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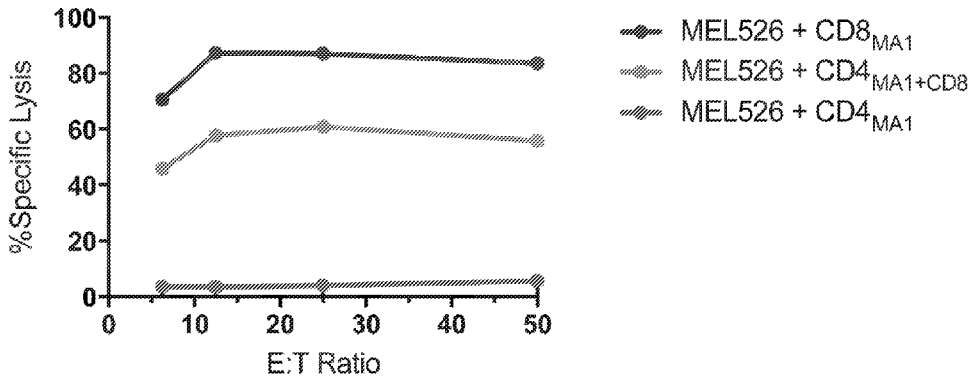

FIG. 8C

(57) Abstract: The present disclosure provides TCRs with high or enhanced affinity against various tumor associated antigens (including human MAGE-A1 epitopes), T cells expressing such high affinity antigen specific TCRs, nucleic acids encoding the same, and compositions for use in treating diseases or disorders in which cells overexpress one or more of these antigens, such as in cancer.

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HIGH AFFINITY MAGE-A1-SPECIFIC TCRS AND USES THEREOF

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 360056_446WO_SEQUENCE_LISTING.txt. The text file is 86.3 KB, was created on March 14, 2018, and is being submitted electronically via EFS-Web.

BACKGROUND

Adoptive transfer of tumor-specific T-cells is an appealing strategy to eliminate existing tumors and requires the establishment of a robust population of antigen-specific T cells *in vivo* to eliminate existing tumor and prevent recurrences (Stromnes *et al.*, *Immunol. Rev. 257*:145, 2014). Although transfer of tumor-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) is safe and can mediate direct anti-tumor activity in select patients (Chapuis *et al.*, *Cancer Res. 72*:LB-136, 2012; Chapuis *et al.*, *Sci. Transl. Med. 5*:174ra127, 2013; Chapuis *et al.*, *Proc. Nat'l. Acad. Sci. U.S.A. 109*:4592, 2012),[2-4] the variability in the avidity of the CTLs isolated from each patient or donor limits the anti-tumor efficacy in clinical trials (Chapuis *et al.*, 2013). Since TCR affinity is an important determinant of CTL avidity (Zoete *et al.*, *Frontiers Immunol. 4*:268, 2013), strategies have been developed to redirect the antigen specificity of donor or patient T cells using high affinity TCRα/β genes isolated from a well-characterized T cell clone specific for a tumor-specific antigen (Stromnes *et al.*, *Immunol. Rev. 257*:145, 2014; Robbins *et al.*, *J. Clin. Oncol. 29*:917, 2011). Such high affinity self/tumor-reactive T cells are rare since T cells that express self/tumor-reactive TCRs are subject to central and peripheral tolerance (Stone and Kranz, *Frontiers Immunol. 4*:244, 2013), with relative TCR affinities varying widely between donors. Therefore, many matched donors must be screened to identify a sufficiently high-affinity tumor-specific T cell clone from which a TCRα/β gene therapy construct can be generated. For example, isolation of a naturally elicited Wilms' Tumor antigen 1 (WT1)-specific TCR with high functional avidity for a single HLA-allele required screening of hundreds of WT-

specific T cell lines representing thousands of individual T cell clones from the peripheral repertoires of greater than 75 normal donors, a very time and labor intensive process (Chapuis *et al.*, 2013; Schmitt *et al.*, *Hum. Gene Ther. 20:*1240, 2009; Ho *et al.*, *J. Immunol. Methods 310*:40, 2006).

There is a need for alternative antigen-specific TCR immunotherapies directed against various cancers, such as leukemia and tumors. Presently disclosed embodiments address these needs and provide other related advantages.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show representative data illustrating that high-affinity T cells for viral antigens are found at higher frequencies (A) than high-affinity T cells for self-antigens, which are found at very low frequencies (B).

Figures 2A and 2B show, respectively, (A) a schematic of a T cell enrichment assay performed by the inventors of the present disclosure, (B) flow cytometry data from a series of sorting experiments used to enrich for antigen-specific $CD8^+$ T cells.

Figure 3 shows exemplary data from a TCRβ CDR3 enrichment scheme of the present disclosure using MAGE-A1:HLA tetramers.

Figures 4A and 4B show, respectively, (A) specific binding of MAGE-A1:HLA tetramers by TCRs identified using methods of the present disclosure and (B) enrichment of MAGE-A1-specific TCRs.

Figures 5A-5C provide, respectively, (A) flow cytometry data showing MAGE-A1-specific $CD8^+$ T cells of the present disclosure binding MAGE-A1:HLA tetramers, (B) cytokine production by MAGE-A1-specific $CD8^+$ T cells in the absence (left) or presence (right) of antigen-expressing U266 myeloma cells, and (C) specific lysis data showing that high-affinity MAGE-A1 TCR-transduced $CD8^+$ T cells of this disclosure bind antigen:MHC tetramers and kill cells presenting MAGE-A1: MHC (A*0201). Data in (C) was from a standard Cr^{51}-release assay in which the $CD8^+$ T cells were co-cultured with U266 cells alone, with exogenous interferon-gamma (IFNγ) or with exogenous MAGE-A1 peptide.

Figure 6A illustrates an immunotherapy approach according to the present disclosure in which $CD4^+$ T cells are transduced to express a TCR and a CD8 co-

receptor, both from a CD8+ T cell that is specific for a peptide antigen. Activation of the transduced CD4+ T cell can augment or improve the antigenic response of CD8+ T cells, such as infused CTLs in an immunotherapy setting. Figure 6B shows the design of an experiment performed by the inventors of the present disclosure in which a CD4+ T cell was transduced to express a CDS-independent MHC Class I-restricted TCR, but not a CD8 co-receptor.

Figure 7 A shows flow cytometry data from an experiment in which T cells (CD8+ and CD4+) expressing high-affinity CD8 anti-MAGE-Al TCR were assayed for binding to MAGE-Al :MHC tetramers.

Figure 7B shows specific binding by the MAGE-Al-specific T cells to MAGE-Al :MHC tetramers.

Figure 7C shows target cell lysis (Cr51 release) by CD8+ T cells expressing MAGE-Al-specific TCR of this disclosure and the lack of killing by comparable CD4+ T cells.

Figure 8A shows a schematic illustrating an experiment conducted by the inventors of the present disclosure in which CD4+ T cells were transduced to express the high-affinity MAGE Al Class I TCR plus a CD8αβ, co-receptor and examined for functionality in the presence of cells expressing peptide:MHC. Figure 8B shows that a higher proportion of the CD4+ T cells transduced with both MAGE-Al TCR and CD8 co-receptor produced cytokines as compared to CD4+ T cells expressing the MAGE-Al TCR alone. Figure 8C shows specific lysis of antigen-presenting MEL526 melanoma functionality in the presence of cells expressing peptide:MHC. Figure 8B shows that a higher proportion of the CD4+ T cells transduced with both MAGE-Al TCR and CD8 co-receptor produced cytokines as compared to CD4+ T cells expressing the MAGE-Al TCR alone. Figure 8C shows specific lysis of antigen-presenting MEL526 melanoma target cells by the indicated T cells. Figure 8D shows expansion of the two groups of transduced CD4+ T cells following stimulation with antigen.

