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(71) Applicants: **THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; National Institute of Health, Office of Technology Transfer, 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, MD 20852-7660 (US). **LOYOLA UNIVERSITY CHICAGO** [US/US]; 2160 South First Avenue, Maywood, IL 60153 (US).

(72) Inventors *(for US only)*: **CHILDS, Richard W.**; NHLBI/NIH, 10 Center Drive, Room 3E-3532, Bethesda, MD 20892 (US). **NISHIMURA, Michael I.**; Loyola University Chicago, 2160 South First Avenue. CBCC Rm 301, Maywood, IL 60153 (US).

(72) Inventor: **CHERKASOVA, Elena A.**; NHLBI/NIH, 10 Center Drive, Room 5E-5264, Bethesda, MD 20892 (US).

(74) Agent: **GRAF, Susan W.**; Klarquist Sparkman LLP, One World Trade Center, Suite 1600, 121 SW Salmon Street, Portland, OR 97204 (US).

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(54) Title: HERV-E REACTIVE T CELL RECEPTORS AND METHODS OF USE

(57) Abstract: Disclosed herein are T cell receptors (TCRs) capable of binding an antigen expressed by renal cell carcinoma cells. In some examples, the TCRs include an a chain (such as SEQ ID NO: 2) and a β chain (such as SEQ ID NO: 3). Also disclosed herein are vectors including nucleic acids encoding the disclosed TCR a and/or β chains. Further disclosed are modified T cells expressing the TCRs. In some examples, the modified T cells are prepared by transducing T cells with a vector including nucleic acids encoding the TCR a chain and the TCR β chain. In some embodiments, methods include treating a subject with RCC, by obtaining a population of T cells, transducing the population of T cells with a vector including a nucleic acids encoding the TCR a chain and the TCR β chain, and administering a composition comprising the modified T cells to the subject.

HERV-E REACTIVE T CELL RECEPTORS AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/357,265, filed June 30, 2016, which is herein incorporated by reference in its entirety.

FIELD

This disclosure relates to cancer immunotherapy, particularly T cells expressing a renal cell carcinoma-reactive T cell receptor, and methods of making and using the T cells.

BACKGROUND

Renal cell carcinoma (RCC) is responsible for approximately 12,000 deaths every year in the United States alone. As with most cancer, when detected at early stages, surgical intervention is highly effective. Despite progress in treating RCC with targeted inhibitors and inhibitors of immune checkpoints (such as anti-CTLA-4 and anti-PD-1 monoclonal antibodies), metastatic RCC is generally lethal, with mean survival being less than a year. Thus, there remains a need for more effective therapies for RCC.

SUMMARY

Disclosed herein are T cell receptors (TCRs) recognizing an antigen expressed on RCC cells. T cells can be transduced with a nucleic acid encoding the TCR (*e.g.*, TCR α and β chains) and administered to a subject with RCC in order to treat or inhibit RCC in the subject.

Disclosed herein are TCRs that are capable of binding a human endogenous retrovirus-E (HERV-E) antigen expressed by RCC cells (*e.g.*, a peptide having the sequence ATWLGSKTWK; SEQ ID NO: 1). In some examples, the TCRs are HLA-A11 restricted TCRs expressed by clear cell renal cell carcinoma (ccRCC) cells. In some examples, the TCRs include an α chain (such as an α chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 4) and a β chain (such as a β chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 5). In some examples, the TCR α chain is encoded by a nucleic acid having at least 90% sequence identity to SEQ ID NO: 2 and the TCR β chain is encoded by a nucleic acid having at least 90% sequence identity to SEQ ID NO: 3.

Also disclosed herein are vectors (such as viral vectors) including nucleic acids encoding the disclosed TCR α and/or β chains, for example, operably linked to an expression control

sequence (such as a promoter). In some examples, the vector also includes a nucleic acid encoding a truncated CD34 protein, such as a CD34 protein including the extracellular and transmembrane domains, but lacking the intracellular domain. In one non-limiting example, the vector is a retroviral vector (such as a SAMEN vector) including nucleic acids encoding the TCR α chain (such as SEQ ID NO: 2), the TCR β chain (such as SEQ ID NO: 3), and the truncated CD34.

Further disclosed are modified T cells expressing the TCRs capable of binding the HERV-E antigen expressed by RCC cells (such as ccRCC cells), such as nucleic acids encoding the TCR α chain (for example, SEQ ID NO: 2) and the TCR β chain (for example, SEQ ID NO: 3). In some examples, the modified T cells are prepared by transducing T cells (such as T cells obtained from a subject with RCC or a donor) with a vector including nucleic acids encoding the TCR α chain and the TCR β chain, and optionally the truncated CD34 protein.

In some embodiments, methods include treating a subject with RCC (for example, ccRCC or metastatic ccRCC), by obtaining a population of T cells from the subject or a donor, transducing the population of T cells with vector including a nucleic acids encoding the TCR α chain (such as SEQ ID NO: 2) and the TCR β chain (such as SEQ ID NO: 3), producing a population of modified T cells, and administering a composition including the modified T cells to the subject. In some examples, the population of T cells is activated *in vitro* prior to transduction with the nucleic acid molecule. In other examples, the population of modified T cells is expanded and/or enriched prior to administering to the subject.

The foregoing and other features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram of an exemplary retroviral vector for expression of the TCR α and β chains described herein. CMV, human cytomegalovirus promoter/enhancer; ψ, packaging signal; SD, splice donor; SA, splice acceptor; TCRα, HERV-E antigen specific TCR α chain (*e.g.*, SEQ ID NO: 2); P2A, self-cleaving 2A peptide derived from porcine teschovirus; TCRβ, HERV-E antigen specific TCR β chain (*e.g.*, SEQ ID NO: 3); T2A, self-cleaving 2A peptide of *Thosea asigna* virus; CD34t, truncated CD34 with extracellular and transmembrane regions of the protein; LTR, 3' LTR.

FIG. 2 is a schematic diagram of an exemplary protocol for collecting and producing modified T cells for treating a subject with RCC

FIGS. 3A and 3B are graphs showing reactivity of T cells transduced with a retroviral vector encoding the HLA-A11 restricted TCR against ccRCC cells from two donors (FIG. 3A) and from one donor (FIG. 3B).

FIGS. 4A-4C are a series of plots showing CD34 expression in transduced T cells pre- and post-CD34 selection step (FIG. 4A) and CD3 (FIG. 4B) and HERV-E tetramer (FIG. 4C) expression in CD34-selected transduced T cells.

FIGS. 5A and 5B are plots showing CD8 (FIG. 5A) and CD4 (FIG. 5B) cells in $CD34^+$-HERV-E $tetramer^+$ transduced T cells.

FIGS. 6A and 6B are graphs showing chromium cytotoxicity of T cells transduced with a retroviral vector encoding the HLA-A11 restricted TCR against ccRCC cells from two donors. The T cell population from donor 1 was 39.9% $CD8^+$ (FIG. 6A) and the T cell population from donor 2 was 52.8% $CD8^+$ (FIG. 6B).

FIG. 7 is a graph showing chromium release cytotoxicity of T cells transduced with a retroviral vector encoding the HLA-A11 restricted TCR against RCC or LCL cells from two different donors and against T cells and activated T cells from a HLA-A11 negative donor.

FIG. 8 is a graph showing interferon-γ (IFNγ) secretion using CD8+CD34+ T cells from a healthy donor transduced with a retroviral vector encoding the HLA-A11 restricted TCR contacted with various cell lines. The HERV-E/HLA-A11 status of each cell line is as follows: SAUJ: HERV-E +/ HLA-A11+; LYO WT: HERV-E +/ HLA-A11neg; SNY A11+: HERV-E neg/ HLA-A11-transduced; URB A11+: HERV-E neg/ HLA-A11-transduced; WHI A11+: HERV-E neg/ HLA-A11-transduced; ORT A11+: HERV-E neg/ HLA-A11-transduced; ORT WT: HERV-E neg/ HLA-A11neg; SEA WT: HERV-E neg/ HLA-A11neg. A standard curve is shown in the inset.

FIG. 9 is a schematic diagram showing an exemplary phase I clinical trial for determining safety and tolerability of HERV-E TCR transduced autologous T cells in HLA-A11 positive patients with advanced ccRCC.

FIG. 10 is a schematic diagram showing an exemplary protocol for treating patients with metastatic ccRCC with HERV-E TCR transduced T cells.

SEQUENCE LISTING

Any nucleic acid and amino acid sequences listed herein or in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases and amino acids, as defined in 37 C.F.R. § 1.822. In at least some cases, only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

SEQ ID NO: 1 is the amino acid sequence of an HLA-A11 RCC-specific HERV-E antigenic peptide.

SEQ ID NO: 2 is the nucleic acid sequence of an exemplary RCC HERV-reactive TCR alpha chain.

SEQ ID NO: 3 is the nucleic acid sequence of an exemplary RCC HERV-reactive beta chain.

SEQ ID NO: 4 is the amino acid sequence of an exemplary RCC HERV-reactive TCR alpha chain.

SEQ ID NO: 5 is the amino acid sequence of an exemplary RCC HERV-reactive beta chain.

SEQ ID NO: 6 is the nucleic acid sequence of an exemplary SAMEN vector for expression of the RCC HERV-reactive TCR and truncated CD34.

SEQ ID NO: 7 is the amino acid sequence of an exemplary truncated CD34 (CD34t) protein.

DETAILED DESCRIPTION

An allogeneic T cell clone was previously isolated from a RCC patient who showed prolonged tumor regression after an allogeneic transplant (Takahashi *et al., J. Clin. Invest.* 118:1099-1109, 2008). This HLA-A11 restricted CD8+ T cell clone was highly cytotoxic to ccRCC cell lines that were HLA-A11 positive, but did not kill non-malignant cells (Takahashi *et al.*, 2008). Using cDNA expression cloning, the antigen recognized by this clone, which is encoded by an endogenous retrovirus type E (HERV-E), was identified (Takahashi *et al.*, 2008). This antigen was expressed in ccRCC but not observed in normal tissues or other tumor types and is expressed by about 80% of ccRCC tumors.

The present inventors have identified the T cell receptor expressed by the T cell clone isolated from the RCC patient. As described herein, this TCR can be used for gene transfer immunotherapy for treating RCC patients. T cells are transduced with genes encoding the TCR α and β chains and are administered to a subject with RCC to redirect specificity of normal T cells from the subject to the RCC cells.

I. Abbreviations

ccRCC	clear cell renal cell carcinoma
CD34t	truncated CD34
CTL	cytotoxic T lymphocyte
HERV	human endogenous retrovirus

HLA	human leukocyte antigen
LTR:	long terminal repeat
MMLV	Moloney murine leukemia virus
PBMC	peripheral blood mononuclear cells
RCC	renal cell carcinoma
TCR	T cell receptor

II. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in *Lewin's Genes X*, ed. Krebs *et al.*, Jones and Bartlett Publishers, 2009 (ISBN 0763766321); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Publishers, 1994 (ISBN 0632021829); Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and George P. Rédei, E*ncyclopedic Dictionary of Genetics, Genomics, Proteomics and Informatics,* 3rd Edition, Springer, 2008 (ISBN: 1402067534); and other similar references.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Hence "comprising A or B" means including A, or B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. Sequences associated with GenBank Accession Numbers are herein incorporated by reference as present in GenBank on June 30, 2016, unless otherwise noted. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

Antigen: A compound, composition, or substance that can stimulate the production of antibodies or a T cell response in a subject. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. The term "antigen" includes all related antigenic epitopes. "Epitope" or "antigenic determinant" refers to a site on an antigen to which B and/or T cells respond. In one embodiment, T cells respond to the epitope, when the epitope is presented in conjunction with an MHC molecule. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5, about 9, about 7-11, or about 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

An antigen can be a tissue-specific antigen, or a disease-specific antigen. These terms are not exclusive, as a tissue-specific antigen can also be a disease-specific antigen. A tissue-specific antigen is expressed in a limited number of tissues, such as a single tissue. A disease-specific antigen is expressed coincidentally with a disease process. A specific non-limiting examples of a disease-specific antigen is an antigen whose expression correlates with, or is predictive of, tumor formation, for example, RCC.

Autologous: Refers to tissues, cells or nucleic acids taken from an individual's own tissues. For example, in an autologous transfer or transplantation of T cells, the donor and recipient are the same person. Autologous (or "autogeneic" or "autogenous") is related to self, or originating within an organism itself.

CD34: A cell surface glycoprotein that functions as a cell-cell adhesion molecule. CD34 is a single-pass transmembrane protein with a highly glycosylated extracellular domain, a transmembrane domain, and an intracellular signaling domain. CD34 is expressed on hematopoietic cells and plays a role in cell migration. Exemplary human CD34 sequences include GenBank Accession Nos. NM_001025109 and NM_001773 (nucleic acid sequences) and NP_001020280 and NP_001764 (amino acid sequences), all of which are incorporated herein by reference as present in GenBank on June 30, 2016.

HLA-A11: An human leukocyte antigen (HLA) serotype within the HLA A group. HLA-A11 is an MHC class I molecule that includes an α chain encoded by HLA-A*11 allele group and a β chain encoded by β2-microglobulin. MHC class I molecules such as HLA-A11 bind peptides

(antigens) that are typically 7-11 amino acids long and are involved in presenting the antigen to T cells via binding to a TCR.

