" HLA-DRB1*0101-restricted NY-ESO-1_{95-106}-specific tumor-recognizing $CD4^+$ T cell clone

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

TCR alpha
ATGCTCCTGCTGCTCGTCCCAGTGCTCGAGGTGATTTTTACCCTGGGAGGAACCAGAGCCCA
GTCGGTGACCCAGCTTGGCAGCCACGTCTCTGTCTCTGAGGGAGCCCTGGTTCTGCTGAGGT
GCAACTACTCATCGTCTGTTCCACCATATCTCTTCTGGTATGTGCAATACCCCAACCAAGGA
CTCCAGCTTCTCCTGAAGCACACAACAGGGGCCACCCTGGTTAAAGGCATCAACGGTTTTGA
GGCTGAATTTAAGAAGAGTGAAACCTCCTTCCACCTGACGAAACCCTCAGCCCATATGAGC
GACGCGGCTGAGTACTTCTGTGCTGTGAGTGATTCTAGGGCTGCAGGCAACAAGCTAACTTT
TGGAGGAGGAACCAGGGTGCTAGTTAAACCAAATATCCAGAACCCTGACCCTGCCGTGTAC
CAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCA
AACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGAC
ATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTG
CATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAA
AGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTCA
AAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCA
TGACGCTGCGGCTGTGGTCCAGC**TGA** (SEQ ID NO:9)

TCR beta
ATGGGAATCAGGCTCCTCTGTCGTGTGGCCTTTTGTTTCCTGGCTGTAGGCCTCGTAGATGT
GAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGGAGAGAAAGTTTTTCTGGAA
TGTGTCCAGGATATGGACCATGAAAATATGTTCTGGTATCGACAAGACCCAGGTCTGGGGCT
ACGGCTGATCTATTTCTCATATGATGTTAAAATGAAAGAAAAAGGAGATATTCCTGAGGGG
TACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGTCCGCCAGCACCA
ACCAGACATCTATGTACCTCTGTGCCAGCAGATTCCCCGGGACAGCCTATAATTCACCCCTC
CACTTTGGGAATGGGACCAGGCTCACTGTGACAGAGGACCTGAACAAGGTGTTCCCACCCG
AGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACACTGGT
GTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAAG
GAGGTGCACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATG
ACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCAGAACCCCCGC
AACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGG
ATAGGGCCAAACCCGTCACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGG
CTTTACCTCGGTGTCCTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCT

AGGGAAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGA
GAAAGGATTTCTGA (SEQ ID NO:10)

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

TCR α chain
MLLLLVPVLEVIFTLGGTRAQSVTQLGSHVSVSEGALVLLRCNYSSSVPP 50
YLFWYVQYPNQGLQLLLKHTTGATLVKGINGFEAEFKKSETSFHLTKPSA 100
*HMSDAAEYF**CAVSDSRAAGNKLTF**GGGTRVLVKPN*IQNPDPAVYQLRDSK 150
SSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWS 200
NKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLS 250
VIGFRILLLKVAGFNLLMTLRLWSS (SEQ ID NO:11)

TCR β chain
MGIRLLCRVAFCFLAVGLVDVKVTQSSRYLVKRTGEKVFLECVQDMDHEN 50
MFWYRQDPGLGLRLIYFSYDVKMKEKGDIPEGYSVSREKKERFSLILESA 100
*STNQTSMYL**CASRFPGTAYNSPLH**FGNGTRLTVTE*DLNKVFPPEVAVFEP 150
SEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQ 200
PALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKP 250
VTQIVSAEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSAL 300
VLMAMVKRKDF (SEQ ID NO:12)

[0040] **Description of TCR expression vector.** Viral transduction was performed using a murine stem cell virus vector pMSCV-derived plasmid such as pMIG-II and pMIG-w (Fig. 6A). TCR-expressing constructs were inserted into multiple cloning site (MCS) of pMIG plasmid. pMIG plasmids have IRES-GFP after multiple cloning sites so that transduction efficacy is monitored by GFP expression.