DETAILED DESCRIPTION

According to a first aspect, the present invention provides an isolated modified cell comprising a heterologous polynucleotide encoding a binding protein, wherein the encoded binding protein comprises:

a T cell receptor (TCR) α-chain variable (V_α) domain having CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:48-50, respectively, and a TCR β-chain variable (V_β) domain having CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:45-47, respectively,

wherein the binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123):human leukocyte antigen (HLA)-A*0201 complex on a cell surface independent of CD8 or in the absence of CD8.

According to a second aspect, the present invention provides a composition comprising a modified cell according to the first aspect and a pharmaceutically acceptable carrier, diluent, or excipient, wherein, optionally,the composition comprises modified CD4+ T cells, modified CD8+ T cells, or modified CD4+ T cells and modified CD8+ T cells.

According to a third aspect, the present invention provides a unit dose, comprising an effective amount of (i) the modified cell according to the first aspect or (ii) a composition according to the second aspect, wherein, optionally,(a) the unit dose comprises (i) at least about 30% modified CD4+ T cells, combined with (ii) a composition comprising at least about 30% modified CD8+ T cells, in about a 1:1 ratio; and/or(b) the unit dose comprises (i) at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% engineered CD4+ T cells, combined with (ii) at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% engineered CD8+ T cells, in about a 1:1 ratio, wherein, further optionally, the unit dose comprises:

(1) at least about 30% modified CD4+ T cells, combined with a composition comprising at least about 30% modified CD8+ T cells, in about a 1:1 ratio;

(2) at least about 40% modified CD4+ T cells, combined with a composition comprising at least about 40% modified CD8+ T cells, in about a 1:1 ratio;

(3) at least about 50% modified CD4+ T cells, combined with a composition comprising at least about 50% modified CD8+ T cells, in about a 1:1 ratio;

(4) at least about 60% modified CD4+ T cells, combined with a composition comprising at least about 60% modified CD8+ T cells, in about a 1:1 ratio;

(5) at least about 70% modified CD4+ T cells, combined with a composition comprising at least about 70% modified CD8+ T cells, in about a 1:1 ratio;

(6) at least about 70% modified CD4+ T cells, combined with a composition comprising at least about 70% modified CD8+ T cells, in about a 1:1 ratio;

(7) at least about 80% modified CD4+ T cells, combined with a composition comprising at least about 80% modified CD8+ T cells, in about a 1:1 ratio;

(8) at least about 85% modified CD4+ T cells, combined with a composition comprising at least about 85% modified CD8+ T cells, in about a 1:1 ratio;

(9) at least about 90% modified CD4+ T cells, combined with a composition comprising at least about 90% modified CD8+ T cells, in about a 1:1 ratio; or

(10) at least about 95% modified CD4+ T cells, combined with a composition comprising at least about 95% modified CD8+ T cells, in about a 1:1 ratio; and/or(c) the unit dose contains substantially no naïve T cells; and/or(d) the unit dose comprises equal, or approximately equal numbers, of modified $CD45RA^{-}$ $CD3^{+}$ $CD8^{+}$ and modified $CD45RA^{-}$ $CD3^{+}$ $CD4^{+}$ TM cells.

According to a fourth aspect, the present invention provides an isolated polynucleotide encoding:

(1) a binding protein having a TCR V_{α} domain and a TCR V_{β} domain, wherein:

(i) the encoded V_{α} domain comprises CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:48-50, respectively, and the encoded V_{β} domain comprises CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:45-47, respectively; and

(ii) the encoded binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123): human leukocyte antigen (HLA)-A*0201 complex on a cell surface independent of or in the absence of CD8; and

(2) at least an extracellular portion of a CD8 co-receptor.

According to a fifth aspect, the present invention provides an expression vector, comprising a polynucleotide according to the fourth aspect operably linked to an expression control sequence, wherein, optionally, (1) the vector is capable of delivering the polynucleotide to a host cell, wherein, further optionally, the host cell is a hematopoietic progenitor cell or a human immune system cell, wherein, even further optionally, the human immune system cell is a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a natural killer cell, a dendritic cell, or any combination thereof, wherein, even further optionally, the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof, and/or

(2) the vector is a viral vector, wherein, further optionally,

the viral vector is a lentiviral vector or a γ-retroviral vector.

According to a sixth aspect, the present invention provides a method for treating a hyperproliferative disorder associated with MAGE-A1 expression, comprising administering to human subject in need thereof a modified cell according to the first aspect, a composition according to the second aspect, or a unit dose according to the third aspect.

According to a seventh aspect, the present invention provides use of a modified cell according to the first aspect, a composition according to the second aspect, or a unit dose according to the third aspect, in the preparation of a medicament for treating a hyperproliferative disorder associated with MAGE-A1 expression.

According to an eighth aspect, the present invention provides a method for making a modified cell of the first aspect, comprising introducing into the cell or a cell culture a polynucleotide encoding the binding protein, wherein, optionally, (1) the polynucleotide is comprised in a vector and/or (2) the polynucleotide is according to the fourth aspect.

In certain aspects, the present disclosure provides compositions comprising binding proteins specific for MAGE-Al peptide antigens associated with a major histocompatibility complex (MHC) (e.g., human leukocyte antigen, HLA), which can be used in, for example, treating diseases or disorders associated with MAGE-Al expression (e.g., cancer) or adoptive immunotherapy to treat cancer. In certain embodiments, the instant disclosure provides polynucleotides encoding such MAGE-Al-specific binding proteins, as well as host cells modified to express MAGE-Al- specific binding proteins (e.g., TCRs)

In other aspects, the present disclosure provides modified $CD4^+$ T cells comprising a heterologous polynucleotide encoding a TCR from a $CD8^+$ T cell that is capable of specifically binding to a peptide antigen (*e.g.*, MAGE-A1) and optionally comprising a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor molecule.

By way of background, most tumor targets for T cell-based immunotherapies are self-antigens since tumors arise from previously normal tissue. For example, such tumor-associated antigens (TAAs) may be expressed at high levels in a cancer cell, but may not be expressed or may be minimally expressed in other cells. During T cell development in the thymus, T cells that bind weakly to self-antigens are allowed to survive in the thymus, and can undergo further development and maturation, while T cells that bind strongly to self-antigens are eliminated by the immune system since such cells would mount an undesirable autoimmune response. Hence, T cells are sorted by their relative ability to bind to antigens to prepare the immune system to respond against a foreign invader (*i.e.*, recognition of non-self-antigen) while at the same time preventing an autoimmune response (*i.e.*, recognition of self-antigen). This tolerance mechanism limits naturally occurring T cells that can recognize tumor (self) antigens with high affinity and, therefore, eliminates the T cells that would effectively eliminate tumor cells. Consequently, isolating T cells having high affinity TCRs specific for tumor antigens is difficult because most such cells are essentially eliminated by the immune system.

The instant disclosure provides TCRs specific for MAGE-A1 (also called MAGE-1, MAGE family member A1, CT 1.1, and Melanoma-Antigen Gene 1) peptides, such as high affinity TCRs specific for MAGE-A1 peptides, wherein a cell expressing such a TCR is capable of binding to a MAGE-A1:HLA complex independent of CD8. In addition, such TCRs may optionally be capable of more efficiently associating with a CD3 protein as compared to endogenous TCRs.

A method was developed to quickly and simultaneously screen and rank T cell clonotypes (based on affinity) from a large cohort of HLA-matched donors in a short time (about 6-8 weeks). In certain embodiments, the instant disclosure provides methods for enriching for cells with high-affinity TCRs by using limiting

concentrations of antigen-specific pMHC multimers in the presence of a subject's immune cells (*e.g.*, PBMCs). The TCRβ repertoire and frequency analysis, coupled with bioinformatics, was used to accurately identify TCR α-chain and β-chain pairs. An advantage of these methods is that they allow for a quick comparison of the TCR affinity of thousands of clones from multiple donors as opposed to cloning individual TCRs.

