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DESCRIPTION

FIELD

[0001] 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

[0002] 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.

[0003] WO 2012/038055 discloses antigen-specific T cell receptors and T cell epitopes and their use in immunotherapy.

SUMMARY

[0004] The present invention provides modified human T cells and related subject matter, as defined by the appended claims.

[0005] More generally, 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).

[0006] 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.

[0007] 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.

[0008] 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.

[0009] 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.

[0010] 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

[0011]

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-γELISPOT assay.

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.

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.

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.

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.

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.

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

[0012] 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).

[0013] 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.

[0014] 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.

[0015] 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 disclosure 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 disclosure includes compositions and methods that are useful for creating and using TR-CD4 cells for improved care of cancer patients.

[0016] 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 disclosure because, as described above, the recombinant TCRs of the present disclosure are class II restricted. Moreover, and as also described above, unlike canonical class II restriction, cells engineered to express a recombinant TCR of the disclosure 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 disclosure recognize peptides by those disclosed in the '438 patent. Accordingly, the present disclosure 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.

[0017] In one embodiment, the disclosure includes transforming any $CD4^+$ T cell into a TR-CD4 by introducing a polynucleotide encoding a recombinant TCR of the disclosure into polyclonally expanded $CD4^+$ T cells and allowing expression of the TCR polypeptide coding region(s) of the polynucleotide.

[0018] In various embodiments, the present disclosure 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 disclosure 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.

[0019] The TCRs provided by the disclosure 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 disclosure 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.

[0020] The disclosure includes each and every polynucleotide sequence that encodes one or more TCR polypeptides of the disclosure and disclosed herein, including DNA and RNA sequences, and including isolated and/or recombinant polynucleotides comprising and/or consisting of such sequences. The disclosure 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 disclosure. 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 disclosure 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 disclosure.

[0021] 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.

[0022] With respect to the polypeptides that are encoded by the polynucleotides described above, in certain aspects the disclosure 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 disclosure includes a P2A amino acid sequence. In embodiments, a fusion protein of the disclosure 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.

[0023] 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.

[0024] Figure 6 provides representative configurations of TCR polypeptides of the disclosure 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 disclosure 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.

[0025] In connection with the present disclosure, 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 disclosure 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 disclosure.

[0026] With respect to use of the engineered $CD4^+$ T cells of the present disclosure, 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 disclosure 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.

[0027] 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.

[0028] We demonstrate herein 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).

[0029] 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.

[0030] The following description provides illustrative examples of materials and methods used to make and use various embodiments of the disclosure.

[0031] 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. 1. HLA-DRB1*01-restricted NY-ESO-1:96-106-specific TR-CD4 (referred to herein as Clone: "SB95")
2. 2. HLA-DPB1*04-restricted NY-ESO-1:157-170-specific TR-CD4 (referred to herein as Clone: "5B8")
3. 3. HLA-DPB1*04-restricted NY-ESO-1:157-170-specific TR-CD4 (referred to herein as Clone: "JM")

[0032] 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.

[0033] 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 disclosure. The disclosure 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 disclosure 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 disclosure 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 disclosure 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

ATGAAGTTGGTGACAAGCATTACTGTACTCCTATCTTTGGGTATTATGGGTGATGCTAAGAC
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GTACGTGATTCATGGTCTTACAAGCAATGTGAACAACAGAATGGCCTCTCTGGCAATCGCTG
AAGACAGAAAGTCCAGTACCTTGATCCTGCACCGTGCTACCTTGAGAGATGCTGCTGTGTAC
TACTGCATCCCTAATAACAATGACATGCGCTTTGGAGCAGGGACCAGACTGACAGTAAAAC
CAAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAA
GTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTG

ATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAG
TGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTA
TTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAA
AGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCT
CCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGC**TGA** (SEQ ID NO:1)

have no effect on the function of the TCR.