Human endogenous retrovirus E (HERV-E): HERVs are remnants of ancient exogenous retroviruses integrated into the human genome. HERVs are estimated to comprise 5-8% of the human genome. Most HERVs have accumulated mutations or are transcriptionally silenced and do not produce full-length proteins. However, some HERVs are transcriptionally active in contexts such as tumors. HERV-E is a HERV subtype located on human chromosome 6q. At least three transcripts from HERV-E (*e.g.*, GenBank Accession Nos. EU137846, EU137847, and JQ733905) have been identified and are expressed in RCC cells, but not in other tumors or non-tumor cells (Takahashi *et al., J. Clin. Oncol.* 118:1099-1109, 2008).

Operably linked: A first nucleic acid is operably linked with a second nucleic acid when the first nucleic acid is placed in a functional relationship with the second nucleic acid. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked nucleic acids are contiguous and, where necessary to join two protein coding regions, the open reading frames are aligned.

Recombinant: A recombinant nucleic acid molecule is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or by the artificial manipulation of isolated segments of nucleic acid molecules, such as by genetic engineering techniques.

Similarly, a recombinant virus is a virus with a nucleic acid sequence that is non-naturally occurring (such as including a heterologous sequence that is not from the virus) or made by artificial combination of at least two sequences of different origin. The term "recombinant" also includes nucleic acids, proteins and viruses that have been altered solely by addition, substitution, or deletion of a portion of a natural nucleic acid molecule, protein or virus.

Renal cell carcinoma (RCC): A tumor originating in the cells of the kidney. RCC is the most common type of kidney cancer in adults. There are multiple histological subtypes of RCC, including clear cell renal cell carcinoma (ccRCC), which accounts for 60-70% of RCC and originates in the cells of the proximal tubule. ccRCC cells exhibit clear cytoplasm with acinar or sarcomatoid growth pattern. Additional subtypes include but are not limited to papillary RCC (also originating in cells of the proximal tubule), chromophobic RCC (originating in cells of the cortical collecting duct), oncolytic RCC (a benign neoplasm originating in cells of the cortical collecting duct), and collecting duct RCC (originating in cells of the medullary collecting duct).

T cell: A white blood cell (lymphocyte) that is an important mediator of the immune response. T cells include, but are not limited to, $CD4^+$ T cells and $CD8^+$ T cells. A $CD4^+$ T lymphocyte is an immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. $CD8^+$ T cells carry the "cluster of differentiation 8" (CD8) marker. In one embodiment, a $CD8^+$ T cell is a cytotoxic T lymphocyte (CTL). In another embodiment, a $CD8^+$ cell is a suppressor T cell.

Activated T cells can be detected by an increase in cell proliferation and/or expression of or secretion of one or more cytokines (such as IL-2, IL-4, IL-6, IFNγ, or TNFα). Activation of CD8+ T cells can also be detected by an increase in cytolytic activity in response to an antigen.

In some examples, a **"modified T cell"** is a T cell transduced with a heterologous nucleic acid (such as one or more of the nucleic acids or vectors disclosed herein) or expressing one or more heterologous proteins. The terms "modified T cell" and "transduced T cell" are used interchangeably in some examples herein.

T cell receptor (TCR): A heterodimeric protein on the surface of a T cell that binds an antigen (such as an antigen bound to an MHC molecule, for example, on an antigen presenting cell). TCRs include α and β chains, each of which is a transmembrane glycoprotein. Each chain has variable and constant regions with homology to immunoglobulin variable and constant domains, a hinge region, a transmembrane domain, and a cytoplasmic tail. Similar to immunoglobulins, TCR gene segments rearrange during development to produce complete variable domains.

T cells are activated by binding of an antigen to a TCR and co-stimulatory signals. For example, a $CD8^+$ T cell bears T cell receptors that recognize a specific epitope when presented by a particular HLA molecule on a cell. When a CTL precursor that has been stimulated by an antigen presenting cell to become a cytotoxic T lymphocyte contacts a cell that bears such an HLA-peptide complex, the CTL forms a conjugate with the cell and destroys it.

Transduce: Transferring nucleic acid into a cell, such as transfer of a heterologous nucleic acid into a host cell. As used herein, the term transduce (or transfect or transform) include all techniques by which a nucleic acid is introduced into a cell, including but not limited to transformation with plasmid vectors, infection with viral vectors, and introduction of naked DNA by electroporation, nucleofection, lipofection, or particle gun acceleration.

A "heterologous" nucleic acid or protein refers to a nucleic acid or protein originating from a different genetic source. For example, a nucleic acid or protein that is heterologous to a cell originates from an organism or individual other than the cell in which it is expressed. In other

examples, a heterologous nucleic acid or protein originates from a cell type other than the cell in which it is expressed.

Vector: A nucleic acid molecule allowing insertion of foreign nucleic acid without disrupting the ability of the vector to replicate and/or integrate in a host cell. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements. An expression vector is a vector that contains the necessary regulatory sequences to allow transcription and translation of an inserted gene or genes. In some non-limiting examples, the vector is a viral vector, such as a retroviral vector.

III. T Cell Receptors, Vectors, and Host Cells

Disclosed herein are T cell receptors (*e.g.* TCR α and β chains) cloned from a RCC HERV-E reactive T cell line. Also disclosed are vectors (such as expression vectors) including the disclosed TCRs and host cells including at least one heterologous nucleic acid encoding the disclosed TCR α and/or β chains.

A. TCRs

In some embodiments, the TCR recognizes a HERV-E peptide expressed on RCC cells, such as ATWLGSKTWK (SEQ ID NO: 1). The TCR includes α and β chain nucleic acids or polypeptides.

In some examples, the TCR α chain is encoded by a nucleic acid including or consisting of the nucleic acid sequence of SEQ ID NO: 2. In some examples, the TCR β chain is encoded by a nucleic acid including or consisting of the nucleic acid sequence of SEQ ID NO: 3. In some examples, the TCR α chain polypeptide includes or consists of the amino acid sequence of SEQ ID NO: 4. In some examples, the TCR β chain polypeptide includes or consists of the amino acid sequence of SEQ ID NO: 5.

In some embodiments, the TCR-encoding nucleic acids disclosed herein have a sequence at least 90% (for example at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, such as 100%) identical to the nucleic acid sequence of SEQ ID NO: 2 or SEQ ID NO: 3. In other embodiments, the TCR polypeptides disclosed herein have an amino acid sequence at least 95% (such as at least 96%, 97%, 98%, 99%, or 100%) identical to the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5. Exemplary sequences can be obtained using computer programs that are readily available on the internet and the nucleic acid and amino acid sequences set forth herein. In one example, the polypeptide retains at least one activity of the disclosed TCR polypeptides, such

as binding to an RCC-specific antigenic epitope (for example, SEQ ID NO: 1), for example when expressed by a T cell in the context of both TCR α and β chains.

Minor modifications of a TCR α and/or β chain encoding nucleic acid or primary amino acid sequence may result in polypeptides which have substantially equivalent activity as compared to the unmodified counterpart polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein. Thus, a specific, non-limiting example of a TCR α or β chain polypeptide is a conservative variant of the TCR α or β chain polypeptide (such as a single conservative amino acid substitution, for example, one or more conservative amino acid substitutions, for example 1-10 conservative substitutions, 2-5 conservative substitutions, 4-9 conservative substitutions, such as 1, 2, 5 or 10 conservative substitutions). A table of conservative substitutions is provided herein (Table 1). Substitutions of the amino acid sequences shown in SEQ ID NOs: 4 and 5 can be made based on this table. However, it is to be understood that non-conservative amino acid substitutions can also be made without significantly changing the activity of the polypeptide. One of ordinary skill in the art can select amino acids that can be substituted based on sequence alignments and other available sequence analysis tools.

Table 1. Exemplary conservative amino acid substitutions

Original Residue	**Conservative Substitution(s)**
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr

Original Residue	Conservative Substitution(s)
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

B. Vectors

Also disclosed herein are vectors including nucleic acids encoding HERV-E-reactive TCRs. The vectors include a nucleic acid encoding one or both of the α and β chains of the disclosed TCR (such as a nucleic acid at least 90% identical to SEQ ID NO: 2 and/or SEQ ID NO: 3) operably linked to one or more expression control elements. In particular embodiments the vector includes nucleic acids encoding both the TCR α chain (*e.g.*, a nucleic acid encoding SEQ ID NO: 4, such as SEQ ID NO: 2) and the TCR β chain (*e.g.*, a nucleic acid encoding SEQ ID NO: 5, such as SEQ ID NO: 3). However, in other examples, the TCR α and TCR β chains may be expressed from separate vectors. Expression control elements are sequences that control or regulate transcription and/or translation of a nucleic acid, such as promoters, enhancers, leader sequences, transcription terminators, start and/or stop codons, internal ribosome entry sites (IRES), splicing signals, and polyadenylation signals. The vector may also contain additional elements for the transfer and subsequent replication of the vector, such as origins of replication and selectable markers.

In some examples, the vector is a viral vector that includes a nucleic acid encoding at least one of the disclosed TCR α and β chains (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 3). In specific embodiments, the vector is a retroviral vector. Additional viral vectors suitable for gene delivery to T cells include lentivirus, adenovirus, adeno-associated virus, vaccinia virus, alphavirus, herpesvirus, and fowlpox virus vectors. In other examples, the vector is a plasmid or baculovirus vector. One of ordinary skill in the art can select an appropriate vector, for example to stably or transiently transduce T cells with the TCRs described herein.

In some embodiments, the vector is a retroviral vector including nucleic acids encoding one or both of the TCR α and β chain polypeptides disclosed herein. In particular examples, the vector is a modified retroviral vector, from which the virally encoded proteins have been deleted (for example, to prevent production of replication competent virus, reduce unwanted immunogenicity, and/or to accommodate insertion of gene(s) of interest). Exemplary retroviral backbones include those based on Moloney murine leukemia virus (MMLV), such as LXSN and SAMEN vectors (Clay *et al.*, *Pathol. Oncol. Res.* 5:3-15, 1999). Thus, in one example, a vector is a SAMEN

retrovirus vector including a nucleic acid encoding a TCR α chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 4 (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 2). In another example, a vector is a SAMEN retrovirus vector including a nucleic acid encoding a TCR β chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 5 (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 3). In yet another example, a vector is a SAMEN retrovirus vector including a nucleic acid encoding a TCR α chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 4 (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 2) and a nucleic acid encoding a TCR β chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 5 (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 3). In one non-limiting example, the vector is a SAMEN retrovirus vector including a nucleic acid encoding a TCR α chain with the amino acid sequence of SEQ ID NO: 4 (such as the nucleic acid sequence of SEQ ID NO: 2) and a nucleic acid encoding a TCR β chain with the amino acid sequence of SEQ ID NO: 5 (such as the nucleic acid sequence of SEQ ID NO: 3). In vectors where both TCR α chain and β chain nucleic acids are present, the α chain and β chain nucleic acids may be separated by an IRES or a promoter, such that both the α chain and β chain nucleic acids are transcribed and/or translated. In other examples, the α chain and β chain nucleic acids are separated by a nucleic acid encoding a peptide cleavage site or a "self-cleaving" peptide, such as a viral 2A peptide, for example, porcine teschovirus-1 2A or *Thosea asigna* virus self-cleaving peptides (see, *e.g.*, Kim *et al., PLoS One* 6:e18556, 2011).

In additional embodiments, the vector further includes a nucleic acid encoding a selectable marker that allows identification and/or enrichment of cells transduced with the vector. Exemplary selectable markers include antibiotic resistance genes (such as neomycin resistance), thymidine kinase, fluorescent proteins (such as green fluorescent protein), or β-galactosidase. In other examples, a selectable marker includes a cell surface expressed protein that can be used to identify transduced cells (for example, using flow cytometry or immuno-magnetic separation). In one non-limiting example, the vectors disclosed herein include a nucleic acid encoding a truncated CD34 protein (CD34t) lacking the intracellular signaling domain. The CD34t protein includes the extracellular and transmembrane regions of CD34, and as a result, it is expressed on the cell surface, but does not affect activity of cells expressing the truncated protein (Norell *et al.*, *Cancer Immunol. Immunother.* 59:851-862, 2010). Cells expressing CD34t can be identified with an anti-CD34 antibody, and can be isolated using flow cytometry or immuno-magnetic methods.

In one example, a nucleic acid encoding CD34t includes or consists of the sequence of nucleotides 4028-4975 of SEQ ID NO: 6 or a sequence having at least 95% (such as at least 95%,

96%, 97%, 98%, 99%, or more) sequence identity with nucleotides 4028-4975 of SEQ ID NO: 6. In particular examples, the CD34t protein includes or consists of an amino acid sequence having at least 95% (such as at least 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity with the amino acid sequence of SEQ ID NO: 7.

An exemplary retroviral vector (such as a SAMEN vector) for expressing the disclosed TCR α and β chains is shown in FIG. 1. The vector includes a 5′ long terminal repeat (LTR) including a promoter/enhancer (such as a human cytomegalovirus promoter/enhancer fused to a MMLV 5′ LTR), a packaging signal (ψ), a nucleic acid encoding the TCR α chain (*e.g.*, SEQ ID NO: 2), a first self-cleaving 2A peptide (such as a porcine teschovirus self-cleaving 2A (P2A) peptide), a nucleic acid encoding the TCR β chain (*e.g.*, SEQ ID NO: 3), a second self-cleaving 2A peptide (such as a *Thosea asigna* self-cleaving 2A (T2A) peptide), a nucleic acid encoding a truncated CD34 protein, and a 3′ LTR.

In some examples, the vector includes or consists of the nucleic acid sequence of SEQ ID NO: 6. In other examples, the vector includes or consists of a nucleic acid sequence having at least 95% (such as at least 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity with the nucleic acid sequence of SEQ ID NO: 6. In the exemplary vector provided herein, the TCRα chain is encoded by nucleotides 2165-2971 of SEQ ID NO: 6, the P2A peptide is encoded by nucleotides 2972-3037 of SEQ ID NO: 6, the TCRβ chain is encoded by nucleotides 3038-3958 of SEQ ID NO: 6, the T2A peptide is encoded by nucleotides 3959-4027 of SEQ ID NO: 6, and the CD34t receptor is encoded by nucleotides 4028-4975 of SEQ ID NO: 6.