The compositions and methods described herein will in certain embodiments have therapeutic utility for the treatment of diseases and conditions associated with MAGE-A1 expression. Such diseases include various forms of hyperproliferative disorders, such as hematological malignancies and solid cancers. Non-limiting examples of these and related uses are described herein and include *in vitro*, *ex vivo* and *in vivo* stimulation of MAGE-A1 antigen-specific T cell responses, such as by the use of recombinant T cells expressing an enhanced or high affinity TCR specific for a MAGE-A1 peptide.

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein. Additional definitions are set forth throughout this disclosure.

In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, the term "about" means ± 20% of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include," "have" and "comprise" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

In addition, it should be understood that the individual compounds, or groups of compounds, derived from the various combinations of the structures and substituents described herein, are disclosed by the present application to the same extent as if each compound or group of compounds was set forth individually. Thus, selection of particular structures or particular substituents is within the scope of the present disclosure.

The term "consisting essentially of" is not equivalent to "comprising" and refers to the specified materials or steps of a claim, or to those that do not materially affect the basic characteristics of a claimed subject matter. . For example, a protein domain, region, or module (*e.g.*, a binding domain, hinge region, linker module) or a protein (which may have one or more domains, regions, or modules) "consists essentially of" a particular amino acid sequence when the amino acid sequence of a domain, region, module, or protein includes extensions, deletions, mutations, or a combination thereof (*e.g.*, amino acids at the amino- or carboxy-terminus or between domains) that, in combination, contribute to at most 20% (*e.g.*, at most 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2% or 1%) of the length of a domain, region, module, or protein and do not substantially affect (*i.e.*, do not reduce the activity by more than 50%, such as no more than 40%, 30%, 25%, 20%, 15%, 10%, 5%, or 1%) the activity of the domain(s), region(s), module(s), or protein (*e.g.*, the target binding affinity of a binding protein).

As used herein, an "immune system cell" means any cell of the immune system that originates from a hematopoietic stem cell in the bone marrow, which gives rise to two major lineages, a myeloid progenitor cell (which give rise to myeloid cells such as monocytes, macrophages, dendritic cells, megakaryocytes and granulocytes) and a lymphoid progenitor cell (which give rise to lymphoid cells such as T cells, B cells and natural killer (NK) cells). Exemplary immune system cells include a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a regulatory T cell, a natural killer cell, and a dendritic cell. Macrophages and dendritic cells may be referred to as "antigen presenting cells" or "APCs," which are specialized cells that can activate T cells when a major histocompatibility complex (MHC) receptor on the surface of the APC complexed with a peptide interacts with a TCR on the surface of a T cell.

"Major histocompatibility complex" (MHC) refers to glycoproteins that deliver peptide antigens to a cell surface. MHC class I molecules are heterodimers having a membrane spanning α chain (with three α domains) and a non-covalently associated β2 microglobulin. MHC class II molecules are composed of two transmembrane glycoproteins, α and β, both of which span the membrane. Each chain has two domains. MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where a peptide:MHC complex is recognized by $CD8^+$ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by $CD4^+$ T cells. Human MHC is referred to as human leukocyte antigen (HLA).

A "T cell" is an immune system cell that matures in the thymus and produces T cell receptors (TCRs). T cells can be naïve (not exposed to antigen; increased expression of CD62L, CCR7, CD28, CD3, CD127, and CD45RA, and decreased expression of CD45RO as compared to T_{CM}), memory T cells (T_M) (antigen-experienced and long-lived), and effector cells (antigen-experienced, cytotoxic). T_M can be further divided into subsets of central memory T cells (T_{CM}, increased expression of CD62L, CCR7, CD28, CD127, CD45RO, and CD95, and decreased expression of CD54RA as compared to naïve T cells) and effector memory T cells (T_{EM}, decreased expression of CD62L, CCR7, CD28, CD45RA, and increased expression of CD127 as compared to naïve T cells or T_{CM}). Effector T cells (T_E) refers to antigen-experienced CD8+ cytotoxic T lymphocytes that have decreased expression of CD62L ,CCR7, CD28, and are positive for granzyme and perforin as compared to T_{CM}. Other exemplary T cells include regulatory T cells, such as CD4+ CD25+ (Foxp3+) regulatory T cells and Treg17 cells, as well as Tr1, Th3, CD8+CD28-, and Qa-1 restricted T cells.

"T cell receptor" (TCR) refers to an immunoglobulin superfamily member (having a variable binding domain, a constant domain, a transmembrane region, and a short cytoplasmic tail; *see*, *e.g.*, Janeway *et al.*, *Immunobiology: The Immune System in Health and Disease*, 3rd Ed., Current Biology Publications, p. 4:33, 1997) capable of specifically binding to an antigen peptide bound to a MHC receptor. A TCR can be found on the surface of a cell or in soluble form and generally is comprised of a

heterodimer having α and β chains (also known as TCRα and TCRβ, respectively), or γ and δ chains (also known as TCRγ and TCRδ, respectively). Like immunoglobulins, the extracellular portion of TCR chains (*e.g.*, α-chain, β-chain) contain two immunoglobulin domains, a variable domain (*e.g.*, α-chain variable domain or V_α, β-chain variable domain or V_β; typically amino acids 1 to 116 based on Kabat numbering Kabat *et al.*, "Sequences of Proteins of Immunological Interest, US Dept. Health and Human Services, Public Health Service National Institutes of Health, 1991, 5th ed.) at the N-terminus, and one constant domain (*e.g.*, α-chain constant domain or C_α, typically amino acids 117 to 259 based on Kabat, β-chain constant domain or C_β, typically amino acids 117 to 295 based on Kabat) adjacent to the cell membrane. Also like immunoglobulins, the variable domains contain complementary determining regions (CDRs) separated by framework regions (FRs) (*see*, *e.g.*, Jores *et al.*, *Proc. Nat'l Acad. Sci. U.S.A. 87*:9138, 1990; Chothia *et al.*, *EMBO J. 7*:3745, 1988; *see also* Lefranc *et al.*, *Dev. Comp. Immunol. 27*:55, 2003). The V_α and V_β of a native TCR generally have similar structures, with each variable domain comprising four conserved FRs and three CDRs. The V_α domain is encoded by two separate DNA segments, the variable gene segment and the joining gene segment (V-J); the V_β domain is encoded by three separate DNA segments, the variable gene segment, the diversity gene segment, and the joining gene segment (V-D-J). A single V_α or V_β domain may be sufficient to confer antigen-binding specificity. Furthermore, TCRs that bind a particular antigen may be isolated using a V_α or V_β domain from a TCR that binds the antigen to screen a library of complementary V_α or V_β domains, respectively. In certain embodiments, a TCR is found on the surface of T cells (or T lymphocytes) and associates with the CD3 complex. The source of a TCR as used in the present disclosure may be from various animal species, such as a human, mouse, rat, rabbit or other mammal.

As used herein, the term "CD8 co-receptor" or "CD8" means the cell surface glycoprotein CD8, either as an alpha-alpha homodimer or an alpha-beta heterodimer. The CD8 co-receptor assists in the function of cytotoxic T cells ($CD8^+$) and functions through signaling via its cytoplasmic tyrosine phosphorylation pathway (Gao and Jakobsen, *Immunol. Today* 21:630-636, 2000; Cole and Gao, *Cell. Mol. Immunol.* 1:81-88, 2004). There are five (5) different CD8 beta chains (*see* UniProtKB identifier

P10966) and a single CD8 alpha chain (*see* UniProtKB identifier P01732). CD8 generally binds pMHC Class I complexes.