[0034] In specific and illustrative embodiments, the polynucleotide sequences encoding the TCRs of the disclosure, 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

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

   TCR α chain

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

   TCR β chain

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

   *AVYYC**IPNNNDMRF**GAGTRLTVKPN*IQNPDPAVYQLRDSKSSDKSVCLFT 150
   DFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANA 200
   FNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLK 250
   VAGFNLLMTLRLWSS (SEQ ID NO:3)

   TCR β chain

   MGSRLLCWVLLCLLGAGPVKAGVTQTPRYLIKTRGQQVTLSCSPISGHRS 50
   VSWYQQTPGQGLQFLFEYFSETQRNKGNFPGRFSGRQFSNSRSEMNVSTL 100
   *ELGDSALYLC**ASSFPREPNYGYTF**GSGTRLTVVE*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

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

TCR α chain

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

TCR β chain

ATGGGCACCAGGCTCCTCTTCTGGGTGGCCTTCTGTCTCCTGGGGGCAGATCACACAGGAGC
TGGAGTCTCCCAGTCCCCCAGTAACAAGGTCACAGAGAAGGGAAAGGATGTAGAGCTCAGG
TGTGATCCAATTTCAGGTCATACTGCCCTTTACTGGTACCGACAGAGCCTGGGGCAGGGCCT
GGAGTTTTTAATTTACTTCCAAGGCAACAGTGCACCAGACAAATCAGGGCTGCCCAGTGATC
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CTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCA
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TCACCTCCGAGTCTTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTA
GGGAAGGCCACCTTGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAG
AAAGGATTCCAGAGGC**TAG** (SEQ ID NO:6)

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

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
*TQQEDSAVYLC**ASSLVPDSAYEQYF**GPGTRLTVTE*DLKNVFPPEVAVFEP 150
SEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQ 200
PALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKP 250
VTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSAL 300
VLMAMVKRKDSRG (SEQ ID NO:8)

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

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

TCR alpha

ATGCTCCTGCTGCTCGTCCCAGTGCTCGAGGTGATTTTTACCCTGGGAGGAACCAGAGCCCA
GTCGGTGACCCAGCTTGGCAGCCACGTCTCTGTCTCTGAGGGAGCCCTGGTTCTGCTGAGGT
GCAACTACTCATCGTCTGTTCCACCATATCTCTTCTGGTATGTGCAATACCCCAACCAAGGA
CTCCAGCTTCTCCTGAAGCACACAACAGGGGCCACCCTGGTTAAAGGCATCAACGGTTTTGA
GGCTGAATTTAAGAAGAGTGAAACCTCCTTCCACCTGACGAAACCCTCAGCCCATATGAGC
GACGCGGCTGAGTACTTCTGTGCTGTGAGTGATTCTAGGGCTGCAGGCAACAAGCTAACTTT
TGGAGGAGGAACCAGGGTGCTAGTTAAACCAAATATCCAGAACCCTGACCCTGCCGTGTAC
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CATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAA
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AAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCA
TGACGCTGCGGCTGTGGTCCAGC**TGA** (SEQ ID NO:9)

TCR beta

ATGGGAATCAGGCTCCTCTGTCGTGTGGCCTTTTGTTTCCTGGCTGTAGGCCTCGTAGATGT
GAAAGTAACCCAGAGCTCGAGATATCTAGTCAAAAGGACGGGAGAGAAAGTTTTTCTGGAA
TGTGTCCAGGATATGGACCATGAAAATATGTTCTGGTATCGACAAGACCCAGGTCTGGGGCT
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TACAGTGTCTCTAGAGAGAAGAAGGAGCGCTTCTCCCTGATTCTGGAGTCCGCCAGCACCA
ACCAGACATCTATGTACCTCTGTGCCAGCAGATTCCCCGGGACAGCCTATAATTCACCCCTC
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AGGGAAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGA
GAAAGGATTTC**TGA** (SEQ ID NO:10)

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

TCR α chain

MLLLVPVLEVIFTLGGTRAQSVTQLGSHVSVSEGALVLLRCNYSSSVPP 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)

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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.

[0039] 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 (3-4 × 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.

Results

[0040] High-titer retrovirus-producing PT67 clones were established. The following retrovirus-producing clones were established.