[0041] To induce equimolar expression of TCR α and β chain proteins, cDNAs encoding TCR α and β chain were connected by a linker sequence including P2A translation skipping site (Fig. 6B (I)). Using this sequence, mRNA is transcribed as one sequence. Because of the ribosomal skipping by P2A sequence, two proteins were translated from the mRNA, to produce TCRβ-P2A fusion protein and P(Pro)-TCRα chain protein.

[0042] To avoid potential functional inhibition by P2A peptides added after the TCR β chain protein in TCR-expressing cassette (I), another TCR-expressing cassette that introduces the furin protease recognition site (RAKR) after TCR β chain gene was constructed (Fig 6B (II). In this expression cassette, additional peptide after the TCR β chain protein is removed by furin protease-mediated cleavage, resulting in expression of TCR α and β chain proteins with minimal modification. In particular, in expression cassettes with or without RAKR sequences, no amino acid is removed relative to the sequences presented herein. However, for a cassette without RAKR (Fig 6B(I)), GSG linker and P2A sequences are attached to the C-terminus of beta chain, and a Proline (from P2A) is attached to the N-terminus of alpha chain. For a cassette with

RAKR (Fig. 6B(II)), Arginine (from RAKR) is attached to the C-terminus of the beta chain and Proline (from P2A) is attached to the N-teminus of alpha chain. Thus, in embodiments, the expression vector encodes a fusion protein comprising TCR amino acid sequences. In embodiments, the only TCR amino acid sequence is selected from the TCR amino acid sequences presented herein.

[0043] The TCR-expressing sequences were cloned into multiple cloning site of pMIG plasmid. Retrovirus was produced transiently or stably using GP2-293 and PT67 packaging cell lines purchased from Clontech. Briefly, GP2-293 stably expresses viral gag-pol gene and they transiently produce after co-transfection with pMIG and pVSV-G VSV-G viral envelope-expressing plasmids. PT67 stably expresses viral gag-pol and 10A1 viral envelope genes. After infection with retrovirus produced from GP2-293, PT67 is integrated with the expression construct from pMIG, and therefore stably (continuously) produces retrovirus. In an embodiment, promoter activity of 5'-LTR (long terminal repeat) is used to drive transgene expression. However, other promoters such as EF-1α promoter can be introduced for enhancement of transgene expression.

[0044] **Infection of retrovirus to PBMC-derived T cells.** Whole PBMC were obtained by a density gradient separation method and stored in a liquid nitrogen tank in 90% fetal bovine serum (FBS) and 10% dimethyl sulfoxide (DMSO) until use. PBMC ($3\text{-}4 \times 10^6$ cells / well in a 24-well culture plate) were polyclonally activated by 10 μg/ml phytohemaglutinin (PHA) for 2 days in culture medium (RPMI1640 medium containing 10% FBS, L-Glutamine, Streptomycin, Penicillin and human recombinant IL-2). 1×10^5 preactivated PBMC in 100 μl culture medium were added to wells of a 96-well culture plate pre-coated with 20-25 μg/ml retronectin in PBS and blocked with 2% bovine serum albumin (BSA) in PBS. In some experiments, 5 μg/ml anti-CD3 monoclonal antibody (Clone:OKT3) was co-coated with retronectin. 100 μl supernatant containing retrovirus was added to PBMC and incubated for 24 hours. Retrovirus infection was performed 2-3 times every 24 hours. After infection, cells were expanded for 10-14 days and used for functional assays.

[0045] **Results**

[0046] High-titer retrovirus-producing PT67 clones were established. The following retrovirus-producing clones were established.

(1) pMIG-II/JM-TCR(II)

(2) pMIG-II/SB95-TCR(II)

(3) pMIG-w/JM-TCR(I)

(4) pMIG-w/SB95-TCR(I)

(5) pMIG-w/JM-TCR(II)

(6) pMIG-w/SB95-TCR(II)

[0047] In the enumerated list above, (I) and (II) refer to expression cassettes without and with the furin protease recognition site (RAKR), respectively, as shown in Fig. 6B. The transduction efficacy measured by GFP expression after a single infection to Jurkat cells was: 60% for (1); 55% for (2); 75% for (3); 75% for (4); 64% for (5); and 62% for (6).