C. Host Cells

Also disclosed herein are host cells that include a nucleic acid encoding a disclosed TCR α chain and/or a disclosed TCR β chain, such as a vector encoding the TCR α chain, the TCR β chain, or both. In some examples, the host cell is a cell capable of producing recombinant virus including the vector (*e.g.*, a producer cell). In other examples, the host cell is a lymphocyte (for example, a T cell). Methods of introducing a vector into a host cell are known to one of ordinary skill in the art and include transformation (*e.g.* with plasmid vectors), infection (*e.g.*, with viral vectors), and electroporation, nucleofection, lipofection, or particle gun acceleration (*e.g.,* naked DNA).

In examples where the TCR α and/or β chains are expressed from a retroviral vector (such as the disclosed SAMEN vectors), production of recombinant virus requires viral proteins expressed from a helper virus or a packaging cell line. Therefore, in some examples, a viral vector disclosed herein is introduced into a host cell (such as a 293 cell line) with a helper virus expressing viral proteins (such as gag, pol, and/or env). In other examples, a viral vector disclosed herein is transduced into a packaging cell line that stably expresses viral gag, pol, and env proteins.

Exemplary packaging cell lines include NIH-3T3 cell lines, such as GP&E 86, PG13, and PA317 cell lines (Markowitz *et al., J. Virol.* 62:1120-1124, 1988; Miller *et al., J. Virol.* 65:2220-2224, 1991; Miller *et al., Mol. Cell Biol.* 6:2895-2902, 1986) or 293 cell lines, such as 293GPG cells, GP2-293 cells. Thus, in one embodiment, a host cell is a producer cell, such as packaging cell line transduced with a viral vector described herein. In one non-limiting example, the viral vector is a SAMEN vector encoding a HERV-E specific TCR α chain and β chain and a truncated CD34 protein (such as a vector with the nucleic acid of SEQ ID NO: 6). In some examples, the producer cell line is GMP qualified. In one example, a producer cell line is a PG13 packaging cell line including a SAMEN vector encoding a HERV-E specific TCR α chain and β chain and a truncated CD34 protein (such as a vector with the nucleic acid of SEQ ID NO: 6).

In some examples, the host cell is a lymphocyte, such as a T cell. In some embodiments, the lymphocytes are T cells (such as a population of enriched or expanded T cells) that include a heterologous nucleic acid encoding at least one of the disclosed TCR α and β chains. In some examples, the lymphocytes include a heterologous nucleic acid encoding a TCR α chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 4 (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 2). In another example, the lymphocytes include a heterologous nucleic acid encoding a TCR β chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 5 (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 3). In yet another example, the lymphocytes include a heterologous nucleic acid encoding a TCR α chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 4 (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 2) and a nucleic acid encoding a TCR β chain having an amino acid sequence with at least 95% sequence identity to SEQ ID NO: 5 (such as a nucleic acid with at least 90% sequence identity to SEQ ID NO: 3). In one non-limiting example, the lymphocytes include a heterologous nucleic acid encoding a TCR α chain with the amino acid sequence of SEQ ID NO: 4 (such as the nucleic acid sequence of SEQ ID NO: 2) and a nucleic acid encoding a TCR β chain with the amino acid sequence of SEQ ID NO: 5 (such as the nucleic acid sequence of SEQ ID NO: 3). In additional examples, the lymphocytes also include a heterologous nucleic acid encoding a truncated CD34 protein (for example, a CD34 protein lacking the intracellular or signaling domain).

In some embodiments, the lymphocytes (such as a population of lymphocytes) are transduced with a vector disclosed herein. Following transduction, expression of the TCR α chain and/or β chain can be determined by methods known to one of ordinary skill in the art, such as flow cytometry using a labeled antibody or detecting reactivity to the cognate peptide (such as SEQ ID NO: 1). In some examples, if the TCR α and/or β chain is co-expressed with CD34t, transduced

cells can also be detected and/or enriched using an anti-CD34 antibody, for example, utilizing flow cytometry or immuno-magnetic techniques (*e.g.*, CliniMACS® CD34 reagent system, Miltenyi Biotec Inc., San Diego, CA or Isolex® 300 magnetic cell selection system, Nexell Therapeutics Inc., Irvine, CA).

In some embodiments, modified (*e.g.*, transduced) T cells expressing the disclosed TCR α and β chains are produced by obtaining a population of lymphocytes (such as a population of PBMCs) from a subject, for example by apheresis. Naïve or quiescent T cells in the population of lymphocytes are activated prior to transduction, for example, by contacting the lymphocytes with one or more cytokines (such as one or more of IL-2, IL-4, IL-6, IL-7, IL-12, IL-15, and IL-23). In some examples, the lymphocytes are contacted with anti-CD3 antibody and IL-2 for 1-4 days (such as 1 day, about 2 days, about 3 days, or about 4 days) to produce activated T cells. In some examples, the lymphocytes are contacted with 30 ng/ml anti-CD3 antibody and 300 IU/ml IL-2 for 2 or 3 days.

The activated T cells are transduced with a vector disclosed herein, for example, by infection (in the case of a viral vector) or by transfection or transformation (in the case of a plasmid or naked DNA vector). In some examples, the transduced T cells are enriched and/or expanded. For example, if the vector includes a nucleic acid encoding a truncated CD34 molecule, transduced T cells can be selected or enriched by contacting the population of transduced T cells with an anti-CD34 antibody and purifying CD34-expressing cells (for example, using flow cytometry or immuno-magnetic beads), for example about 2-4 days after transduction. The transduced T cells can also be expanded by culturing the transduced T cells with anti-CD3 (*e.g.*, about 30 ng/ml), anti-CD28 (*e.g.*, about 30 ng/ml), and/or IL-2 (*e.g.*, about 300 IU/ml) for a period of time (such as about 7-14 days or 9-11 days). In some examples, the transduced T cells are expanded by culture on irradiated PBMC cells.

IV. Methods of Treating or Inhibiting Renal Cell Carcinoma

Disclosed herein are methods of treating or inhibiting RCC in a subject by administering to the subject a T cell (or population of T cells) expressing a TCR (for example, TCR α and β chains) that bind to an antigen or epitope expressed by RCC cells. In some examples, the methods include administering the modified T cells described herein to a subject with RCC (such as ccRCC, advanced ccRCC, or metastatic ccRCC). In particular examples, the subject is HLA-A11 positive and has ccRCC.

The modified lymphocytes (*e.g.*, modified or transduced T cells) described herein can be incorporated into pharmaceutical compositions. In some examples, the composition includes about

10^4 to 10^{12} of the modified T cells (for example, about 10^4-10^7 cells, about 10^6-10^9 cells, or about 10^8-10^{12} cells). For example, the composition may be prepared such that about $5x10^6$ to $5x10^8$ modified T cells/kg are administered to a subject. Such compositions typically include a population of modified T cells and a pharmaceutically acceptable carrier. A "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. Supplementary active compounds can also be incorporated into the compositions. Actual methods for preparing administrable compositions are known or apparent to those skilled in the art and are described in more detail in such publications as *Remington: The Science and Practice of Pharmacy*, The University of the Sciences in Philadelphia, Editor, Lippincott, Williams, & Wilkins, Philadelphia, PA, 21st Edition (2005).

In vivo treatment of a subject is initiated by administration of the modified T cells disclosed herein. Administration is typically via intravenous or intraperitoneal infusion, although direct injection into solid tumors or other such focal lesions can also be used. The efficacy of the treatment is generally assessed by lesion reduction/clearance (for example, using RECIST criteria). Lesion size and number can be evaluated by imaging (such as MRI, PET, and/or CT imaging). In some examples, staging is done every month, every 3 months, or every 6 months. In some examples, blood samples from the subject are also analyzed at one or more time points following infusion to quantitate the number of modified T cells present (for example, by assessing absolute number and/or percentage of CD3+ cells expressing CD34, in the case of CD34t-expressing modified T cells).

Multiple doses of the population of modified T cells can be administered. For example, the population of modified T cells can be administered daily, every other day, twice per week, weekly, every other week, every three weeks, monthly, or less frequently. In one specific example, a single infusion of modified T cells is administered; however, a skilled clinician can select alternative schedules based on the subject, the condition being treated, the previous treatment history, and other factors.

In some embodiments, the subject has RCC, such as ccRCC. Methods of identifying a subject with RCC or ccRCC are known to one of ordinary skill in the art and include radiographic evidence of RCC (for example, imaging by ultrasound, MRI, CT scan, or PET scan) and/or a biopsy (such as a fine needle aspirate or needle core biopsy) confirming presence of RCC. In some examples, the subject has metastatic RCC. In additional examples, the subject with RCC also is

HLA A11+ and the tumor expresses a HERV-E provirus (for example, expresses a protein comprising the amino acid sequence of SEQ ID NO: 1). In some embodiments, the methods include selecting a patient with RCC (such as ccRCC) who is HLA $A11^+$ and whose tumor expresses a protein comprising SEQ ID NO: 1 for treatment with the modified T cells.

In particular embodiments, the methods include obtaining a population of cells including lymphocytes from a subject with RCC (such as a subject with ccRCC who is HLA $A11^+$ and whose tumor expresses HERV-E provirus, such as a protein comprising SEQ ID NO: 1). In other examples, a population of cells including lymphocytes are obtained from an HLA-matched donor to the subject to be treated (such as a subject with ccRCC who is HLA $A11^+$ and whose tumor expresses HERV-E provirus, such as a protein comprising SEQ ID NO: 1).

An exemplary protocol for collecting and transducing T cells from a subject is shown in FIG. A population of cells including lymphocytes (such as PBMCs) can be obtained by any method, including, but not limited to apheresis. All or a portion of the population of cells can be utilized immediately or all or a portion of the cells can be cryopreserved for future use. When ready for use, all or a portion of the population of cells is thawed (if previously cryopreserved) and T cells are activated by incubation with an anti-CD3 antibody (such as OKT3). In some examples, about 10^7-10^9 PBMCs are incubated with an anti-CD3 monoclonal antibody (*e.g.*, about 30 ng/ml) and optionally also IL-2 (*e.g.,* about 300 IU/ml) and/or IL-15 (about 10-100 ng/ml). In one specific example, about $6x10^8$ PBMCs are incubated with anti-CD3 antibody OKT3 and IL-2 for about 1-5 days (such as about 1 day, about 2 days, about 3 days, about 4 days, or about 5 days). In another specific example, about $6x10^8$ PBMCs are incubated with anti-CD3 antibody OKT3, IL-2, and IL-15 for about 1-5 days (such as about 1 day, about 2 days, about 3 days, about 4 days, or about 5 days).

In some examples, following T cell activation, the cells are optionally depleted of $CD4^+$ cells. In some examples, $CD4^+$ cells are removed using an anti-CD4 antibody, for example, utilizing flow cytometry or immuno-magnetic techniques (*e.g.*, CliniMACS® CD4 reagent system, Miltenyi Biotec Inc., San Diego, CA) or erythrocyte resetting of $CD4^+$ T-cells bound by anti-CD4 antibodies, to produce a CD4-depleted cell population. In other examples, CD4 depletion can be carried out after transduction or after expansion of the transduced T cells. In some examples, the CD4-depleted cell population is a $CD8^+$ population of T cells (for example, a population of T cells that is substantially $CD8^+$ T cells, such as a population of T cells that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more $CD8^+$ T cells).

Following T cell activation (and optional $CD4^+$ cell depletion) the cells are transduced with a vector including a heterologous nucleic acid encoding the HERV-E reactive TCR α chain, T cell

β chain, or both (such as one or more of the vectors described in Section IIIB, above). In particular examples, about 10^7-10^9 cells are transduced (for example, about $1x10^7$, $5x10^7$, $1x10^8$, $5x10^8$, or $1x10^9$ cells). In one non-limiting example, about $2x10^8$ cells are transduced. In some examples, the vector also includes a heterologous nucleic acid encoding a truncated CD34 protein. Thus, in some examples, transduced T cells are enriched using a CD34-specific antibody (such as flow cytometry or immuno-magnetic purification).

Transduced T cells (or optionally, CD34-enriched transduced T cells and/or CD34-enriched, CD4-depleted transduced T cells) are expanded *ex vivo* and can be cryopreserved at appropriate dosage amounts (for example, about 10^6 to 10^{12} cells) following expansion. In one specific example, the transduced T cells are expanded on irradiated allogeneic PBMC feeder cells (40 million cells per 250,000 T cells) in medium containing 300 IU/ml IL-2, 30 ng/ml anti-CD3, and 30 ng/ml anti-CD28. The expansion can be for a sufficient time to obtain the desired number of T cells, for example, about 4-14 days (such as 4-9 days, 7-10 days, 8-12 days, 9-14 days, 9-11 days). In some examples, the T cells are supplemented with fresh IL-2 on days 5, 8, and 11. In some non-limiting examples, the expansion can optionally be carried out in a WAVE bioreactor (GE Healthcare Life Sciences, Pittsburgh, PA) for at least a portion of the expansion, such as for 1-5 days, for example from days 9-14 of the expansion protocol. One of ordinary skill in the art can identify other methods for expanding T cells *ex vivo*, which can also be used with the transduced T cells described herein (see, *e.g.*, U.S. Pat. No. 5,827,642 and Riddell and Greenberg, *J. Immunol. Meth.* 128:189-201, 1990, incorporated herein by reference in their entirety).