1. (1) pMIG-II/JM-TCR(II)
2. (2) pMIG-II/SB95-TCR(II)
3. (3) pMIG-w/JM-TCR(I)
4. (4) pMIG-w/SB95-TCR(I)
5. (5) pMIG-w/JM-TCR(II)
6. (6) pMIG-w/SB95-TCR(II)

[0041] 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).

[0042] 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.

SEQUENCE LISTING

[0043]

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<120> COMPOSITIONS AND METHODS FOR USE OF RECOMBINANT T CELL RECEPTORS FOR DIRECT RECOGNITION OF TUMOR ANTIGEN

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Thr Arg Gly Gln Gln Val Thr Leu Ser Cys Ser Pro Ile Ser Gly His
        35                  40                  45

Arg Ser Val Ser Trp Tyr Gln Gln Thr Pro Gly Gln Gly Leu Gln Phe
    50                  55                  60

Leu Phe Glu Tyr Phe Ser Glu Thr Gln Arg Asn Lys Gly Asn Phe Pro
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Gly Arg Phe Ser Gly Arg Gln Phe Ser Asn Ser Arg Ser Glu Met Asn
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                85                  90                  95

Val Ser Thr Leu Glu Leu Gly Asp Ser Ala Leu Tyr Leu Cys Ala Ser
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Ser Phe Pro Arg Glu Pro Asn Tyr Gly Tyr Thr Phe Gly Ser Gly Thr
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Arg Leu Thr Val Val Glu Asp Leu Asn Lys Val Phe Pro Pro Glu Val
    130                 135                 140

Ala Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala
145                 150                 155                 160

Thr Leu Val Cys Leu Ala Thr Gly Phe Phe Pro Asp His Val Glu Leu
                165                 170                 175

Ser Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Asp
            180                 185                 190

Pro Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys
        195                 200                 205

Leu Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg
    210                 215                 220

Asn His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp
225                 230                 235                 240

Glu Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala
                245                 250                 255

Glu Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Val Ser Tyr Gln
            260                 265                 270

Gln Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys
        275                 280                 285

Ala Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met
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Val Lys Arg Lys Asp Phe
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acggagaatc gtttctctgt gaacttccag aaagcagcca aatccttcag tctcaagatc 240

tcagactcac agctggggga cactgcgatg tatttctgtg ctttctcgag agggagtgga 300

ggtagcaact ataaactgac atttggaaaa ggaactctct taaccgtgaa tccaaatatc 360

cagaaccctg accctgccgt gtaccagctg agagactcta aatccagtga caagtctgtc 420

tgcctattca ccgattttga ttctcaaaca aatgtgtcac aaagtaagga ttctgatgtg 480

tatatcacag acaaaactgt gctagacatg aggtctatgg acttcaagag caacagtgct 540

gtggcctgga gcaacaaatc tgactttgca tgtgcaaacg ccttcaacaa cagcattatt 600

ccagaagaca ccttcttccc cagcccagaa agttcctgtg atgtcaagct ggtcgagaaa 660

agctttgaaa cagatacgaa cctaaacttt caaaacctgt cagtgattgg gttccgaatc 720

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        35                  40                  45

Val Ile Arg Gln Glu Ala Tyr Lys Gln Gln Asn Ala Thr Glu Asn Arg
    50                  55                  60

Phe Ser Val Asn Phe Gln Lys Ala Ala Lys Ser Phe Ser Leu Lys Ile
65                  70                  75                  80

Ser Asp Ser Gln Leu Gly Asp Thr Ala Met Tyr Phe Cys Ala Phe Ser
                85                  90                  95

Arg Gly Ser Gly Gly Ser Asn Tyr Lys Leu Thr Phe Gly Lys Gly Thr
            100                 105                 110

Leu Leu Thr Val Asn Pro Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr
        115                 120                 125

Gln Leu Arg Asp Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr
    130                 135                 140

Asp Phe Asp Ser Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val
145                 150                 155                 160

Tyr Ile Thr Asp Lys Thr Val Leu Asp Met Arg Ser Met Asp Phe Lys
                165                 170                 175