"CD4 co-receptor" or "CD4" refers to an immunoglobulin co-receptor glycoprotein that assists the TCR in communicating with antigen-presenting cells (*see,* Campbell & Reece, Biology 909 (Benjamin Cummings, Sixth Ed., 2002)). CD4 is found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells, and includes four immunoglobulin domains (D1 to D4) that are expressed at the cell surface. During antigen presentation, CD4 is recruited, along with the TCR complex, to bind to different regions of the MHCII molecule (CD4 binds MHCII β2, while the TCR complex binds MHCII α1/β1). Without wishing to be bound by theory, it is believed that close proximity to the TCR complex allows CD4-associated kinase molecules to phosphorylate the immunoreceptor tyrosine activation motifs (ITAMs) present on the cytoplasmic domains of CD3. This activity is thought to amplify the signal generated by the activated TCR in order to produce various types of T helper cells. CD4 generally binds pMHC Class II complexes.

"CD3" is a multi-protein complex of six chains (*see*, Abbas and Lichtman, 2003; Janeway *et al.*, p172 and 178, 1999). In mammals, the complex comprises a CD3γ chain, a CD3δ chain, two CD3ε chains, and a homodimer of CD3ζ chains. The CD3γ, CD3δ, and CD3ε chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3γ, CD3δ, and CD3ε chains are negatively charged, which is a characteristic that allows these chains to associate with the positively charged T cell receptor chains. The intracellular tails of the CD3γ, CD3δ, and CD3ε chains each contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM, whereas each CD3ζ chain has three. Without wishing to be bound by theory, it is believed the ITAMs are important for the signaling capacity of a TCR compelx. CD3 as used in the present disclosure may be from various animal species, including human, mouse, rat, or other mammals.

As used herein, "TCR complex" refers to a complex formed by the association of CD3 with TCR. For example, a TCR complex can be composed of a CD3γ chain, a CD3δ chain, two CD3ε chains, a homodimer of CD3ζ chains, a TCRα chain, and a

TCRβ chain. Alternatively, a TCR complex can be composed of a CD3γ chain, a CD3δ chain, two CD3ε chains, a homodimer of CD3ζ chains, a TCRγ chain, and a TCRδ chain.

A "component of a TCR complex," as used herein, refers to a TCR chain (*i.e.*, TCRα, TCRβ, TCRγ or TCRδ), a CD3 chain (*i.e.*, CD3γ, CD3δ, CD3ε or CD3ζ), or a complex formed by two or more TCR chains or CD3 chains (*e.g.*, a complex of TCRα and TCRβ, a complex of TCRγ and TCRδ, a complex of CD3ε and CD3δ, a complex of CD3γ and CD3ε, or a sub-TCR complex of TCRα, TCRβ, CD3γ, CD3δ, and two CD3ε chains).

A "binding domain" (also referred to as a "binding region" or "binding moiety"), as used herein, refers to a molecule or portion thereof (*e.g.*, peptide, oligopeptide, polypeptide, protein) that possesses the ability to specifically and non-covalently associate, unite, or combine with a target (*e.g.*, MAGE-A1, MAGE-A1 peptide:MHC complex). A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule, a molecular complex (*i.e.*, complex comprising two or more biological molecules), or other target of interest. Exemplary binding domains include single chain immunoglobulin variable regions (*e.g.*, scTCR, scFv), receptor ectodomains, ligands (*e.g.*, cytokines, chemokines), or synthetic polypeptides selected for their specific ability to bind to a biological molecule, a molecular complex or other target of interest.

As used herein, "specifically binds" or "specific for" refers to an association or union of a binding protein (*e.g.*, TCR receptor) or a binding domain (or fusion protein thereof) to a target molecule with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10^5 M^{-1} (which equals the ratio of the on-rate [k_{on}] to the off-rate [k_{off}] for this association reaction), while not significantly associating or uniting with any other molecules or components in a sample. Binding proteins or binding domains (or fusion proteins thereof) may be classified as "high affinity" binding proteins or binding domains (or fusion proteins thereof) or as "low affinity" binding proteins or binding domains (or fusion proteins thereof). "High affinity" binding proteins or binding domains refer to those binding proteins or binding domains having a K_a of at least 10^7

M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, or at least 10^{13} M^{-1}. "Low affinity" binding proteins or binding domains refer to those binding proteins or binding domains having a K_a of up to 10^7 M^{-1}, up to 10^6 M^{-1}, up to 10^5 M^{-1}. Alternatively, affinity may be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (*e.g.*, 10^{-5} M to 10^{-13} M).

In certain embodiments, a receptor or binding domain may have "enhanced affinity," which refers to selected or engineered receptors or binding domains with stronger binding to a target antigen than a wild type (or parent) binding domain. For example, enhanced affinity may be due to a K_a (equilibrium association constant) for the target antigen that is higher than the wild type binding domain, due to a K_d (dissociation constant) for the target antigen that is less than that of the wild type binding domain, due to an off-rate (k_{off}) for the target antigen that is less than that of the wild type binding domain, or a combination thereof. In certain embodiments, enhanced affinity TCRs may be codon optimized to enhance expression in a particular host cell, such as T cells (Scholten *et al.*, *Clin. Immunol. 119*:135, 2006).

A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, as well as determining binding domain or fusion protein affinities, such as Western blot, ELISA, analytical ultracentrifugation, spectroscopy and surface plasmon resonance (Biacore®) analysis (*see*, *e.g.*, Scatchard *et al.*, *Ann. N.Y. Acad. Sci. 51*:660, 1949; Wilson, *Science 295*:2103, 2002; Wolff *et al.*, *Cancer Res. 53*:2560, 1993; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

The term "MAGE-A1-specific binding protein" refers to a protein or polypeptide that specifically binds to MAGE-A1 or a peptide or fragment thereof. In some embodiments, a MAGE-A1-specific binding protein or polypeptide binds to MAGE-A1 or a peptide thereof, such as a MAGE-A1 peptide complexed with an MHC or HLA molecule, *e.g.*, on a cell surface, with at least, or at least about, a particular affinity. In certain embodiments, a MAGE-A1-specific binding protein binds a MAGE-A1-derived peptide:HLA complex (or MAGE-A1-derived peptide:MHC complex) with a K_d of less than about 10^{-8} M, less than about 10^{-9} M, less than about 10^{-10} M, less than about 10^{-11} M, less than about 10^{-12} M, or less than about 10^{-13} M, or with an affinity

that is about the same as, at least about the same as, or is greater than at or about the affinity exhibited by an exemplary MAGE-A1 specific binding protein provided herein, such as any of the MAGE-A1-specific TCRs provided herein, for example, as measured by the same assay. In certain embodiments, a MAGE-A1-specific binding protein comprises a MAGE-A1-specific immunoglobulin superfamily binding protein or binding portion thereof.

Assays for assessing affinity or apparent affinity or relative affinity include, for example, measuring apparent affinity for a TCR (or for a binding protein comprising a binding domain derived from a TCR) by assessing binding to various concentrations of tetramers, for example, by flow cytometry using labeled tetramers. In some examples, apparent K_D of a TCR is measured using 2-fold dilutions of labeled tetramers at a range of concentrations, followed by determination of binding curves by non-linear regression, apparent K_D being determined as the concentration of ligand that yielded half-maximal binding.

The term "MAGE-A1 binding domain" or "MAGE-A1 binding fragment" refer to a domain, or portion of a MAGE-A1-specific binding protein, responsible for the specific MAGE-A1 binding. A MAGE-A1-specific binding domain alone (*i.e.*, without any other portion of a MAGE-A1-specific binding protein) can be soluble and can bind to MAGE-A1 with a K_d of less than about 10^{-8} M, less than about 10^{-9} M, less than about 10^{-10} M, less than about 10^{-11} M, less than about 10^{-12} M, or less than about 10^{-13} M. Exemplary MAGE-A1-specific binding domains include MAGE-A1-specific scTCR (*e.g.*, single chain αβTCR proteins such as Vα-L-Vβ, Vβ-L-Vα, Vα-Cα-L-Vα, or Vα-L-Vβ-Cβ, wherein Vα and Vβ are TCRα and β variable domains respectively, Cα and Cβ are TCRα and β constant domains, respectively, and L is a linker) and scFv fragments as described herein, which can be derived from an anti-MAGE-A1 TCR or antibody.