The transduced (modified) T cells are thawed (if previously frozen), prior to administration to the subject. The subject may undergo an immunosuppressive regimen (*e.g.*, lymphodepletion) prior to administering the modified T cells. In one example, the subject is administered cyclophosphamide and/or fludarabine prior to administering the modified T cells. In one non-limiting example, the subject is administered cyclophosphamide (*e.g.*, 60 mg/kg) on days -5 and -4 and/or fludarabine (*e.g.*, 25 mg/m^2) on days -5 through -1 (where day 0 is administration of the modified T cells). In another non-limiting example, the subject is administered cyclophosphamide (1,000 mg/m^2 IV) on day -5 and fludarabine (30mg/m^2) on days -5 through -3 (where day 0 is administration of the modified T cells). The modified T cells are administered to the subject, for example by infusion. In some examples, the T cells are administered at a dose of about 10^4 to 10^{12} of the modified T cells (for example, about 10^4-10^7 cells, about 10^6-10^9 cells, or about 10^8-10^{12} cells or about $1x10^6$ to $1x10^9$ modified T cells/kg (such as about $1x10^6$, $5x10^6$, $1x10^7$, $5x10^7$, $1x10^8$, $5x10^8$, or $1x10^9$ cells/kg). Immune system supportive therapies may also be administered to the subject, for example to promote expansion of the modified T cells in the subject and/or to support

recovery of neutrophils. In one non-limiting example, the subject is administered IL-2 (*e.g.*, 72,000 iu/kg iv every 8 hours) for 10 days and/or G-CSF (*e.g.*, 300-480 μg sc) daily from day +1 until absolute neutrophil count is greater than 500. In another example, the immune system supportive therapy includes administering $2x10^6$ i.u./m^2 every 12 hours for seven days following administration of the modified T cells.

Treatment efficacy is monitored by standard methods such as tumor size, number of lesions, tumor stage, response rate, or other criteria known to one of ordinary skill in the art. In some examples, a decrease in size of primary tumor or metastases (for example, as defined by standard RECIST or irRECIST criteria) indicates inhibition of RCC in the subject. See, *e.g.,* Eisenhauer *et al., Eur. J. Cancer* 45:228-247, 2009; Wolchock *et al., Clin. Cancer Res.* 15:7412-7420, 2009; both of which are incorporated herein by reference. In other examples, progression-free survival and/or overall survival (for example, for 1 month, 3 months, 6 months, 9 months, 12 months, 18 months 2 years, or more, such as 1-12 months, 6-18 months, 1-2 years, or more) indicates inhibition of RCC in the subject. In other examples, one or more of persistence of circulating HERV-E TCR transduced CD34+ or CD8+/CD34+ T cells, changes in immune cell subsets and activation status of T cells, as well as other immunologic determinants are evaluated, with clinical outcomes at baseline, at different time points during treatment, and at the time of disease progression.

In some examples, the subject is also administered one or more additional treatments, such as one or more therapeutic agents, surgical resection, and/or radiation therapy before, concurrently, or after treatment with the transduced T cells. One of skill in the art can select therapeutic agents for administration to a subject with RCC in combination with the modified T cells disclosed herein. Such agents include anti-VEGF agents (such as pazopanib, sorafenib, sunitinib, axitinib, cabozantinib, sorafenib, lenvatinib, and/or bevacizumab), mTOR inhibitors (such as temsirolimus and/or everolimus), immune checkpoint inhibitors (such as nivolumab), and/or cytokine therapy (such as IFNα and/or IL-2).

EXAMPLES

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

Example 1

Cloning of T Cell Receptor from HERV-E CT-RCC1 Reactive CD8+ T Cells

A CD8+ HLA-A11 restricted RCC-reactive T cell clone was previously identified (Takahashi *et al., J. Clin. Invest.* 118:1099-1109, 2008). This RCC reactive CTL recognizes the HERV-E peptide ATWLGSKTWK (SEQ ID NO: 1) (Takahashi *et al.*, 2008). Total RNA was extracted from the T cell clone with HLA-A11 restricted recognition of ccRCC cells and full length TCR chains were identified by 5' RACE. The nucleic acids encoding the TCR α and β chains are disclosed herein as SEQ ID NOs: 2 and 3, respectively. The amino acid sequence of the TCR α and β chains are disclosed herein as SEQ ID NOs: 4 and 5, respectively.

The TCR α and β chain-encoding nucleic acids (SEQ ID NOs: 2 and 3) were cloned into a retroviral construct including a truncated CD34 molecule linked to the β chain (FIG. 1). The truncated CD34 cassette includes the extracellular and transmembrane domains but lacks the intracellular signaling domain so it does not function in cells, but its expression allows enrichment of transduced T cells based on their surface expression of CD34. The sequence of the vector is provided as SEQ ID NO: 6.

Example 2

Reactivity of T Cells Transduced with HLA-A11 Restricted TCR Against ccRCC Cells

PBMCs from two healthy donors were transduced with the vector described in Example 1. The transduced T cells specifically killed HLA-A11+ and HERV-E+ ccRCC cells (FIGS. 3A and 3B). Addition of the CT-RCC-1 peptide (SEQ ID NO: 1) increased reactivity against both HERV-E negative and HERV-E positive cells, as expected (FIGS. 3A and 3B).

Example 3

Development of Producer Clones

The retroviral vector containing this TCR described in Example 1 was introduced into the PG13 packaging cell line to isolate high titer retroviral producer clones for clinical use. The PG13 producer cell line was cloned in limiting dilution to generate high titer clones. Then, the clones were screened for their ability to efficiently transduce and transfer anti-HERV-E reactivity to human T cells. Two clones were selected (7G1 and 27A7) for further testing.

For this purpose, retrovirus was prepared from each clone and two separate full scale validation runs were performed. T cells from 2 healthy donors were transduced with retrovirus from clone 7G1 or 27A7. They were 63.2% and 67.5% ($\bar{X}$=65.4%) or 65.2% and 61.6% ($\bar{X}$=63.4%) $CD34^+$, respectively, indicating both clones transduced T cells with equal efficiency. Following

CD34 purification of the transduced cells using immune-magnetic beads, the cultures were 99.3% and 98.4% ($\bar{X}$=98.9%) or 99.4% and 99.7% ($\bar{X}$=99.6%) $CD34^+$ respectively indicating pure TCR transduced T cell cultures.

Master Cell banks of $5x10^6$ cells per vial were generated for both clones (212 vials for 7G1 and 220 vials 27A7). A large 3-liter batch of virus was prepared from the 7G1 clone and both the 7G1 clone and 7G1 virus were sent to the Vector Production Facility/National Gene Vector Biorepository at Indiana University for GMP qualification.

Example 4

Evaluation of HERV-E TCR Transduced T Cells

Supernatants from the PG13 retroviral producer clone 7G1 were used to transduce normal PBL-derived T cells. Three days post-transduction, the cells were incubated with anti-CD34 coated immunomagnetic particles and purified using the CliniMACS and stained for CD34 expression. Prior to selection 63.2% of the cells expressed CD34; this was increased to 99.3% post-selection (FIG. 4A). The CD34+ cells expressed CD3 (FIG. 4B) and stained positively with the HERV-E tetramer (FIG. 4C). The CD34-selected cells were primarily $CD8^+$ cells (FIG. 5A), with only a small fraction of $CD4^+$ cells (FIG. 5B).

Transduced T cells as described in Example 2 were evaluated. As determined by chromium release cytotoxicity, the transduced T cells specifically killed HLA-A11+ and HERV-E+ ccRCC cells (FIGS. 6A and 6B). Addition of the CT-RCC-1 peptide (SEQ ID NO: 1) increased reactivity against HERV-E negative cells (FIGS. 6A and 6B). The T cell population from donor 1 was 39.9% $CD8^+$ (FIG. 6A) and the T cell population from donor 2 was 52.8% $CD8^+$ (FIG. 6B).

PBMC from a healthy donor transduced with the vector described in Example 1 were also tested for specific killing of RCC cells and T cells. HLA-$A11^+$ and HERV-E expressing SAUJ-RCC cells were specifically killed, while HLA-$A11^-$ HERV-E negative cells (SAUJ-LCL) were not (FIG. 7). HLA-$A11^-$ cells also were not killed whether they expressed HERV-E (LYO-RCC) or not (LYO-LCL). Finally, T cells from an HLA-$A11^+$ donor were not killed (FIG. 7).

Antigen specificity of HERV-E TCR transduced T cells was evaluated by measuring interferon-y (INF-γ) secretion by enzyme-linked immunosorbent assay (ELISA) using a panel of ccRCC cell lines as targets. FIG. 8 shows that transduced T cells produce INF-γ only when co-culturing with both HLA-A11-positive and CT-RCC HERV-E-expressing ccRCC cells (>twice background and >1 ng/ml).

Example 5

Determining Safety and Tolerability of HERV-E TCR Transduced T Cells

This example describes methods that can be used to determine safety and tolerability of treating ccRCC with HERV-E TCR transduced T cells. However, one skilled in the art will appreciate that methods that deviate from these specific methods can also be used to successfully determine safety and tolerability of the HERV-E TCR transduced T cells.

FIG. 9 is a schematic diagram showing an exemplary protocol for determining safety and tolerability of HERV-E TCR transduced T cells (for example prior to clinical implementation).

Subjects with metastatic HERV-E positive ccRCC that is not amenable to complete surgical resection and that is progressive bi-dimensionally evaluable clinically or radio-graphically and are HLA-A11 positive are selected for this study. Subjects undergo a 15-20 liter leukocyte apheresis to collect (and optionally cryopreserve) $1x10^{10}$ PBMCs. Subsequently, PBMCs ($6x10^8$ to $2x10^9$ cells) are thawed and activated in media containing anti-CD3 (*e.g.*, 50 ng/ml OKT3) and IL-2 (*e.g.*, 300 IU/ml) (and optionally, IL-15, *e.g.*, 100 ng/ml) for 2-3 days followed by depletion for CD4 expressing cells, using immunomagnetic methods. Transduction of about $200x10^6$ T cells with a retrovirus encoding the HLA-A11 restricted HERV-E TCR and CD34t (such as that described in Example 1) is carried out. Following transduction, $0.5\text{-}1x10^6$ transduced T cells/kg are enriched using CD34+ immuno-magnetic bead selection and then expanded *ex vivo* for 9-11 days using irradiated allogeneic PBMC feeder cells (pooled from 3 healthy donors) in IL-2/IL-15 containing media (*e.g.*, 300 IU/ml IL-2 and 100 ng/ml IL-15).

In some embodiments of the protocol, the positive selected $CD34^+$ T cells are placed into a REP to rapidly generate the number of cells needed for infusion. The number of transduced cells that are placed in the REP is determined by the patient's weight and the infusion cohort. To initiate the REP, $1x10^6$ HERV-E TCR transduced cells are combined with $200x10^6$ feeder cells in an upright T175cm^2 flask containing 150 ml complete cytokine media (rhIL2/rhIL15) with 30ng/mL of soluble anti hCD3 antibody (OKT3). The feeder cells consist of PBMC from a minimum of 3 separate normal donors which are mixed together and irradiated with 5000 RAD of irradiation. The flasks are placed upright in a humidified 5% CO_2 incubator for 5-6 days. The flasks are removed from the incubator, the cells collected, counted, and re-suspended in fresh media containing 300 IU/mL-IL2 and 100 ng/mL IL15. The cell suspension is transferred into Wave bioreactor bag(s) for further expansion. The culture media is replenished daily with fresh medium containing cytokines by utilizing the Wave bioreactor perfusion system.

Transduced and expanded T cells are cryopreserved at the appropriate dose level (see below) for subsequent infusion into individual subjects following treatment with immune-suppressive chemotherapy.

Three subjects are enrolled sequentially into each of 4 different T cell dose escalating cohorts ($5x10^6$ T cells/kg , $5x10^6$ T cells/kg, $1x10^7$ T cells/kg, or $5x10^7$ T cells/kg). Subjects undergo a non-myeloablative immunosuppressive conditioning regimen with cyclophosphamide 1,000 mg/m^2 IV (day -5) and fludarabine 30 mg/m^2 i.v. over 30 minutes daily x 3 days (days -5 through -3) followed by infusion of HERV-E TCR transduced T cells on day 0 to deliver the targeted T cell dose. Prior to infusion, T cells undergo a final cell count, and have viability and sterility assessment. Following the T cell infusion, the subject is monitored for up to 4 hours for signs of infusion-related toxicities. Premedication prior to T cell infusion is acetaminophen and i.v. Benadryl. Following the T cell infusion, subjects receive IL-2 i.v. 12 hours at a dose of 2,000,000 IU/m^2 for 7 days (day 0 through day +6) and G-CSF 300 μg daily from day 1 until neutrophil recovery (ANC >500) occurs. Subjects are discharged from the clinical center following neutrophil recovery and return for weekly visits for 6-8 weeks, where they undergo standard evaluations including physical exams and body weight, and routine clinical labs (hematology and electrolytes). Restaging using PET and CT imaging using RECIST criteria is performed 30 days following the T cell infusion, then every 3 months for the first year, and then every 6 months thereafter until evidence for tumor progression occurs.

The decision to escalate, de-escalate or suspend the dose escalations in the study will follow the rules outlined in Table 2.

Table 2.

Outcome: No of DLTs out of No. of Patients at a Given Dose Level	**Decision Rule**
0 DLT out of 3 patients	Enter up to 3 patients at the next dose level
2 DLTs out of 2-3 patients	Stop dose escalation: Enter up to 3 additional patients at the previous dose level if only 3 patients have been treated at that dose.
1 DLT out of 3 patients	Enter up to 3 more patients at the same dose level .
1 DLT out of 6 patients	Enter up to 3 patients at the next dose level

Outcome: No of DLTs out of No. of Patients at a Given Dose Level	Decision Rule
2 DLTs out of 4-6 patients	Stop dose escalation: Enter up to 3 additional patients at the previous dose level if only 3 patients have been treated at that dose.