Ser Asn Ser Ala Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala
            180                 185                 190

Asn Ala Phe Asn Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser
        195                 200                 205

Pro Glu Ser Ser Cys Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr
    210                 215                 220

Asp Thr Asn Leu Asn Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile
225                 230                 235                 240

Leu Leu Leu Lys Val Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu
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Trp Ser Ser
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            20                  25                  30

Glu Lys Gly Lys Asp Val Glu Leu Arg Cys Asp Pro Ile Ser Gly His
        35                  40                  45

Thr Ala Leu Tyr Trp Tyr Arg Gln Ser Leu Gly Gln Gly Leu Glu Phe
    50                  55                  60

Leu Ile Tyr Phe Gln Gly Asn Ser Ala Pro Asp Lys Ser Gly Leu Pro
65                  70                  75                  80

Ser Asp Arg Phe Ser Ala Glu Arg Thr Gly Gly Ser Val Ser Thr Leu
                85                  90                  95

Thr Ile Gln Arg Thr Gln Gln Glu Asp Ser Ala Val Tyr Leu Cys Ala
            100                 105                 110

Ser Ser Leu Val Pro Asp Ser Ala Tyr Glu Gln Tyr Phe Gly Pro Gly
        115                 120                 125

Thr Arg Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu
    130                 135                 140

Val Ala Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys
145                 150                 155                 160

Ala Thr Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu
                165                 170                 175

Leu Ser Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr
            180                 185                 190

Asp Pro Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr
        195                 200                 205

Cys Leu Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro
    210                 215                 220

Arg Asn His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn
225                 230                 235                 240

Asp Glu Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser
                245                 250                 255

Ala Glu Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr
            260                 265                 270

Gln Gln Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly
        275                 280                 285
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ggttttgagg ctgaatttaa gaagagtgaa acctccttcc acctgacgaa accctcagcc      300

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aacaaatctg actttgcatg tgcaaacgcc ttcaacaaca gcattattcc agaagacacc      660

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gatacgaacc taaactttca aaacctgtca gtgattgggt tccgaatcct cctcctgaaa      780

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gaatgtgtcc aggatatgga ccatgaaaat atgttctggt atcgacaaga cccaggtctg      180

gggctacggc tgatctattt ctcatatgat gttaaaatga aagaaaaagg agatattcct      240

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            20                  25                  30

Ser Glu Gly Ala Leu Val Leu Leu Arg Cys Asn Tyr Ser Ser Ser Val
        35                  40                  45

Pro Pro Tyr Leu Phe Trp Tyr Val Gln Tyr Pro Asn Gln Gly Leu Gln
    50                  55                  60

Leu Leu Leu Lys His Thr Thr Gly Ala Thr Leu Val Lys Gly Ile Asn
65                  70                  75                  80

Gly Phe Glu Ala Glu Phe Lys Lys Ser Glu Thr Ser Phe His Leu Thr
                85                  90                  95

Lys Pro Ser Ala His Met Ser Asp Ala Ala Glu Tyr Phe Cys Ala Val
            100                 105                 110

Ser Asp Ser Arg Ala Ala Gly Asn Lys Leu Thr Phe Gly Gly Gly Thr
        115                 120                 125

Arg Val Leu Val Lys Pro Asn Ile Gln Asn Pro Asp Pro Ala Val Tyr
    130                 135                 140

Gln Leu Arg Asp Ser Lys Ser Ser Asp Lys Ser Val Cys Leu Phe Thr
145                 150                 155                 160

Asp Phe Asp Ser Gln Thr Asn Val Ser Gln Ser Lys Asp Ser Asp Val
                165                 170                 175

Tyr Ile Thr Asp Lys Thr Val Leu Asp Met Arg Ser Met Asp Phe Lys
            180                 185                 190

Ser Asn Ser Ala Val Ala Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala
        195                 200                 205
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Asn Ala Phe Asn Asn Ser Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser
    210                 215                 220

Pro Glu Ser Ser Cys Asp Val Lys Leu Val Glu Lys Ser Phe Glu Thr
225                 230                 235                 240