Principles of antigen processing by antigen presenting cells (APC) (such as dendritic cells, macrophages, lymphocytes or other cell types), and of antigen presentation by APC to T cells, including major histocompatibility complex (MHC)-restricted presentation between immunocompatible (*e.g.*, sharing at least one allelic form of an MHC gene that is relevant for antigen presentation) APC and T cells, are

well established (*see*, *e.g.*, Murphy, Janeway's Immunobiology (8th Ed.) 2011 Garland Science, NY; chapters 6, 9 and 16). For example, processed antigen peptides originating in the cytosol (*e.g.*, tumor antigen, intrcellular pathogen) are generally from about 7 amino acids to about 11 amino acids in length and will associate with class I MHC molecules, whereas peptides processed in the vesicular system (*e.g.*, bacterial, viral) will vary in length from about 10 amino acids to about 25 amino acids and associate with class II MHC molecules.

"MAGE-A1 antigen" or "MAGE-A1 peptide antigen" refer to a naturally or synthetically produced portion of a MAGE-A1 protein ranging in length from about 7 amino acids to about 15 amino acids, which can form a complex with a MHC (*e.g.*, HLA) molecule and such a complex can bind with a TCR specific for a MAGE-A1 peptide:MHC (*e.g.*, HLA) complex.

A "linker" refers to an amino acid sequence that connects two proteins, polypeptides, peptides, domains, regions, or motifs and may provide a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity (*e.g.*, scTCR) to a target molecule or retains signaling activity (*e.g.*, TCR complex). In certain embodiments, a linker is comprised of about two to about 35 amino acids, for instance, or about four to about 20 amino acids or about eight to about 15 amino acids or about 15 to about 25 amino acids.

"Junction amino acids" or "junction amino acid residues" refer to one or more (*e.g.*, about 2-10) amino acid residues between two adjacent motifs, regions or domains of a polypeptide, such as between a binding domain and an adjacent constant domain or between a TCR chain and an adjacent self-cleaving peptide. Junction amino acids may result from the construct design of a fusion protein (*e.g.*, amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a fusion protein).

An "altered domain" or "altered protein" refers to a motif, region, domain, peptide, polypeptide, or protein with a non-identical sequence identity to a wild type motif, region, domain, peptide, polypeptide, or protein (*e.g.*, a wild type TCRα chain, TCRβ chain, TCRα constant domain, TCRβ constant domain) of at least 85% (*e.g.*,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%).

As used herein, "nucleic acid" or "nucleic acid molecule" or "polynucleotide" refers to any of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), oligonucleotides, fragments generated, for example, by the polymerase chain reaction (PCR) or by *in vitro* translation, and fragments generated by any of ligation, scission, endonuclease action, or exonuclease action. In certain embodiments, the nucleic acids of the present disclosure are produced by PCR. Nucleic acids may be composed of monomers that are naturally occurring nucleotides (such as deoxyribonucleotides and ribonucleotides), analogs of naturally occurring nucleotides (*e.g.,* α-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have modifications in or replacement of sugar moieties, or pyrimidine or purine base moieties. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. Nucleic acid molecules can be either single stranded or double stranded.

The term "isolated" means that the material is removed from its original environment (*e.g.*, the natural environment if it is naturally occurring). For example, a naturally occurring nucleic acid or polypeptide present in a living animal is not isolated, but the same nucleic acid or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such nucleic acid could be part of a vector and/or such nucleic acid or polypeptide could be part of a composition (*e.g.,* a cell lysate), and still be isolated in that such vector or composition is not part of the natural environment for the nucleic acid or polypeptide. The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region "leader and trailer" as well as intervening sequences (introns) between individual coding segments (exons).

As used herein, the terms "modified", "engineered", or "recombinant" refer to a cell, microorganism, nucleic acid molecule, or vector that has been genetically engineered by human intervention – that is, modified by introduction of an exogenous

or heterologous nucleic acid molecule, or refers to a cell or microorganism that has been altered such that expression of an endogenous nucleic acid molecule or gene is controlled, deregulated or constitutive. Human-generated genetic alterations may include, for example, modifications that introduce nucleic acid molecules (which may include an expression control element, such as a promoter) that encode one or more proteins or enzymes, or other nucleic acid molecule additions, deletions, substitutions, or other functional disruption of or addition to a cell's genetic material. Exemplary modifications include those in coding regions or functional fragments thereof of heterologous or homologous polypeptides from a reference or parent molecule.

As used herein, "mutation" refers to a change in the sequence of a nucleic acid molecule or polypeptide molecule as compared to a reference or wild-type nucleic acid molecule or polypeptide molecule, respectively. A mutation can result in several different types of change in sequence, including substitution, insertion or deletion of nucleotide(s) or amino acid(s). In certain embodiments, a mutation is a substitution of one or three codons or amino acids, a deletion of one to about 5 codons or amino acids, or a combination thereof.

A "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are described in, for example: WO 97/09433 at page 10; Lehninger, Biochemistry, 2nd Edition; Worth Publishers, Inc. NY, NY, pp.71-77, 1975; and Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA, p. 8, 1990. Conservative substitutions of amino acids may occur naturally or may be introduced when a binding protein or TCR is recombinantly produced. Amino acid substitutions, deletions, and additions may be introduced into a protein using mutagenesis methods known in the art (*see, e.g.,* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 3d ed., Cold Spring Harbor Laboratory Press, NY, 2001). Oligonucleotide-directed site-specific (or segment specific) mutagenesis procedures may be employed to provide an altered polynucleotide that has particular codons altered according to the substitution, deletion, or insertion desired. Alternatively, random or saturation mutagenesis techniques, such as alanine scanning mutagenesis, error prone polymerase

chain reaction mutagenesis, and oligonucleotide-directed mutagenesis may be used to prepare immunogen polypeptide variants (*see, e.g.,* Sambrook *et al.*, *supra*).

The term "construct" refers to any polynucleotide that contains a recombinant nucleic acid molecule. A construct may be present in a vector (*e.g.*, a bacterial vector, a viral vector) or may be integrated into a genome.

A "vector" is a nucleic acid molecule that is capable of transporting another nucleic acid molecule. Vectors may be, for example, plasmids, cosmids, viruses, a RNA vector or a linear or circular DNA or RNA molecule that may include chromosomal, non-chromosomal, semi-synthetic or synthetic nucleic acid molecules. Exemplary vectors are those capable of autonomous replication (episomal vector) or expression of nucleic acid molecules to which they are linked (expression vectors).

The term "operably linked" or "operatively-linked" refers to the association of two or more nucleic acid molecules on a single nucleic acid molecule or fragment so that the function of one is affected by the other. For example, a promoter is operably-linked with a coding sequence when it is capable of affecting the expression of that coding sequence (*i.e.*, the coding sequence is under the transcriptional control of the promoter). "Unlinked" means that the associated genetic elements are not closely associated with one another and the function of one does not affect the other.

As used herein, "expression vector" refers to a DNA construct containing a nucleic acid molecule that is operably-linked to a suitable control sequence capable of effecting the expression of the nucleic acid molecule in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites, and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, a virus, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself. In the present specification, "plasmid," "expression plasmid," "virus" and "vector" are often used interchangeably.

The term "expression", as used herein, refers to the process by which a polypeptide is produced based on the encoding sequence of a nucleic acid molecule,

such as a gene. The process may include transcription, post-transcriptional control, post-transcriptional modification, translation, post-translational control, post-translational modification, or any combination thereof.

The term "introduced" in the context of inserting a nucleic acid molecule into a cell, means "transfection", or 'transformation" or "transduction" and includes reference to the incorporation of a nucleic acid molecule into a eukaryotic or prokaryotic cell wherein the nucleic acid molecule may be incorporated into the genome of a cell (*e.g.*, chromosome, plasmid, plastid, or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (*e.g.*, transfected mRNA).