[0048] Retrovirus vectors (1) and (2) were transduced to polyclonally activated PBMC. Transduction efficacy as measured by GFP expression was about 40-50%. The reactivity of retrovirally expressed TCR was tested against the same NY-ESO-1-derived cognate peptides (NY-ESO-1:91-110 for SB95-TCR and NY-ESO-1:157-170 for JM-TCR) that were recognized by the original TR-CD4 clones. Significantly more IFN-γ was produced against peptide-pulsed target cells than peptide-unpulsed target cells (Fig. 7), which demonstrates that the cloned TCR genes are functional to transfer the same antigen specificity of original TR-CD4 clones when they are transduced by viral vectors. Functional testing of the remaining TCR expression vectors can be performed in the same manner, such as by infecting activated human peripheral blood mononuclear cells with retrovirus carrying any TCR gene disclosed herein. TCR gene-transduced and untransduced cells can be cocultured for 24 hours with NY-ESO-1-expressing cell lines or tumor samples, and IFN-γ produced by the transduced cells determined using any suitable means, such as by ELISA. IFN-γ level in the supernatant by TCR gene-transduced cells will be higher when co-cultured with cells that express NY-ESO-1 or NY-ESO-1 peptide-pulsed cells, whereas cells cocultured with cells that do not express NY-ESO-1 will have significantly less IFN-γ production. Likewise, negative controls, such as untransduced cells, will have significantly less IFN-γ production. Thus, transfection with a representative recombinant TCR will result in the capability of the cells into which a polynucleotide encoding the TCR to have the same antigen-specificity which directly recognizes NY-ESO-1 antigen on cancer cells.

[0049] Although the invention has been described in detail for the purposes of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

We claim:

1. A modified human T cell comprising a recombinant polynucleotide encoding a T cell receptor (TCR), wherein the T cell is capable of direct recognition of a cancer cell expressing a NY-ESO-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 antigen expressed by the cancer cell.

2. The modified cell of T cell claim 1, wherein the recombinant polynucleotide encodes a TCR alpha chain having the sequence of SEQ ID NO:3 and a TCR beta chain having the sequence of SEQ ID NO:4, or a TCR alpha chain having the sequence of SEQ ID NO:7 and a TCR beta chain having the sequence of SEQ ID NO:8, or a TCR alpha chain having the sequence of SEQ ID NO:11 and a TCR beta chain having the sequence of SEQ ID NO:12.

3. The modified cell of claim 1, wherein the sequence encoding the alpha chain and/or the beta chain does not comprise introns.

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

5. The method of claim 4, wherein the modified human T cells are $CD4^+$ T cells.

6. The method of claim 4, wherein the recombinant polynucleotide encodes a TCR alpha chain having the sequence of SEQ ID NO:3 and a TCR beta chain having the sequence of SEQ ID NO:4, or a TCR alpha chain having the sequence of SEQ ID NO:7 and a TCR beta chain having the sequence of SEQ ID NO:8, or a TCR alpha chain having the sequence of SEQ ID NO:11 and a TCR beta chain having the sequence of SEQ ID NO:12.

7. The method of claim 5, wherein the cancer cells are selected from bladder cancer cells, brain cancer cells, breast cancer cells, gastric cancer cells, esophageal cancer cells, head 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.

8. The method of claim 5, comprising removing CD4+ T cells from the individual prior to the administering, and modifying the CD4+T cells by introducing into the CD4+ T cells the recombinant polynucleotide encoding the TCR.

9. An expression vector encoding a T cell receptor (TCR), wherein the TCR comprises an alpha chain having the sequence of SEQ ID NO:3 and a beta chain having the sequence of SEQ ID NO:4, or an alpha chain having the sequence of SEQ ID NO:7 and a beta chain having the sequence of SEQ ID NO:8, or an alpha chain having the sequence of SEQ ID NO:11 and a beta chain having the sequence of SEQ ID NO:12.