Asp Thr Asn Leu Asn Phe Gln Asn Leu Ser Val Ile Gly Phe Arg Ile
                245                 250                 255

Leu Leu Leu Lys Val Ala Gly Phe Asn Leu Leu Met Thr Leu Arg Leu
            260                 265                 270

    Trp Ser Ser
            275
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<400> 12

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            20                  25                  30

Arg Thr Gly Glu Lys Val Phe Leu Glu Cys Val Gln Asp Met Asp His
        35                  40                  45

Glu Asn Met Phe Trp Tyr Arg Gln Asp Pro Gly Leu Gly Leu Arg Leu
    50                  55                  60

Ile Tyr Phe Ser Tyr Asp Val Lys Met Lys Glu Lys Gly Asp Ile Pro
65                  70                  75                  80

Glu Gly Tyr Ser Val Ser Arg Glu Lys Lys Glu Arg Phe Ser Leu Ile
                85                  90                  95

Leu Glu Ser Ala Ser Thr Asn Gln Thr Ser Met Tyr Leu Cys Ala Ser
            100                 105                 110

Arg Phe Pro Gly Thr Ala Tyr Asn Ser Pro Leu His Phe Gly Asn Gly
        115                 120                 125

Thr Arg Leu Thr Val Thr Glu Asp Leu Asn Lys Val Phe Pro Pro Glu
    130                 135                 140

Val Ala Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys
145                 150                 155                 160

Ala Thr Leu Val Cys Leu Ala Thr Gly Phe Phe Pro Asp His Val Glu
                165                 170                 175
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Leu Ser Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr
            180                 185                 190

Asp Pro Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr
        195                 200                 205

Cys Leu Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro
    210                 215                 220

Arg Asn His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn
225                 230                 235                 240

Asp Glu Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser
                245                 250                 255

Ala Glu Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Val Ser Tyr
            260                 265                 270

Gln Gln Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly
        275                 280                 285

Lys Ala Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala
    290                 295                 300

Met Val Lys Arg Lys Asp Phe
305                 310
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REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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- WO61778673A [0043]

Non-patent literature cited in the description

- Gene Transfer: Delivery and Expression of DNA and RNA, A Laboratory Manual**FRIEDMAN et al.**International Standard Book20070000 [0022]
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- **OKAMOTO S et al.**Molecular Therapy-Nucleic Acids, 2012, vol. 1, e63- [0023]
- **GOFF SL et al.**Cancer ImmunologyImmunotherapy, 2010, vol. 59, 1551-1560 [0033]

Patentkrav

1. Modificeret human T-celle omfattende et rekombinant polynukleotid, der koder for en T-celle-receptor (TCR), hvor T-cellen er i stand til direkte at genkende en cancercelle, der eksprimerer et NY-ESO-1-antigen, hvor den direkte genkendelse af cancercellen omfatter humant leukocytantigen (HLA) klasse II-begrænset binding af TCR'en til NY-ESO-1-antigenet, der eksprimeres af cancercellen; og hvor det rekombinante polynukleotid koder for en TCR-alpha-kæde med sekvensen SEQ ID NO:3 og en TCR-beta-kæde med sekvensen SEQ ID NO:4, eller en TCR-alpha-kæde med sekvensen SEQ ID NO:11 og en TCR-beta-kæde med sekvensen SEQ ID NO:12.

2. Modificeret celle ifølge krav 1, hvor sekvensen, der koder for alpha-kæden og/eller beta-kæden, ikke omfatter introner.

3. Modificerede humane T-celler til anvendelse i en fremgangsmåde til forebyggelse og/eller behandling af et individ, der er diagnosticeret med, formodes at have eller har risiko for at udvikle eller få tilbagefald af en cancer, hvor canceren omfatter cancerceller, der eksprimerer NY-ESO-1-antigen, hvor de modificerede humane T-celler omfatter et rekombinant polynukleotid, der koder for en T-celle-receptor (TCR), hvor T-cellerne er i stand til direkte at genkende cancercellerne, der eksprimerer NY-ESO-1-antigenet, hvor den direkte genkendelse af cancercellerne omfatter humant leukocytantigen (HLA) klasse II-begrænset binding af TCR'en til NY-ESO-1-antigenet, der eksprimeres af cancercellerne; og hvor det rekombinante polynukleotid koder for en TCR-alpha-kæde med sekvensen SEQ ID NO:3 og en TCR-beta-kæde med sekvensen SEQ ID NO:4, eller en TCR-alpha-kæde med sekvensen SEQ ID NO:11 og en TCR-beta-kæde med sekvensen SEQ ID NO:12.