As used herein, "heterologous" or "exogenous" nucleic acid molecule, construct or sequence refers to polynucleotide or portion of a polynucleotide that is not native to a host cell, but may be homologous to a polynucleotide or portion of a polynucleotide from the host cell. The source of the heterologous or exogenous polynucleotide, construct or sequence may be from a different genus or species. In certain embodiments, a heterologous or exogenous polynucleotide is added (*i.e.*, not endogenous or native) to a host cell or host genome by, for example, conjugation, transformation, transfection, electroporation, or the like, wherein the added molecule may integrate into the host genome or exist as extra-chromosomal genetic material (*e.g.*, as a plasmid or other form of self-replicating vector), and may be present in multiple copies. In addition, "heterologous" refers to a non-native enzyme, protein or other activity encoded by an exogenous polynucleotide introduced into the host cell, even if the host cell encodes a homologous protein or activity.

As described herein, more than one heterologous or exogenous nucleic acid molecule can be introduced into a host cell as separate polynucleotides, as a plurality of individually controlled genes, as a polycistronic polynucleotide, as a single nucleic acid molecule encoding a fusion protein, or any combination thereof. For example, as disclosed herein, a host cell can be modified to express two or more heterologous or exogenous polynucleotides encoding desired TCR specific for a MAGE-A1 antigen peptide (*e.g.*, TCRα and TCRβ). When two or more exogenous nucleic acid molecules are introduced into a host cell, it is understood that the two or more exogenous nucleic acid molecules can be introduced as a single polynucleotide (*e.g.*, on a single vector),

on separate vectors, integrated into the host chromosome at a single site or multiple sites, or any combination thereof. The number of referenced heterologous nucleic acid molecules or protein activities refers to the number of encoding nucleic acid molecules or the number of protein activities, not the number of separate polynucleotides introduced into a host cell.

As used herein, the term "endogenous" or "native" refers to a gene, protein, or activity that is normally present in a host cell. Moreover, a gene, protein or activity that is mutated, overexpressed, shuffled, duplicated or otherwise altered as compared to a parent gene, protein or activity is still considered to be endogenous or native to that particular host cell. For example, an endogenous control sequence from a first gene (*e.g.*, promoter, translational attenuation sequences) may be used to alter or regulate expression of a second native gene or nucleic acid molecule, wherein the expression or regulation of the second native gene or nucleic acid molecule differs from normal expression or regulation in a parent cell.

The term "homologous" or "homolog" refers to a molecule or activity found in or derived from a host cell, species or strain. For example, a heterologous or exogenous nucleic acid molecule may be homologous to a native host cell gene, and may optionally have an altered expression level, a different sequence, an altered activity, or any combination thereof.

"Sequence identity," as used herein, refers to the percentage of amino acid residues in one sequence that are identical with the amino acid residues in another reference polypeptide sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. The percentage sequence identity values can be generated using the NCBI BLAST2.0 software as defined by Altschul *et al.* (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402, with the parameters set to default values.

As used herein, a "hematopoietic progenitor cell" is a cell that can be derived from hematopoietic stem cells or fetal tissue and is capable of further differentiation into mature cells types (*e.g.*, immune system cells). Exemplary hematopoietic

progenitor cells include those with a $CD24^{Lo}$ Lin^- $CD117^+$ phenotype or those found in the thymus (referred to as progenitor thymocytes).

As used herein, the term "host" refers to a cell (*e.g.*, T cell) or microorganism targeted for genetic modification with a heterologous or exogenous nucleic acid molecule to produce a polypeptide of interest (*e.g.*, high or enhanced affinity anti-MAGE-A1 TCR). In certain embodiments, a host cell may optionally already possess or be modified to include other genetic modifications that confer desired properties related or unrelated to biosynthesis of the heterologous or exogenous protein (*e.g.*, inclusion of a detectable marker; deleted, altered or truncated endogenous TCR; increased co-stimulatory factor expression). In certain embodiments, a host cell is a human hematopoietic progenitor cell transduced with a heterologous or exogenous nucleic acid molecule encoding a TCRα chain specific for a MAGE-A1 antigen peptide.

As used herein, "hyperproliferative disorder" refers to excessive growth or proliferation as compared to a normal or undiseased cell. Exemplary hyperproliferative disorders include tumors, cancers, neoplastic tissue, carcinoma, sarcoma, malignant cells, pre-malignant cells, as well as non-neoplastic or non-malignant hyperproliferative disorders (*e.g.*, adenoma, fibroma, lipoma, leiomyoma, hemangioma, fibrosis, restenosis, as well as autoimmune diseases such as rheumatoid arthritis, osteoarthritis, psoriasis, inflammatory bowel disease, or the like).

Binding Proteins Specific for MAGE-A1 Antigen Peptides

In certain aspects, the present disclosure provides a modified cell comprising a heterologous polynucleotide that encodes a binding protein (*e.g*, a TCR, a single chain TCR (scTCR), or a CAR) that specifically binds to MAGE-A1 or a MAGE-A1 peptide antigen, such as a MAGE-A1 peptide complexed with an HLA molecule.

By way of background, ideal targets for immunotherapy are immunogenic proteins with high expression in malignant tissues and limited-to-absent expression in normal tissues. A unique group of proteins, known as cancer/testis antigens (CTAs), have been identified as promising immunotherapeutic targets due to their expression in various malignant tissues but low-level expression in healthy adult tissue except for germ cells of the testis (Ademuyiwa *et al. PLoS One*, *7*(6):e38783 (2012); Badovinac Crnjevic *et al.*, *Med Oncol.*, *29*(3):1586-91 (2012); Curigliano, G. *et al.*, *Ann. Oncol.*,

22(1):98-103 (2011). Moreover, CTAs are especially expressed in higher-grade lesions and aggressive malignancies, and associated with poorer clinical outcomes (Barrow *et al.*, *Clin Cancer Res.*, *12*(3 Pt 1):764-71 (2006); Gure, *et al. Clin Cancer Res.*, *11*(22):8055-62 (2005); Velazquez *et al.*, *Cancer Immun.*, *7*: 11 (2007)). MAGE family proteins are CTAs that are broadly expressed in many tumor types such as melanoma, lung, ovarian, multiple myeloma as well as TNBC. Simpson, A.J., *et al.*, Cancer/testis antigens, gametogenesis and cancer, *Nat. Rev. Cancer*, 2005. *5*(8):615-25; Weon, J.L. and P.R. Potts, *Curr Opin Cell Biol*, 2015. 37: 1-8; Park, T.S., *et al.*, *J Immunother*, 2016. *39*(1): 1-7; Li, X., S.C. Hughes, and R. Wevrick, *Cancer Genet*, 2015. 208(1-2):25-34; Kerkar, S.P., *et al.*, *J Immunother*, 2016. *39*(4):181-7. In particular, MAGE-A1 is expressed in 69.1% of TNBC cases overall (n=81) and in 85.7% of Grade III cases. Mrklic, I., *et al.*, *Acta Histochem*, 2014. *116*(5): 740-6. Additionally, evidence from melanoma cell lines suggests that MAGE-A1 directly drives tumorogenesis. Wang, D., *et al.*, *Biochem Biophys Res Commun*, 2016. *473*(4): 959-65.

In certain embodiments, a binding protein of the instant disclosure comprises (a) a T cell receptor (TCR) α-chain variable (V_α) domain having a CDR3 amino acid sequence according to any one of SEQ ID NOS.:26, 32, 38, 44, 50, or 51, and a TCR β-chain variable (V_β) domain; (b) a V_β domain having a CDR3 amino acid sequence according to any one of SEQ ID NOS.:23, 29, 35, 41, or 47, and a V_α domain; or (c)a V_α domain having a CDR3 amino acid sequence according to any one of SEQ ID NOS:26, 32, 38, 44, 50, or 51, and a V_β domain having a CDR3 amino acid sequence according to any one of SEQ ID NOs:23, 29, 35, 41, or 47.