10. A method for making an expression vector encoding a recombinant T cell receptor (TCR), the method comprising obtaining a plurality of T cells from an individual, identifying T cells that are capable of direct recognition of a cancer cell expressing a NY-ESO-1 antigen in an HLA class II-restricted manner without antigen presenting cells presenting the NY-ESO-1 antigen to the T cells, determining the sequence of the alpha chain of the TCR and the sequence of the beta chain of the TCR, and introducing into an expression vector a polynucleotide sequence encoding the alpha chain of the TCR and the beta chain of the TCR.

11. The method of claim 10, further comprising introducing the expression vector into a cell such that the TCR is expressed.

Figure 1

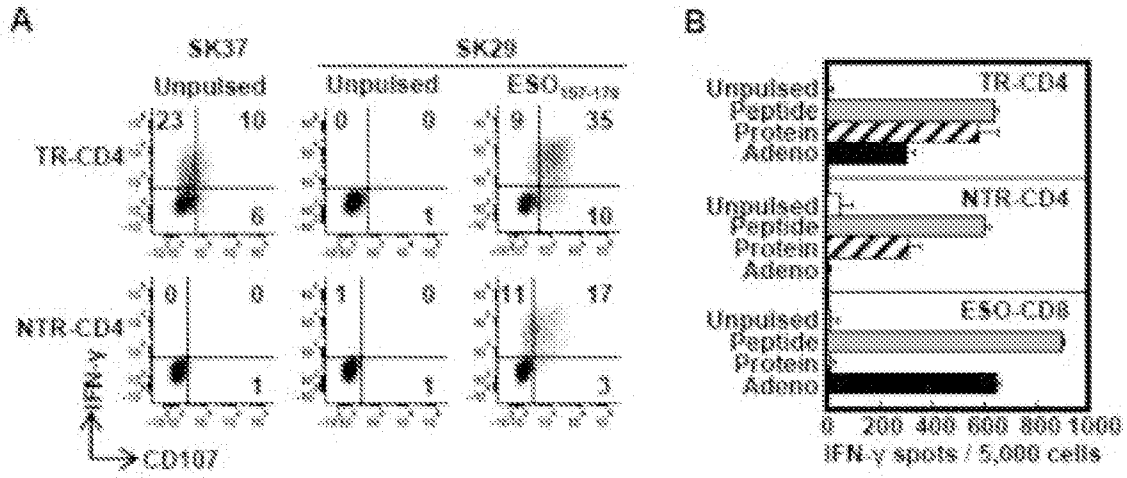


Figure 2

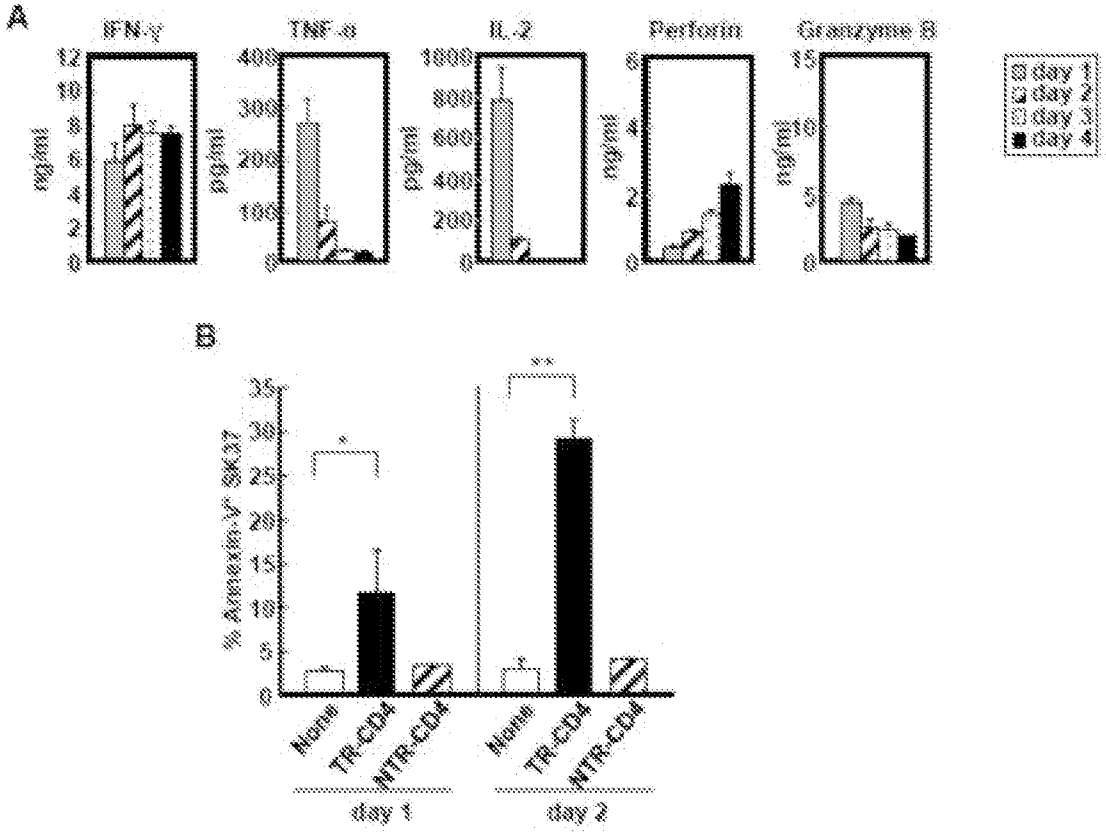


Figure 3

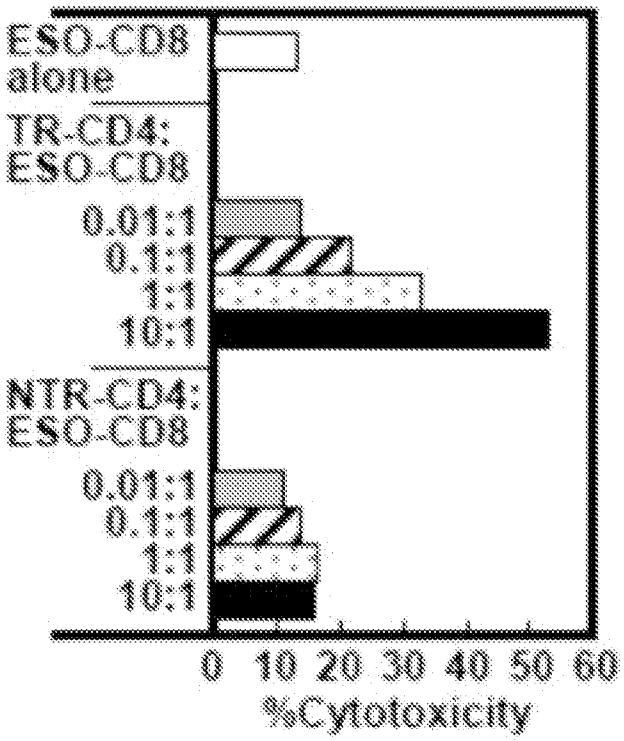

ESO-CD8 alone
TR-CD4: ESO-CD8
0.01:1
0.1:1
1:1
10:1
NTR-CD4: ESO-CD8
0.01:1
0.1:1
1:1
10:1
0 10 20 30 40 50 60
%Cytotoxicity