4. Modificerede humane T-celler til anvendelse ifølge krav 3, hvor de modificerede humane T-celler er $CD4^+$ T-celler.

5. Modificerede humane T-celler til anvendelse ifølge krav 4, hvor cancercellerne er udvalgt blandt blærecancer-celler, hjernecancer-celler, brystcancer-celler, mavecancer-celler, øsofaguscancer-celler, hoved- og halscancer-celler, hepatobiliær cancer-celler, nyrecancer-celler, ovariecancer-celler, ikke-

småcellet lungecancer-celler, myelom, prostatacancer-celler, sarkomceller, testikelcancer-celler, melanomceller eller kombinationer deraf.

6. Modificerede humane T-celler til anvendelse ifølge krav 4, hvor fremgangsmåden omfatter fjernelse af CD4+ T-celler fra individet før indgivelse af de modificerede humane T-celler og modificering af CD4+ T-cellerne ved i CD4+ T-cellerne at indføre det rekombinante polynukleotid, der koder for TCR'en.

7. Ekspressionsvektor, der koder for en T-celle-receptor (TCR), hvor TCR'en omfatter en alpha-kæde med sekvensen SEQ ID NO:3 og en beta-kæde med sekvensen SEQ ID NO:4, eller en alpha-kæde med sekvensen SEQ ID NO:11 og en beta-kæde med sekvensen SEQ ID NO:12.

DRAWINGS

Figure 1

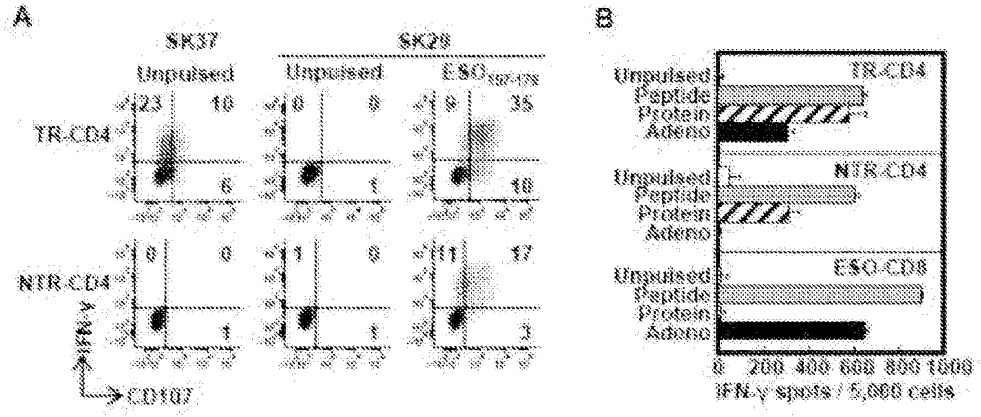
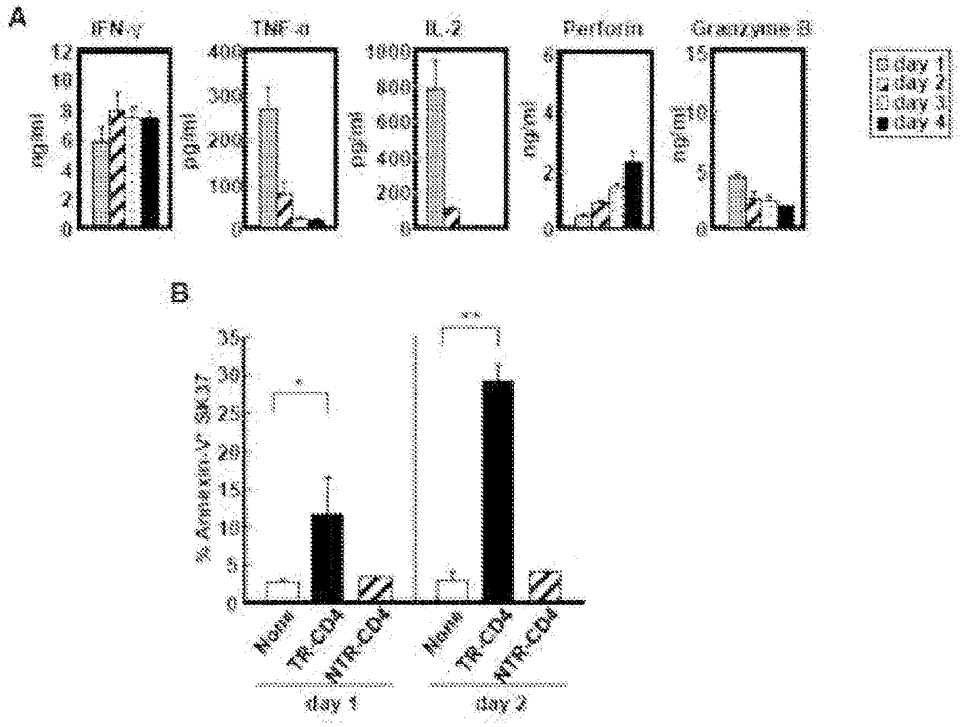