Since adverse events associated with adoptive T cell transfer generally occur within 21 days after the T cell infusion, each subject will be observed for 21 days post T cell infusion before the next subject in the cohort or the first subject in the next cohort is treated. Therefore, there will be a minimum of 21 days between the HERV-E TCR transduced T-cells infusion for each patient before the next patient enrolled starts conditioning chemotherapy. Dose-limiting toxicity (DLT) is defined as any adverse event that leads to a discontinuation of the T-cell infusion and/or all grade 3 and 4 toxicities judged probably or definitely associated to the HERV-E TCR transduced CD8+/CD34+ T-cells infusion except for:

- Myelosuppression, defined as lymphopenia, neutropenia and thrombocytopenia.
- IL-2 expected toxicities. (described section 14.3)
- Fludarabine and Cyclophosphamide expected toxicities
- Immediate hypersensitivity reactions (excluding symptomatic bronchospasm and grade 4 hypotension) occurring within 2 hours of cell infusion (related to cell infusion) that are reversible to a grade 2 or less within 24 hours of cell administration with standard supportive treatments.
- Grade 3 Fever.
- Grade 3 autoimmunity, that resolves to less than or equal to a grade 2 autoimmune toxicity within 10 days.
- Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 within 7 days.

Blood samples are drawn at multiple time points post T cell infusion to assess for circulating HERV-E TCR transduced T cells, which are analyzed by quantitating the percentage and absolute numbers of $CD3^+$ cells expressing CD34. Patients who have tumors which are easily amenable to biopsy may also undergo elective fine needle aspiration of a metastatic tumor lesion to assess for HERV-E TCR transduced T cells using the same methodology as above. Subjects are followed for up to 5 years and are taken off study when disease progression is documented.

Example 6

Treating Renal Cell Carcinoma with HERV-E TCR Transduced T Cells

This example describes methods that can be used to a subject with RCC with HERV-E TCR transduced T cells. However, one skilled in the art will appreciate that methods that deviate from these specific methods can also be used to successfully treat a subject with RCC with HERV-E TCR transduced T cells.

FIG. 10 is a schematic diagram showing an exemplary protocol for treating a subject with RCC.

A subject with RCC (such as metastatic ccRCC) who is HLA-A11 positive and HERV-E positive undergoes apheresis to collect T lymphocytes. T cells are activated with 30 ng/ml anti-CD3 antibody and 300 IU/ml IL-2 *in vitro* for two days. The T cells are transduced with a retroviral vector including the HERV-E specific TCR described herein (*e.g.* SEQ ID NO: 6). Lymphocytes are expanded with 30 mg/ml anti-CD3 and 30 ng/ml anti-CD28 for 7-14 days. The subject undergoes chemotherapy induced lymphodepletion (for example, as described in Example 3) and the transduced T cells are infused with IL-2 support at the MTD (for example, determined as described in Example 5).

In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiments are only examples and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A vector comprising a nucleic acid molecule encoding a T cell receptor α chain comprising at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 2, a nucleic acid molecule encoding a T cell receptor β chain comprising at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 3, or both.

2. The vector of claim 1, wherein the nucleic acid molecule encoding the T cell receptor α chain comprises the nucleic acid sequence of SEQ ID NO: 2, the nucleic acid molecule encoding the T cell receptor β chain comprises the nucleic acid sequence of SEQ ID NO: 3, or both.

3. The vector of claim 2, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 2 and the nucleic acid sequence of SEQ ID NO: 3.

4. The vector of any one of claims 1 to 3, wherein the nucleic acid encoding the T cell receptor α chain, the nucleic acid encoding the T cell receptor β chain, or both, is operably linked to a promoter.

5. The vector of any one of claims 1 to 4, further comprising a nucleic acid molecule encoding a truncated CD34 protein lacking an intracellular domain.

6. The vector of any one of claims 1 to 5, wherein the vector is a retroviral vector.

7. The vector of claim 6, wherein the vector is a SAMEN retroviral vector.

8. The vector of claim 7, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 6.

9. An isolated host cell comprising the vector of any one of claims 1 to 8.

10. The host cell of claim 9, wherein the cell further comprises a nucleic acid encoding a viral gag protein, a viral pol protein, a viral env protein, or a combination of two or more thereof.

11. The host cell of claim 10, wherein the cell is a PG13 cell.

12. The host cell of claim 9, wherein the host cell is a lymphocyte.

13. The host cell of claim 12, wherein the lymphocyte is a T cell.

14. A modified T cell comprising a heterologous nucleic acid encoding a T cell receptor α chain comprising at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 4 and a heterologous nucleic acid molecule encoding a T cell receptor β chain comprising at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 5.

15. The modified T cell of claim 14, wherein the heterologous nucleic acid encoding the T cell receptor α chain encodes a protein comprising or consisting of the amino acid sequence of SEQ ID NO: 4 and the heterologous nucleic acid encoding the T cell receptor β chain encodes a protein comprising or consisting of the amino acid sequence of SEQ ID NO: 5.

16. The modified T cell of claim 14 or claim 15, wherein the heterologous nucleic acid encoding the T cell receptor α chain comprises or consists of the nucleic acid sequence of SEQ ID NO: 2 and the heterologous nucleic acid encoding the T cell receptor β chain comprises or consists of the nucleic acid sequence of SEQ ID NO: 3.

17. The modified T cell of any one of claims 14 to 16, further comprising a heterologous nucleic acid encoding a truncated CD34 protein lacking an intracellular domain.

18. A modified T cell transduced with the vector of any one of claims 1 to 8.

19. The modified T cell of any one of claims 14 to 18, wherein the T cell comprises a genome that is autologous to a subject with a renal cell carcinoma.

20. A pharmaceutical composition comprising the modified T cell of any one of claims 14 to 19 and a pharmaceutically acceptable carrier.

21. A method of treating or inhibiting renal cell carcinoma (RCC), comprising:
obtaining a population of T cells from a subject with RCC;

transducing the population of T cells with a vector comprising a nucleic acid encoding a T cell receptor α chain comprising a nucleic acid with at least 90% sequence identity to SEQ ID NO: 2 and a nucleic acid encoding a T cell receptor β chain comprising a nucleic acid with at least 90% sequence identity to SEQ ID NO: 3, thereby producing a population of modified T cells; and

administering a composition comprising the modified T cells to the subject, thereby treating or inhibiting the RCC.

22. The method of claim 21, wherein the nucleic acid encoding the T cell receptor α chain comprises or consists of the nucleic acid sequence of SEQ ID NO: 2 and the nucleic acid encoding the T cell receptor β chain comprises or consists of the nucleic acid sequence of SEQ ID NO: 3.

23. The method of claim 21 or claim 22, wherein the nucleic acid encoding the T cell receptor α chain encodes a protein comprising or consisting of the amino acid sequence of SEQ ID NO: 4 and the nucleic acid encoding the T cell receptor β chain encodes a protein comprising or consisting of the amino acid sequence of SEQ ID NO: 5

24. The method of claim any one of claims 21 to 23, wherein the vector further comprises a nucleic acid encoding a truncated CD34 protein lacking an intracellular domain.

25. The method of any one of claims 21 to 24, wherein the vector is a retroviral vector.

26. The method of claim 25, wherein the population of modified T cells is enriched by selection with an anti-CD34 antibody prior to administering to the subject.

27. The method of any one of claims 21 to 26, wherein the population of T cells is activated prior to transduction.

28. The method of any one of claims 21 to 27, wherein the population of T cells is depleted of CD4 expressing T cells prior to transduction.

29. The method of any one of claims 21 to 28, wherein the population of modified T cells is expanded prior to administering to the subject.

30. The method of any one of claims 21 to 29, wherein the subject is administered immunosuppressive therapy prior to administering the population of modified T cells to the subject.

31. The method of any one of claims 21 to 30, wherein the RCC is clear cell RCC.

32. The method of any one of claims 21 to 31, wherein the RCC expresses a human endogenous retrovirus-E (HERV-E) protein comprising the amino acid sequence of SEQ ID NO: 1.

33. The method of any one of claims 21 to 32, wherein the subject is positive for human leukocyte antigen (HLA) serotype HLA-A11.

34. The method of any one of claims 21 to 33, further comprising selecting a subject with RCC, wherein the RCC expresses a HERV-E protein comprising SEQ ID NO: 1 and the subject is positive for HLA-A11.

35. A method of making modified T cells expressing a renal cell carcinoma antigen-specific T cell receptor, comprising:

obtaining a population of lymphocytes form a subject;

contacting the population of lymphocytes with an anti-CD3 antibody and interleukin-2 to produce a population of activated T cells;

transducing the population of activated T cells with the vector of any one of claims 1 to 8 to produce a population of transduced T cells; and

expanding the population of transduced T cells, thereby producing the modified T cells.

36. The method of claim 35, wherein expanding the population of transduced T cells comprises contacting the transduced T cells with anti-CD3, anti-CD28, IL-2 and IL-15.

37. The method of claim 36, wherein contacting the transduced T cells with anti-CD3, anti-CD28, IL-2 and IL-15 comprises contacting the cells with anti-CD3, anti-CD28, IL-2 and IL-15 for 9 to 11 days.

38. The method of any one of claims 35 to 37, further comprising enriching the population of transduced T cells by contacting the transduced T cells with an anti-CD34 antibody.

39. The method of any one of claims 35 to 38, wherein the population of lymphocytes is obtained from a subject with renal cell carcinoma (RCC).

40. The method of any one of claims 35 to 39, wherein the population of lymphocytes is obtained from a subject with renal cell carcinoma expressing a protein comprising SEQ ID NO: 1 and the subject is HLA-A11 positive.

41. An isolated nucleic acid encoding a T cell receptor α chain comprising at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 2.

42. The nucleic acid of claim 41, wherein the nucleic acid comprises or consists of the nucleic acid sequence of SEQ ID NO: 2.

43. The nucleic acid of claim 41 or claim 42, wherein the nucleic acid encodes a protein comprising or consisting of the amino acid sequence of SEQ ID NO: 4.

44. An isolated nucleic acid encoding a T cell receptor β chain comprising at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 3.

45. The nucleic acid of claim 44, wherein the nucleic acid comprises or consists of the nucleic acid sequence of SEQ ID NO: 3.

46. The nucleic acid of claim 44 or claim 45, wherein the nucleic acid encodes a protein comprising or consisting of the amino acid sequence of SEQ ID NO: 5.

47. An isolated T cell receptor α chain polypeptide comprising at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 4.

48. The polypeptide of claim 47, wherein the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 4.

49. An isolated T cell receptor β chain polypeptide comprising at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 5.