Figure 4

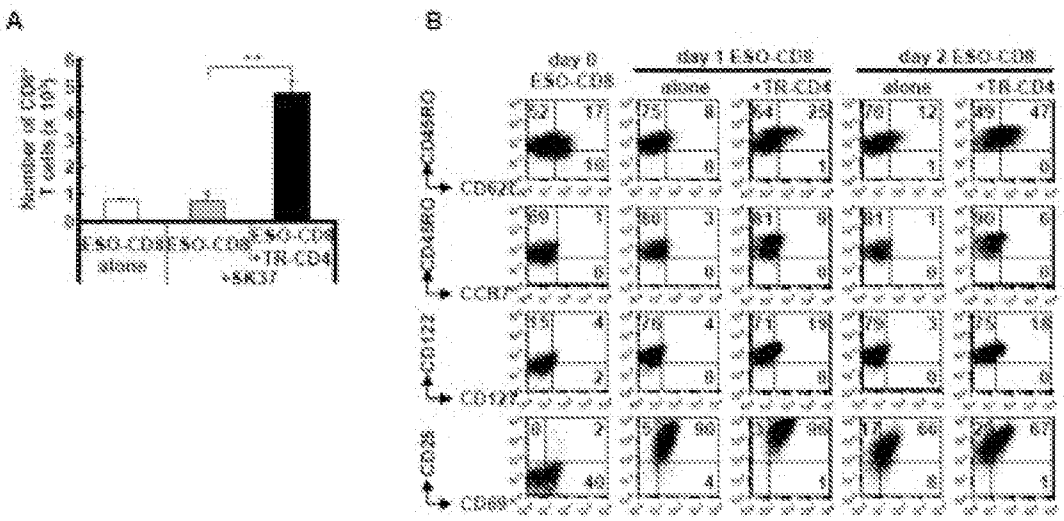


Figure 5

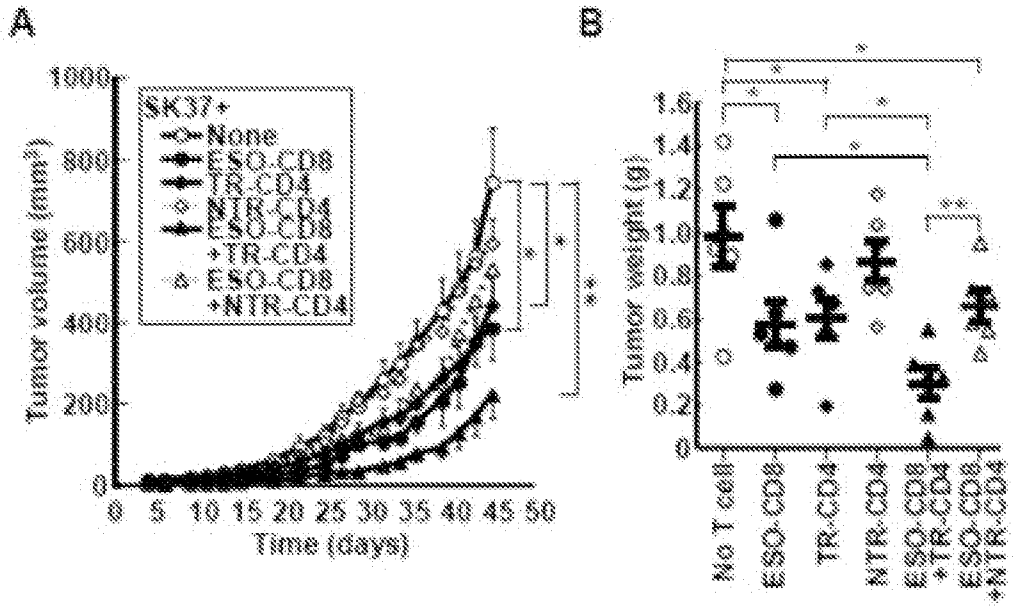