Figure 2



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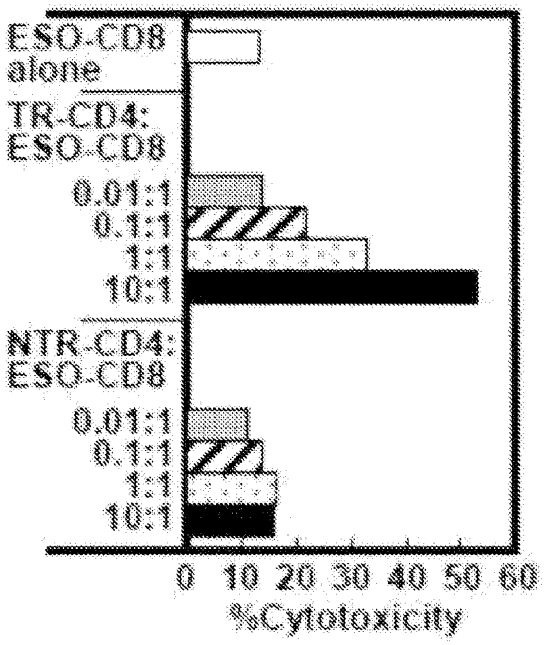
ESO-CD8 alone
TR-CD4: ESO-CD8
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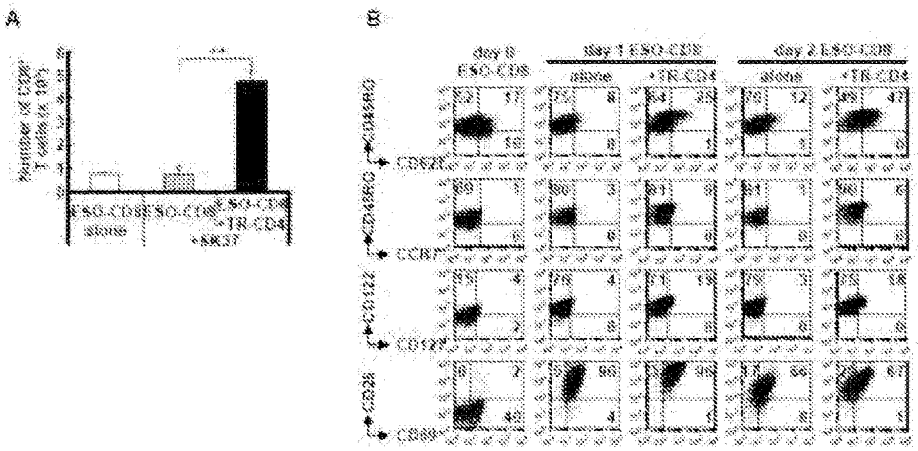
0.1:1
1:1
10:1
NTR-CD4: ESO-CD8
0.01:1
0.1:1
1:1
10:1
0 10 20 30 40 50 60
%Cytotoxicity