50. The polypeptide of claim 49, wherein the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 5.

FIG. 1

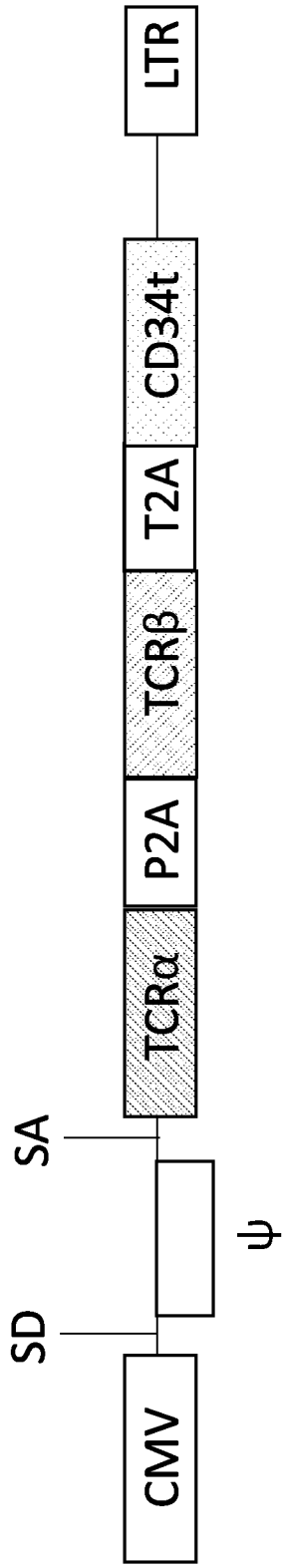


FIG. 2

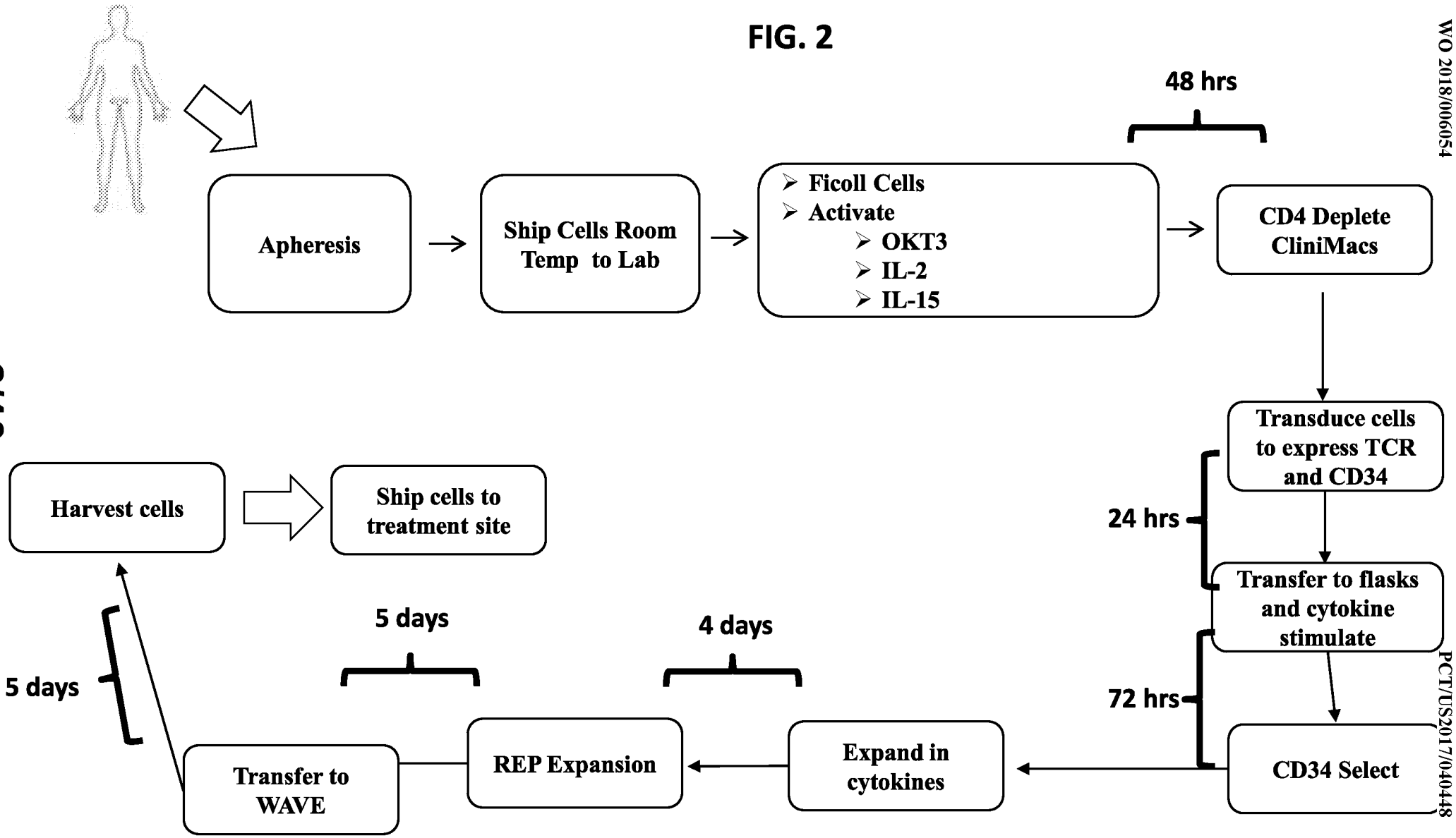
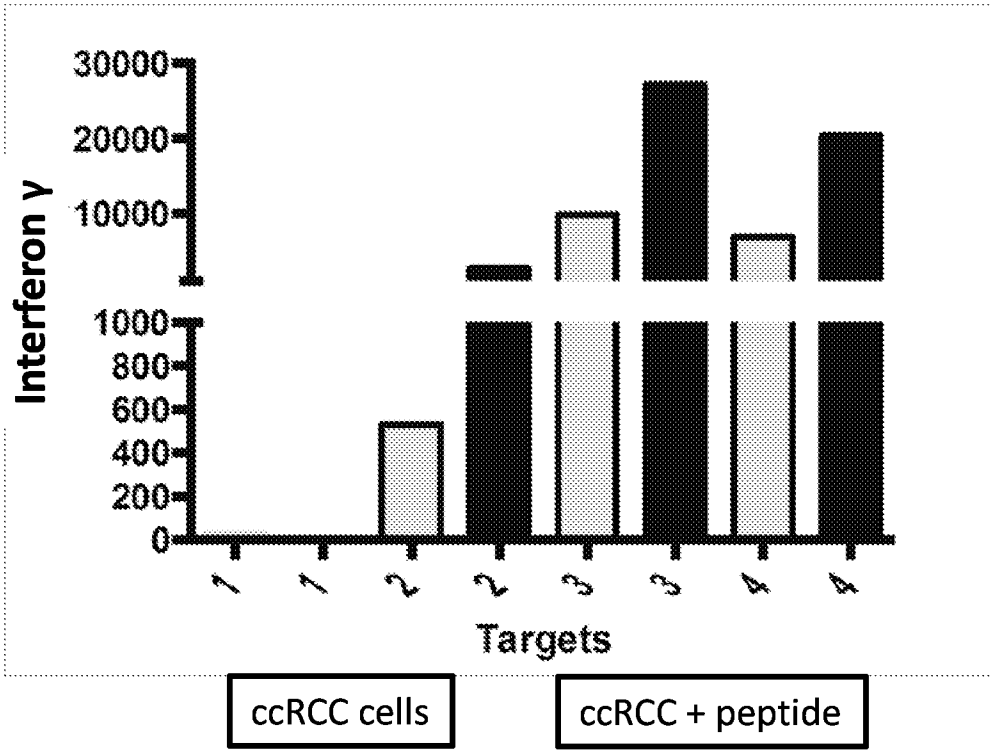

Apheresis
Ship Cells Room Temp to Lab
Ficoll Cells
Activate
OKT3
IL-2
IL-15
48 hrs
CD4 Deplete CliniMacs
Transduce cells to express TCR and CD34
24 hrs
Transfer to flasks and cytokine stimulate
72 hrs
CD34 Select
Expand in cytokines
4 days
REP Expansion
5 days
Transfer to WAVE
5 days
Harvest cells
Ship cells to treatment site

FIG. 3A



Donor 1 – grey columns
Donor 2 – black columns

Targets:

1 – ccRCC HERV-E neg/ HLA-A11+

2 – ccRCC HERV-E +/ HLA-A11+

3 – ccRCC HERV-E neg/ HLA-A11+
+ CT-RCC-1 peptide

4 – ccRCC HERV-E +/ HLA-A11+
+ CT-RCC-1 peptide

FIG. 3B

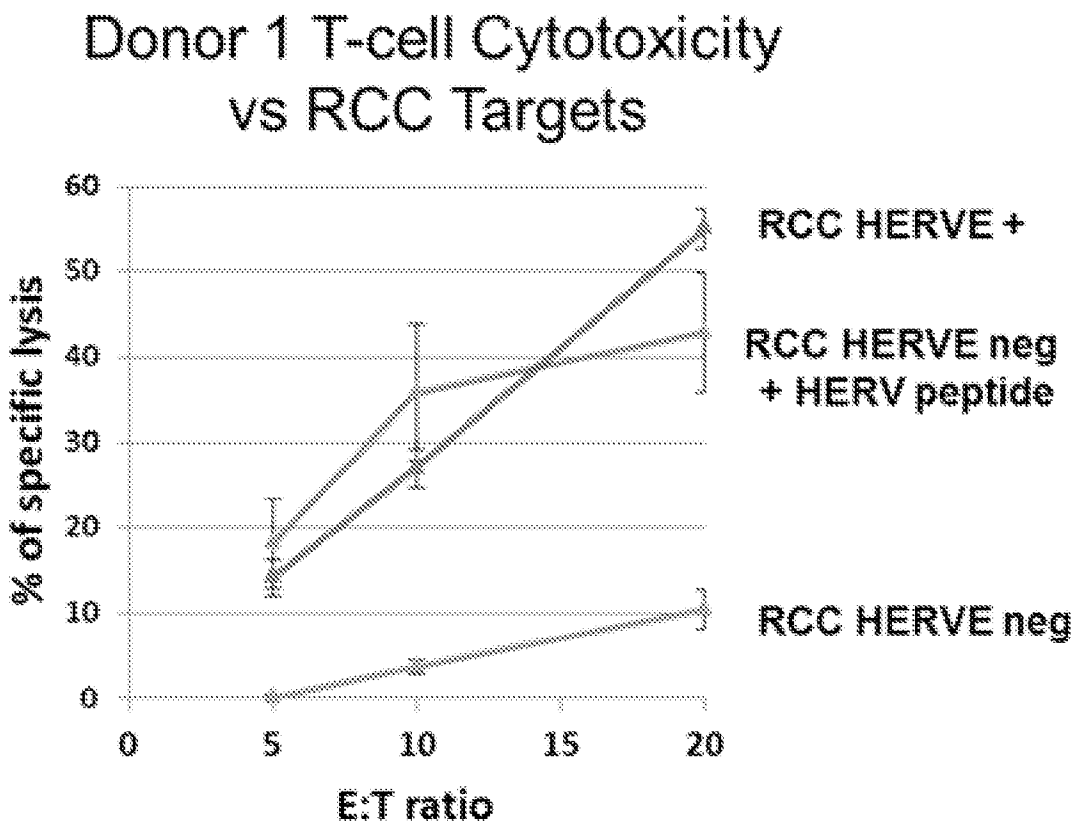
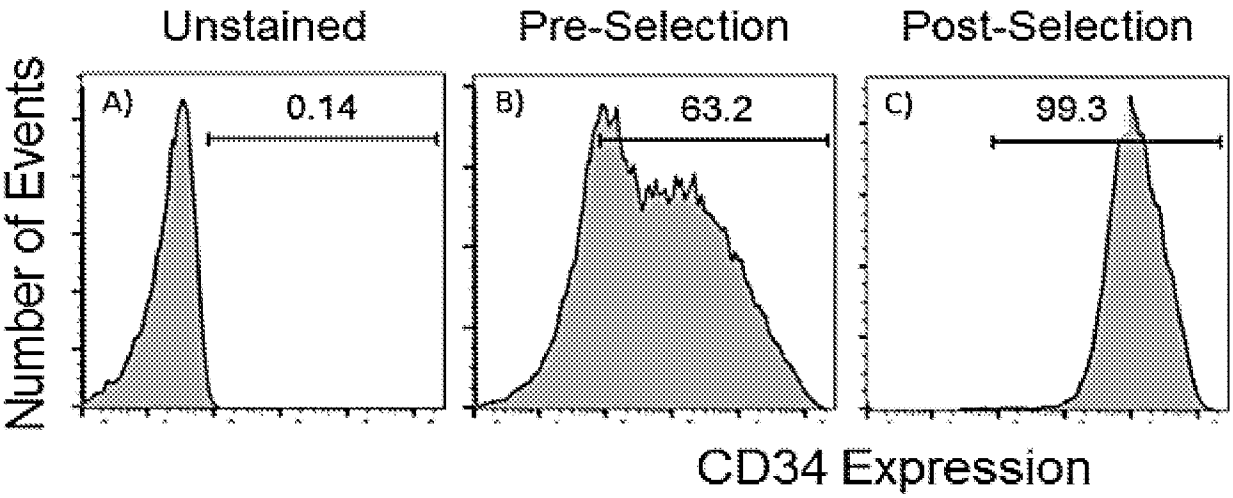

Donor 1 T-cell Cytotoxicity
vs RCC Targets
% of specific lysis
60
50
40
30
20
10
0
0
5
10
15
20
E:T ratio
RCC HERVE +
RCC HERVE neg
+ HERV peptide
RCC HERVE neg