Figure 6

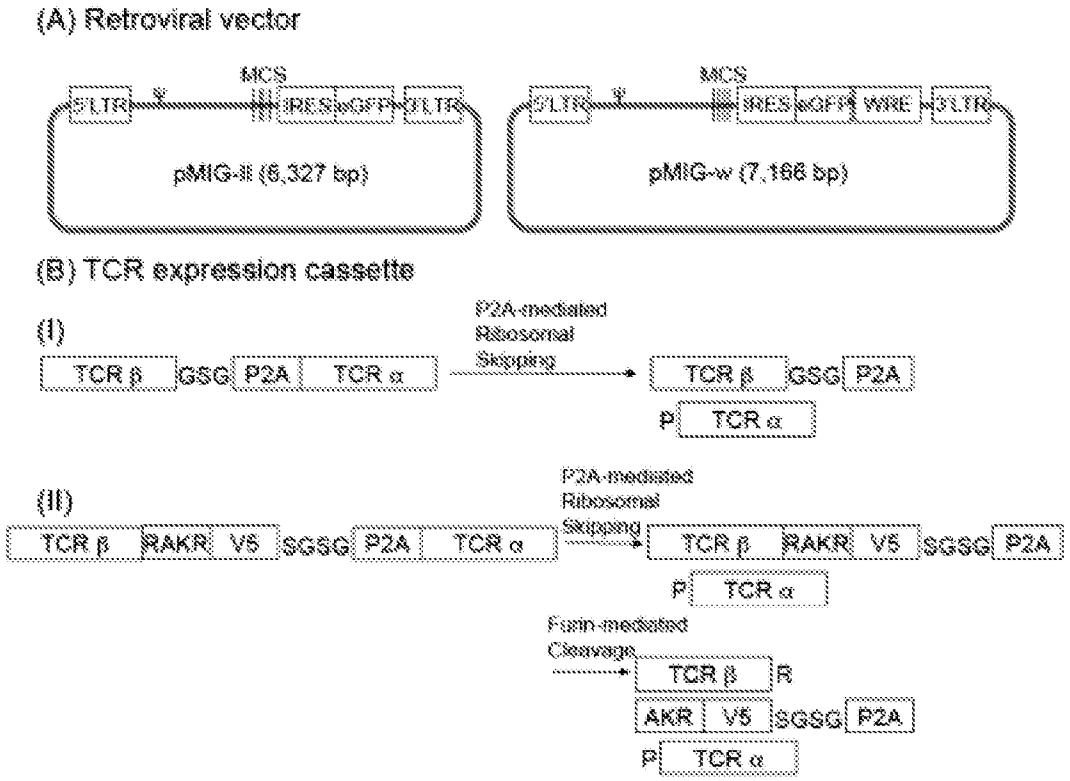
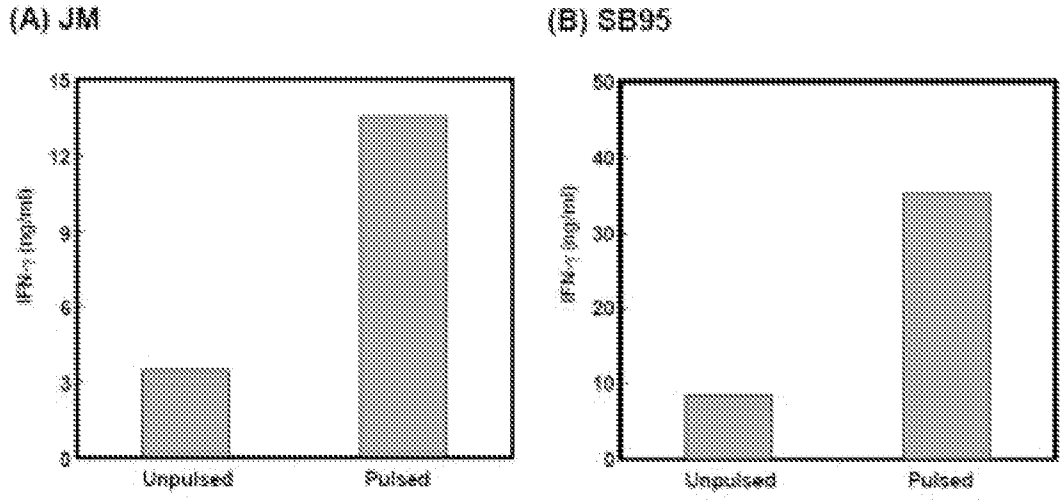



Figure 7