Peptide-MHC complexes, such as MAGE-A1 peptide:MHC complexes are recognized by and bound through the TCR Vα and TCR Vβ domains. During lymphocyte development, Vα exons are assembled from different variable and joining gene segments (V-J), and Vβ exons are assembled from different variable, diversity, and joining gene segments (V-D-J). The TCRα chromosomal locus has 70-80 variable gene segments and 61 joining gene segments. The TCRβ chromosomal locus has 52 variable gene segments, and two separate clusters of each containing a single diversity gene segment, together with six or seven joining gene segments. Functional Vα and Vβ gene exons are generated by the recombination of a variable gene segment with a

joining gene segment for Vα, and a variable gene segment with a diversity gene segment and a joining gene segment for Vβ.

TCR Vα and Vβ domains each comprise three hypervariable loops, also referred to as complementary determining regions (CDRs) that contact the peptide-MHC complex. CDR1 and CDR2 are encoded within the variable gene segment, whereas CDR3 is encoded by the region spanning the variable and joining segments for Vα, or the region spanning variable, diversity, and joining segments for Vβ. Thus, if the identity of the variable gene segment of a Vα or Vβ is known (*e.g.*, by known TRAV or TRVB alleles), the sequences of their corresponding CDR1 and CDR2 can be deduced. Moreover, certain of the presently disclosed high-affinity TCR variable regions specific for MAGE-A1 (*e.g.*, those identified by having high-affinity CDR3 sequences) are encoded by a select TCRα allele or a TCRβ allele. In certain embodiments, an encoded binding domain comprises a V_β domain that is derived from a TRBV30 allele, a TRBV29 allele, or a TRBV9 allele. In some embodiments, an encoded binding domain comprises a V_α domain that is derived from a TRAV38-1 allele, a TRAV34 allele, a TRAV16 allele, or a TRAV5 allele.

TCR variable domain sequences can be aligned to a numbering scheme (International Immunogenetics Information System (IMGT) and Aho), allowing equivalent residue positions to be annotated and for different molecules to be compared using Antigen receptor Numbering And Receptor Classification (ANARCI) software tool (2016, Bioinformatics 15:298-300). A numbering scheme provides a standardized delineation of framework regions and CDRs in the TCR variable domains.

In certain embodiments, a binding protein comprises a functional variant amino acid sequence as compared to a reference amino acid sequence disclosed herein, wherein the encoded binding protein retains binding characteristics as compared to a binding protein comprising a reference amino acid sequence. For example, in some embodiments, an encoded V_α domain comprises an amino acid sequence that is at least about 90% identical (*e.g.*, is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical) to an amino acid sequence according to any one of SEQ ID NOS.:3, 7, 11, 15, and 19, and an encoded V_β domain comprises an amino acid sequence that is

at least about 90% identical to the amino acid sequence according to any one of SEQ ID NOS.:1, 5, 9, 13, 17, provided that (a) at least three or four of the CDRs have no change in sequence, wherein the CDRs that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof, and (b) the encoded binding protein remains capable of specifically binding to a MAGE-A1 peptide:HLA cell surface complex independent, or in the absence, of CD8.

In particular embodiments, (a) a V_α domain comprises (i) a CDR1 amino acid sequence according to any one of SEQ ID NOS:24, 30, 36, 42, and 48, and/or (ii) a CDR2 amino acid sequence according to any one of SEQ ID NOS:25, 31, 37, 43, and 49; and/or (b) an encoded V_β domain comprises (iii) a CDR1 amino acid sequence according to any one of SEQ ID NOS:21, 27, 33, 39, and 45, and/or (iv) a CDR2 amino acid sequence according to any one of SEQ ID NOS:22, 28, 34, 40, and 46. In further embodiments, an encoded binding protein comprises: (a) Vα CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:24-26, respectively, and V β CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:21-23, respectively; (b) Vα CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:30-32, respectively, and V β CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:27-29, respectively; (c) Vα CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:36-38, respectively, and V β CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:33-35, respectively; (d) Vα CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:42-44, respectively, and Vβ CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:39-41, respectively; or (e) Vα CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:48-50, respectively, and V β CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:45-47, respectively.

In certain embodiments, a Vα domain comprises or consists of an amino acid sequence according to SEQ ID NO.:3, 7, 11, 15, or 19. In further embodiments, an encoded Vβ domain comprises or consists of an amino acid sequence according to SEQ ID NO.:1, 5, 9, 13, or 17.

In some embodiments, a binding protein comprises a TCR α-chain constant domain, a TCR β-chain constant domain, or both. In certain embodiments, a a TCR α-

chain constant (Cα) domain has at least 90% sequence identity to the amino acid sequence of any one of SEQ ID NO.:4, 8, 12, 16, or 20. In further embodiments, a a TCR β-chain constant (Cβ) domain has at least 90% sequence identity to any one of the amino acid sequences of SEQ ID NO.:2, 6, 10, 14, or 18.

Accordingly, in some embodiments, a binding of the present disclosure comprises a V_α domain, a V_β domain, a C_α domain, and a C_β domain. In further embodiments, a binding protein comprises Vα domain comprising or consisting of SEQ ID NO.:3, a Vβ domain comprising or consisting of SEQ ID NO.:1, a Cα domain comprising or consisting of SEQ ID NO.:4, and a Cβ domain comprising or consisting of SEQ ID NO.:2. In other embodiments, a binding protein comprises a Vα domain comprising or consisting of SEQ ID NO.:7, a Vβ domain comprising or consisting of SEQ ID NO.:5, a Cα domain comprising or consisting of SEQ ID NO.:8, and a Cβ comprising or consisting of SEQ ID NO.:6. In still further embodiments, a binding protein comprises a Vα domain comprising or consisting of SEQ ID NO.:11, a Vβ domain comprising or consisting of SEQ ID NO.:9, a Cα domain comprising or consisting of SEQ ID NO.:12, and a Cβ domain comprising or consisting of SEQ ID NO.:10. In other embodiments, a binding protein comprises a Vα domain comprising or consisting of SEQ ID NO.:15, a Vβ domain comprising or consisting of SEQ ID NO.: 13, a Cα comprising or consisting of SEQ ID NO.: 16, and a Cβ domain comprising or consisting of SEQ ID NO.:14. In yet other embodiments, a binding protein comprises a Vα domain comprising or consisting of SEQ ID NO.: 19, a Vβ domain comprising or consisting of SEQ ID NO.:17, a Cα domain comprising or consisting of SEQ ID NO.:20, and a Cβ domain comprising or consisting of SEQ ID NO.: 18.

In any of the embodiments disclosed herein, a binding protein (*e.g.*, in soluble form or expressed on a cell surface of a modified cell of the present disclosure) is capable of binding to a MAGE-A1:HLA-A*201 complex (*e.g.*, a KVLEYVIKV (SEQ ID NO.:123):HLA-A*201 complex) on a cell surface independent of or in the absence of CD8.

In certain embodiments, any of the aforementioned MAGE-A1 specific binding proteins are each a T cell receptor (TCR), a chimeric antigen receptor or an antigen-

binding fragment of a TCR, any of which can be chimeric, humanized or human. In further embodiments, an antigen-binding fragment of the TCR comprises a single chain TCR (scTCR) or a chimeric antigen receptor (CAR). In certain embodiments, a MAGE-A1 specific binding protein is a TCR, optionally a scTCR. Methods for producing engineered TCRs are described in, for example, Bowerman *et al., Mol. Immunol., 46*(15):3000 (2009), the techniques of which are herein incorporated by reference. In certain embodiments, a MAGE-A1-specific binding domain is a CAR comprising a MAGE-A1-specific TCR binding domain (*see, e.g.,* Walseng *et al.*, *Scientific Reports 7*:10713 (2017), the TCR CAR constructs of which are hereby incorporated by reference in their entirety). Methods for making CARs are also described, for example, in U.S. Patent No. 6,410,319; U.S. Patent No. 7,446,191; U.S. Patent Publication No. 2010/065818; U.S. Patent No. 8,822,647; PCT Publication No. WO 2014/031687; U.S. Patent No. 7,514,537; and Brentjens *et al.*, 2007, *Clin. Cancer Res.* 13:5426, the techniques of which are herein incorporated by reference.