Figure 4



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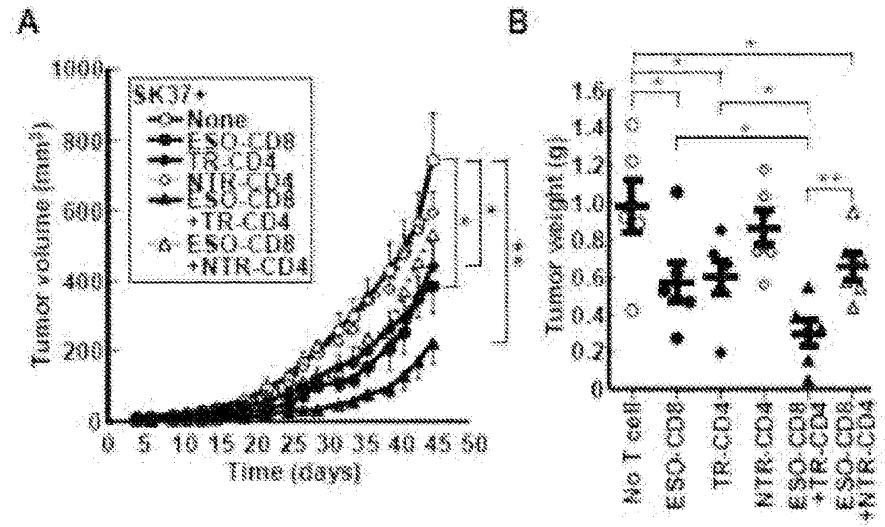

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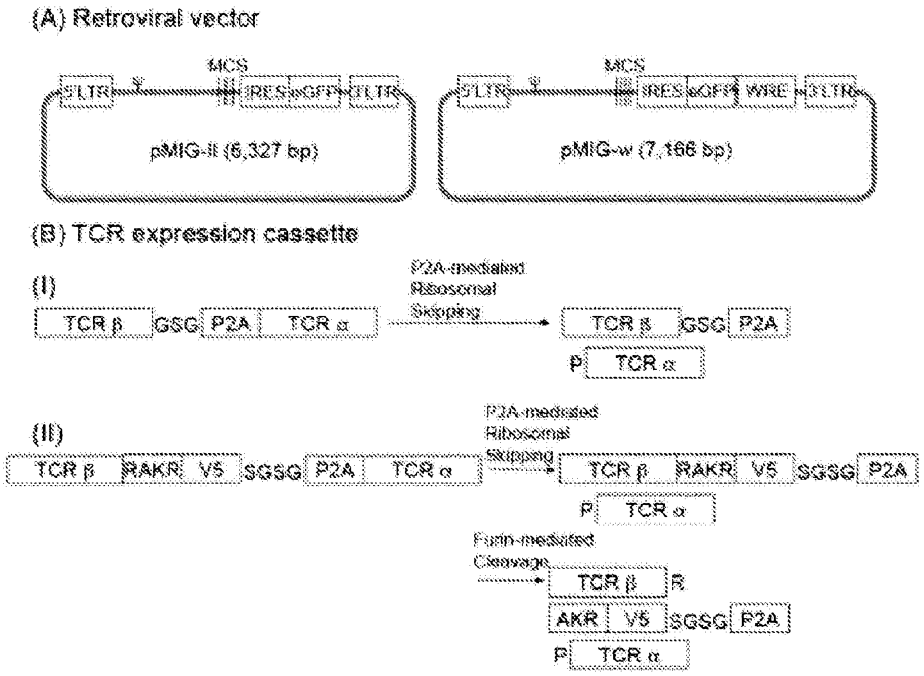

Figure 7