FIG. 4A



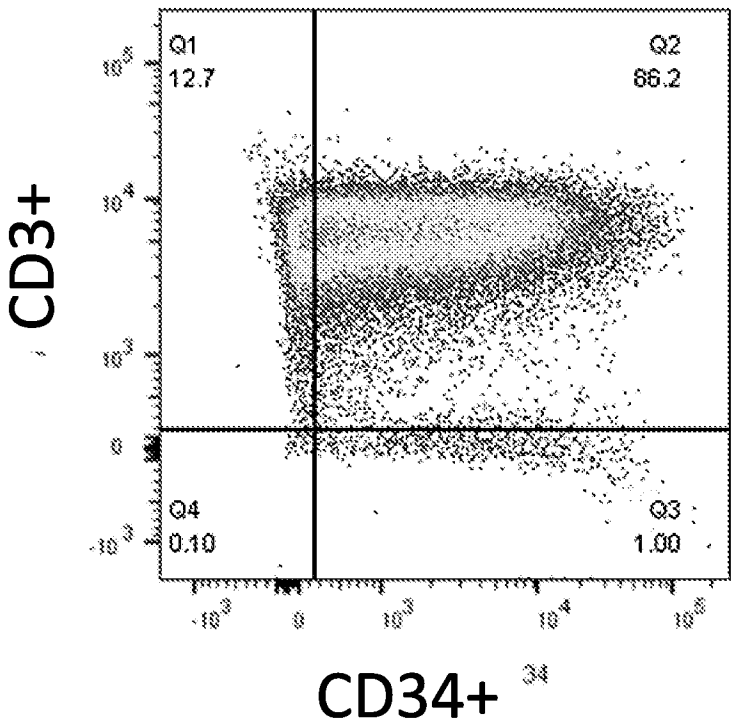


FIG. 4B

FIG. 4C

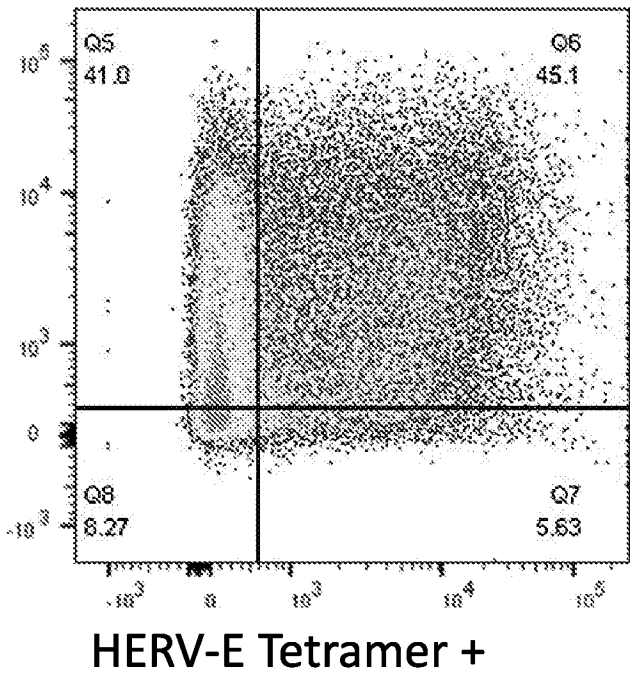


FIG.5A

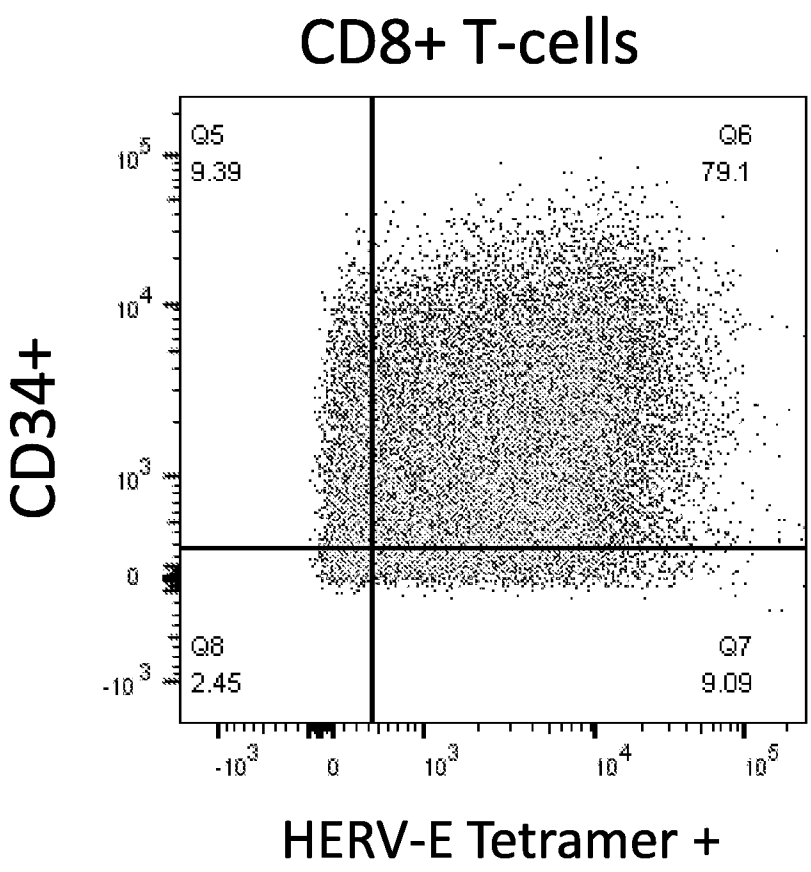

CD8+ T-cells
Q5
9.39
Q6
79.1
Q8
2.45
Q7
9.09
CD34+
HERV-E Tetramer +

FIG.5B

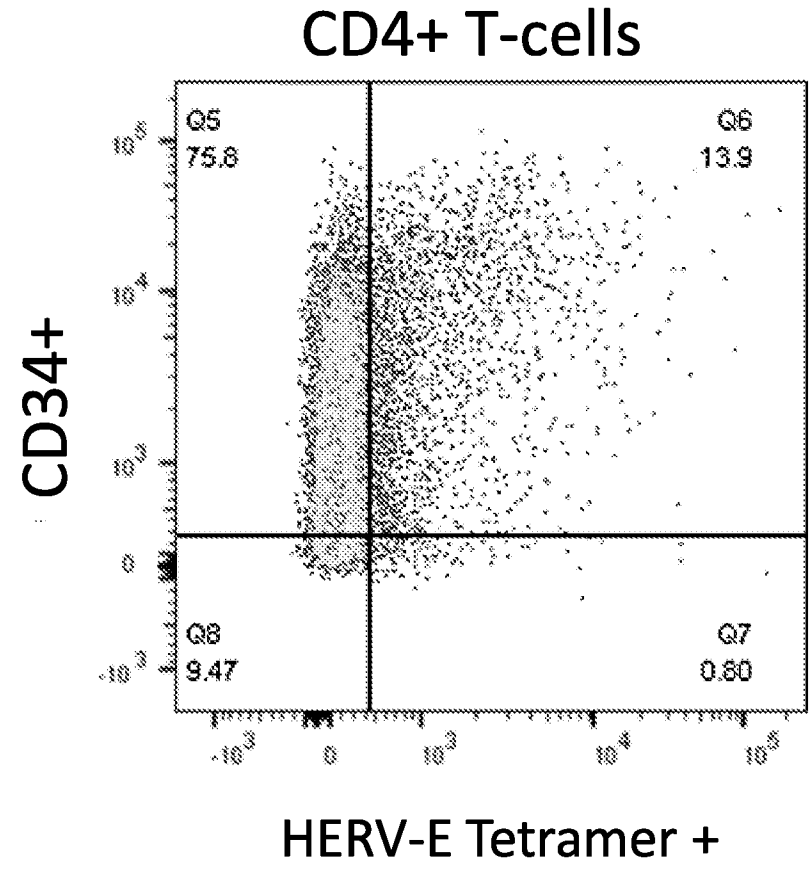

CD4+ T-cells
Q5
75.8
Q6
13.9
Q8
9.47
Q7
0.80
CD34+
HERV-E Tetramer +

FIG.6A

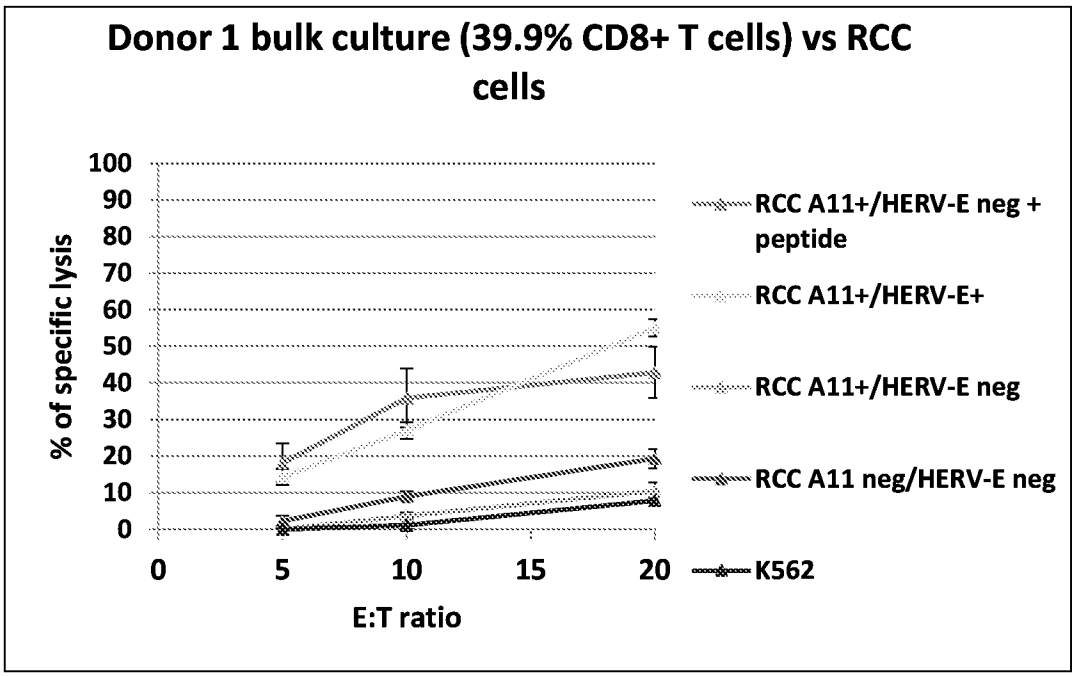

Donor 1 bulk culture (39.9% CD8+ T cells) vs RCC cells
% of specific lysis
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
E:T ratio
RCC A11+/HERV-E neg + peptide
RCC A11+/HERV-E+
RCC A11+/HERV-E neg
RCC A11 neg/HERV-E neg
K562

FIG.6B

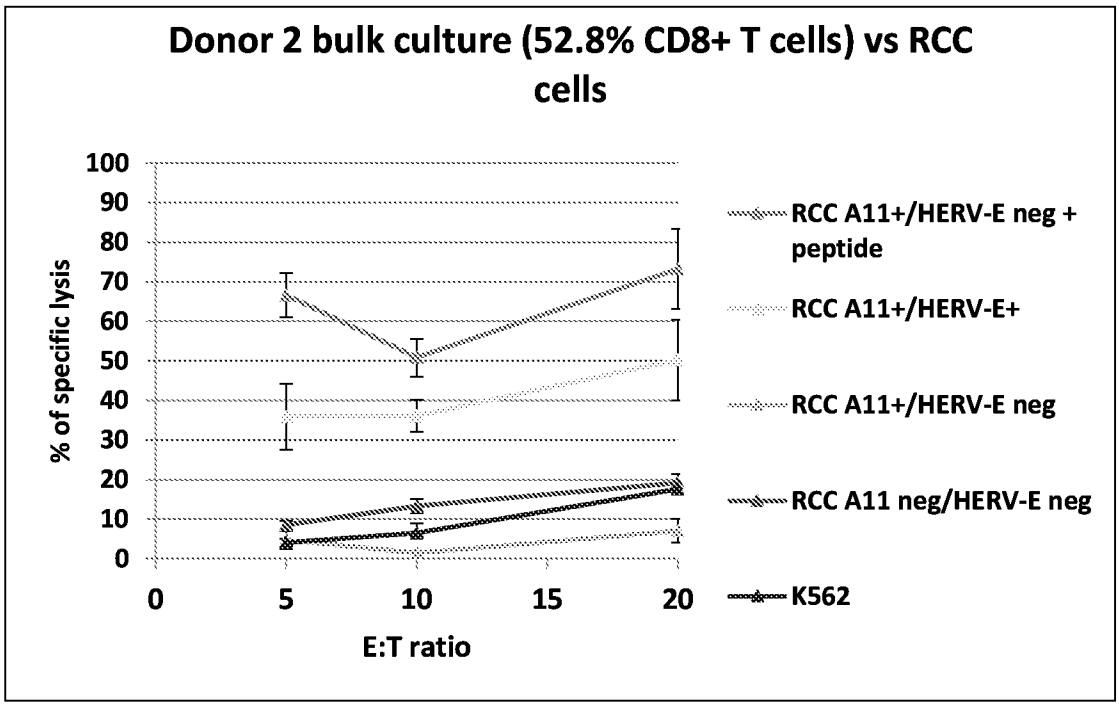

Donor 2 bulk culture (52.8% CD8+ T cells) vs RCC cells
% of specific lysis
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
E:T ratio
RCC A11+/HERV-E neg + peptide
RCC A11+/HERV-E+
RCC A11+/HERV-E neg
RCC A11 neg/HERV-E neg
K562

FIG. 7

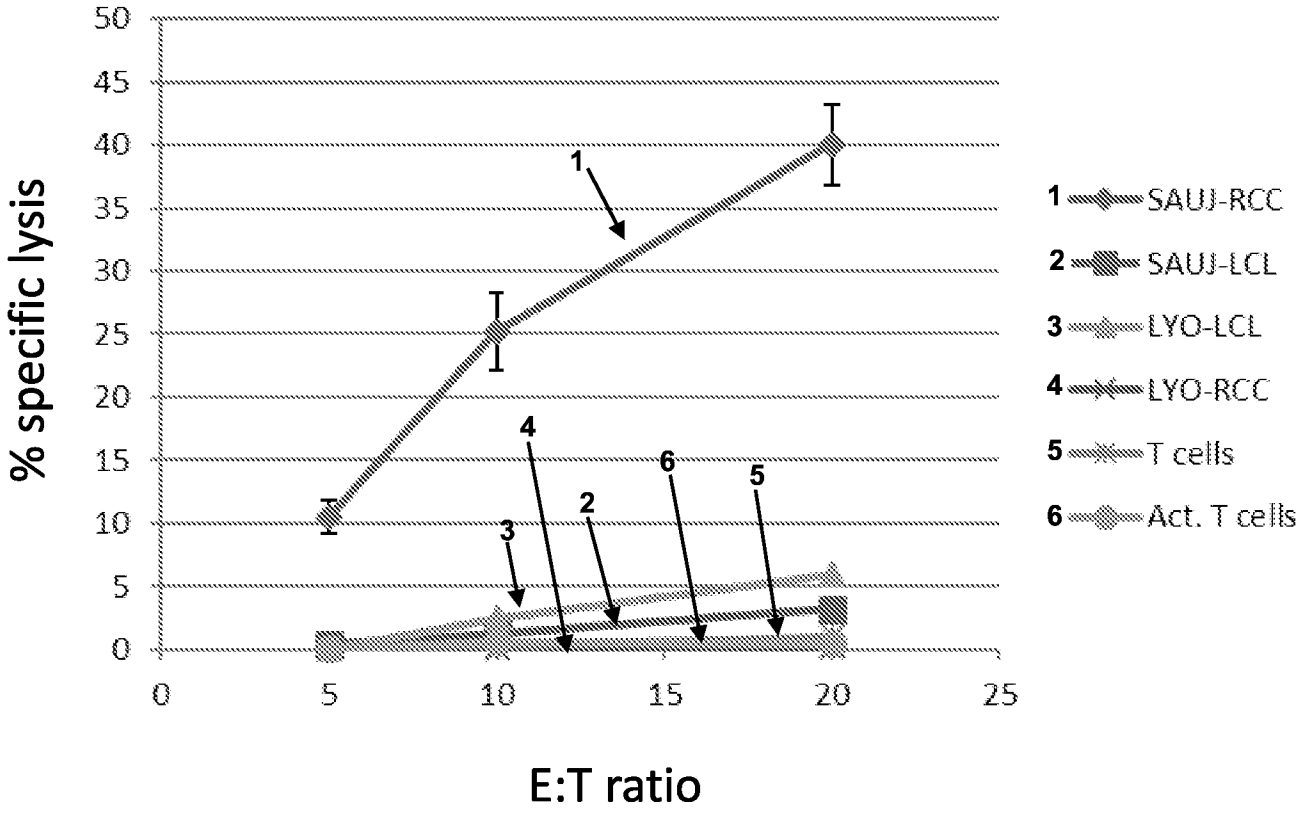

% specific lysis
E:T ratio
1 SAUJ-RCC
2 SAUJ-LCL
3 LYO-LCL
4 LYO-RCC
5 T cells
6 Act. T cells

FIG. 8

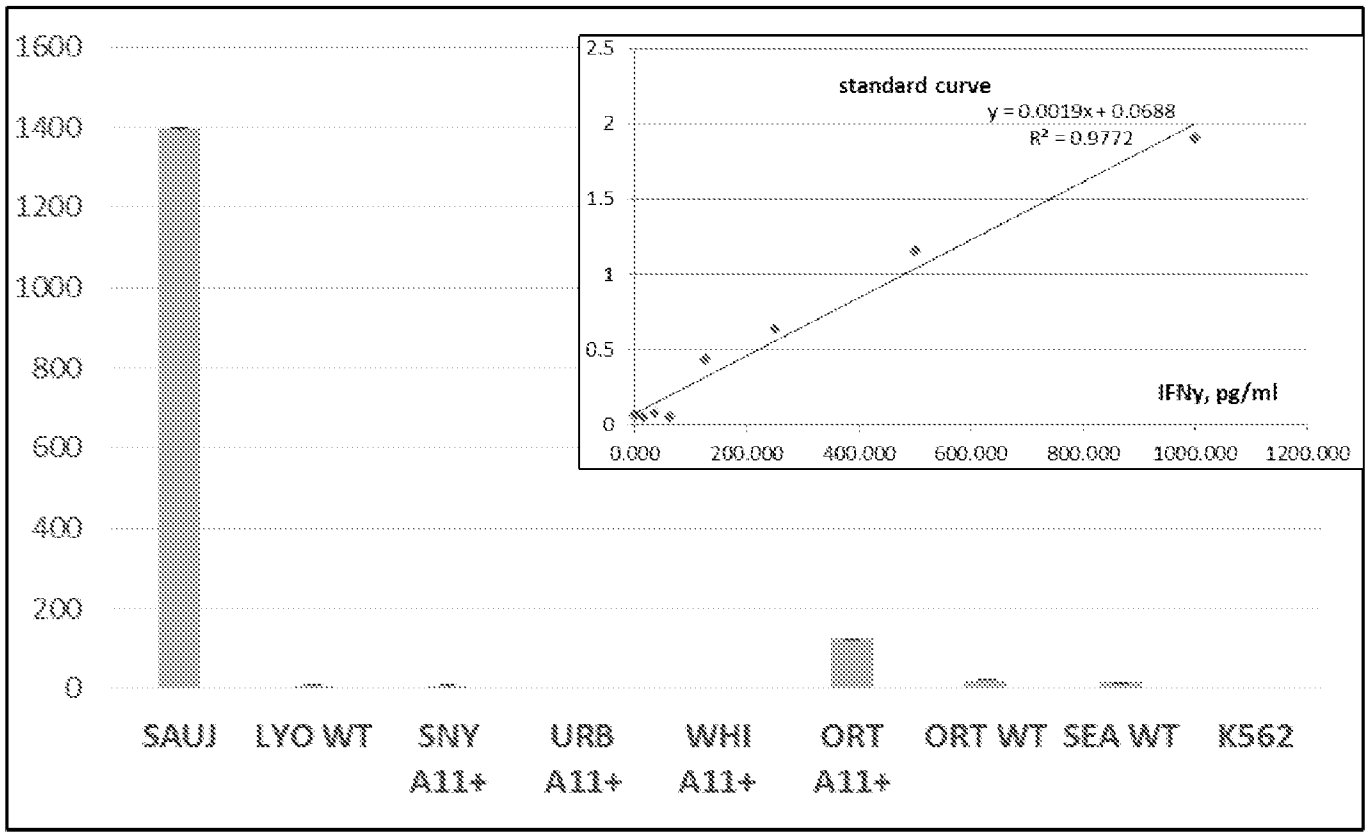

standard curve
y = 0.0019x + 0.0688
R² = 0.9772
IFNγ, pg/ml
SAUJ
LYO WT
SNY A11+
URB A11+
WHI A11+
ORT A11+
ORT WT
SEA WT
K562

FIG. 9

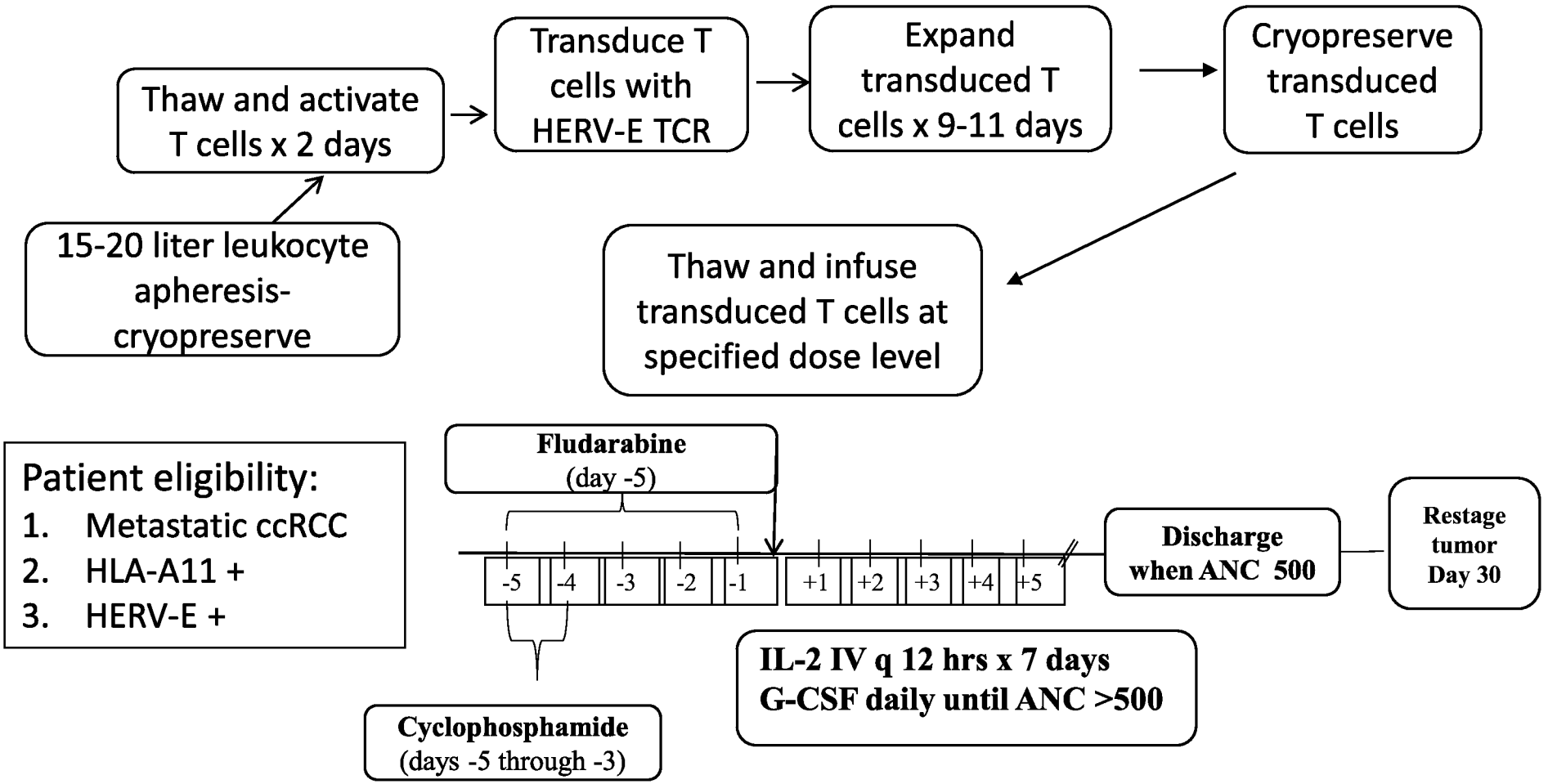
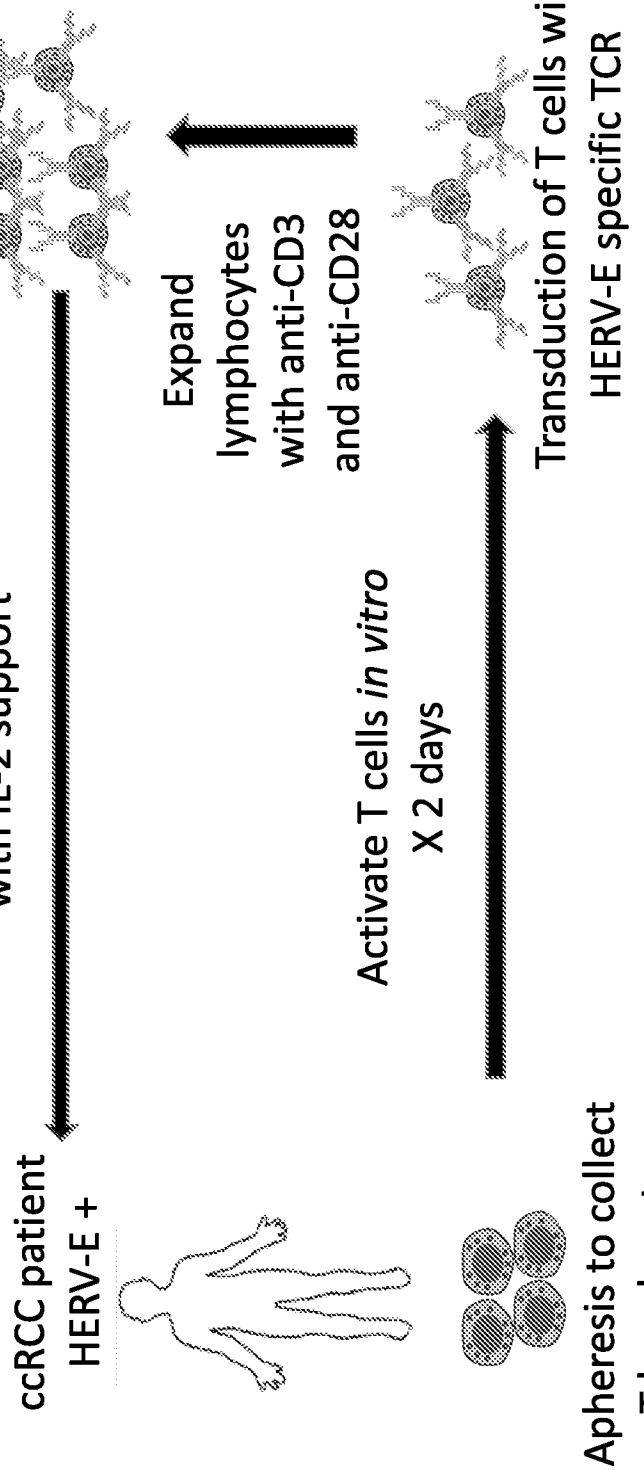

Thaw and activate T cells x 2 days
Transduce T cells with HERV-E TCR
Expand transduced T cells x 9-11 days
Cryopreserve transduced T cells
15-20 liter leukocyte apheresis-cryopreserve
Thaw and infuse transduced T cells at specified dose level
Patient eligibility:
1. Metastatic ccRCC
2. HLA-A11 +
3. HERV-E +
Fludarabine (day -5)
-5 -4 -3 -2 -1 +1 +2 +3 +4 +5
Discharge when ANC 500
Restage tumor Day 30
IL-2 IV q 12 hrs x 7 days
G-CSF daily until ANC >500
Cyclophosphamide (days -5 through -3)
Cyclophosphamide 1000 mg/m2 iv day -5
Fludarabine 30 mg/m^3 days -5,-4,-3
IL-2 2,000,000 iu/m2 iv q 12 hrs x 7 days
G-CSF 300 mcg sq daily day +1 until ANC >500

FIG. 10


ccRCC patient
HERV-E +
Apheresis to collect
T lymphocytes
Activate T cells *in vitro*
X 2 days
Transduction of T cells with
HERV-E specific TCR
Expand
lymphocytes
with anti-CD3
and anti-CD28
After chemotherapy induced
lymphodepletion, T cells are infused
with IL-2 support

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/040448

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/725 A61K35/17 C12N15/85
ADD. C07K14/005 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, FSTA, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2013 176373 A (SHIZUOKA PREFECTURE) 9 September 2013 (2013-09-09) Par.1, Examples 1, 11, Cl.4 & DATABASE Geneseq [Online] 7 November 2013 (2013-11-07), "Human anti-MAGE1 TCR alpha-chain gene, SEQ ID NO: 46.", retrieved from EBI accession no. GSN:BAT10537 Database accession no. BAT10537 sequence -/--	1,4,9, 12,13, 18,20, 41,44,49

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 October 2017	17/10/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bonello, Steve

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/040448

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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