Methods useful for isolating and purifying recombinantly produced soluble TCR, by way of example, may include obtaining supernatants from suitable host cell/vector systems that secrete the recombinant soluble TCR into culture media and then concentrating the media using a commercially available filter. Following concentration, the concentrate may be applied to a single suitable purification matrix or to a series of suitable matrices, such as an affinity matrix or an ion exchange resin. One or more reverse phase HPLC steps may be employed to further purify a recombinant polypeptide. These purification methods may also be employed when isolating an immunogen from its natural environment. Methods for large scale production of one or more of the isolated/recombinant soluble TCR described herein include batch cell culture, which is monitored and controlled to maintain appropriate culture conditions. Purification of the soluble TCR may be performed according to methods described herein and known in the art and that comport with laws and guidelines of domestic and foreign regulatory agencies.

In certain embodiments, nucleic acid molecules encoding a binding protein or high affinity TCR specific for MAGE-A1 are used to transfect/transduce a host cell (*e.g.*, T cells) for use in adoptive transfer therapy. Advances in TCR sequencing have

been described (*e.g.*, Robins *et al.*, *Blood* 114:4099, 2009; Robins *et al.*, *Sci. Translat. Med.* 2:47ra64, 2010; Robins *et al.*, (Sept. 10) *J. Imm. Meth.* Epub ahead of print, 2011; Warren *et al.*, *Genome Res.* 21:790, 2011) and may be employed in the course of practicing the embodiments according to the present disclosure. Similarly, methods for transfecting/transducing T cells with desired nucleic acids have been described (*e.g.*, U.S. Patent Application Pub. No. US 2004/0087025) as have adoptive transfer procedures using T-cells of desired antigen-specificity (*e.g.*, Schmitt *et al.*, *Hum. Gen. 20*:1240, 2009; Dossett *et al.*, *Mol. Ther. 17*:742, 2009; Till *et al.*, *Blood 112*:2261, 2008; Wang *et al.*, *Hum. Gene Ther. 18*:712, 2007; Kuball *et al.*, *Blood 109*:2331, 2007; US 2011/0243972; US 2011/0189141; Leen *et al.*, *Ann. Rev. Immunol. 25*:243, 2007), such that adaptation of these methodologies to the presently disclosed embodiments is contemplated, based on the teachings herein, including those directed to high affinity TCRs specific for MAGE-A1 peptide antigens complexed with an HLA receptor.

MAGE-A1-specific binding proteins or domains as described herein may be functionally characterized according to any of a large number of art accepted methodologies for assaying T cell activity, including determination of T cell binding, activation or induction and also including determination of T cell responses that are antigen-specific. Examples include determination of T cell proliferation, T cell cytokine release, antigen-specific T cell stimulation, MHC restricted T cell stimulation, CTL activity (*e.g.*, by detecting ^{51}Cr release from pre-loaded target cells), changes in T cell phenotypic marker expression, and other measures of T-cell functions. Procedures for performing these and similar assays are may be found, for example, in Lefkovits (*Immunology Methods Manual: The Comprehensive Sourcebook of Techniques*, 1998). *See*, *also*, *Current Protocols in Immunology*; Weir, *Handbook of Experimental Immunology*, Blackwell Scientific, Boston, MA (1986); Mishell and Shigii (eds.) *Selected Methods in Cellular Immunology*, Freeman Publishing, San Francisco, CA (1979); Green and Reed, *Science* 281:1309 (1998) and references cited therein.

"MHC-peptide tetramer staining" refers to an assay used to detect antigen-specific T cells, which features a tetramer of MHC molecules, each comprising an identical peptide having an amino acid sequence that is cognate (*e.g.*, identical or

related to) at least one antigen (*e.g.*, MAGE-A1), wherein the complex is capable of binding T cell receptors specific for the cognate antigen. Each of the MHC molecules may be tagged with a biotin molecule. Biotinylated MHC/peptides are tetramerized by the addition of streptavidin, which can be fluorescently labeled. The tetramer may be detected by flow cytometry via the fluorescent label. In certain embodiments, an MHC-peptide tetramer assay is used to detect or select enhanced affinity TCRs of the instant disclosure.

Levels of cytokines may be determined according to methods described herein and practiced in the art, including for example, ELISA, ELISPOT, intracellular cytokine staining, and flow cytometry and combinations thereof (*e.g.*, intracellular cytokine staining and flow cytometry). Immune cell proliferation and clonal expansion resulting from an antigen-specific elicitation or stimulation of an immune response may be determined by isolating lymphocytes, such as circulating lymphocytes in samples of peripheral blood cells or cells from lymph nodes, stimulating the cells with antigen, and measuring cytokine production, cell proliferation and/or cell viability, such as by incorporation of tritiated thymidine or non-radioactive assays, such as MTT assays and the like. The effect of an immunogen described herein on the balance between a Th1 immune response and a Th2 immune response may be examined, for example, by determining levels of Th1 cytokines, such as IFN-γ, IL-12, IL-2, and TNF-β, and Type 2 cytokines, such as IL-4, IL-5, IL-9, IL-10, and IL-13.

Polynucleotides and Vectors

In another aspect, isolated or recombinant polynucleotides are provided herein, wherein a polynucleotide encodes a binding protein of the present disclosure (*e.g.*, immunoglobulin superfamily binding protein, such as a TCR, scTCR, or CAR) specific for 5T4, and wherein the polynucleotide is codon optimized for expression in a host cell (*e.g.*, an immune cell of the present disclosure). Also provided are vectors (*e.g.*, expression vectors) that comprise a polynucleotide of this disclosure, wherein the polynucleotide is operatively associated or operably linked to an expression control sequence (*e.g.*, a promoter). Construction of an expression vector to produce a binding protein specific for a MAGE-A1 peptide of this disclosure can be made using restriction endonuclease digestion, ligation, transformation, plasmid purification, DNA

sequencing, or a combination thereof, as described in, for example, Sambrook *et al.* (1989 and 2001 editions; *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY) and Ausubel *et al.* (Current Protocols in Molecular Biology, 2003). For efficient transcription and translation, a polynucleotide contained in an expression construct includes at least one appropriate expression control sequence (also called a regulatory sequence), such as a leader sequence and particularly a promoter operably (*i.e.*, operatively) linked to the nucleotide sequence encoding the binding protein of this disclosure.

A nucleic acid may be a single- or a double-stranded DNA, cDNA or RNA in any form, and may include a positive and a negative strand of the nucleic acid which complement each other, including anti-sense DNA, cDNA and RNA. Also included are siRNA, microRNA, RNA—DNA hybrids, ribozymes, and other various naturally occurring or synthetic forms of DNA or RNA.

Isolated or recombinant nucleic acid molecules encoding a binding protein (*e.g.*, immunoglobulin superfamily binding protein) or high affinity recombinant T cell receptor (TCR) specific for MAGE-A1 as described herein may be produced and prepared according to various methods and techniques of the molecular biology or polypeptide purification arts.

In certain embodiments, an isolated polynucleotide is provided that encodes a binding protein having a TCR Vα domain and a TCR Vβ domain, wherein the encoded binding protein is capable of specifically binding to a MAGE-A1 peptide:HLA complex on a cell surface independent of CD8 or in the absence of CD8, the isolated polynucleotide comprising: (a) a Vα CDR3-encoding polynucleotide according to SEQ ID NO:97, 103, 109, 115 or 121, and a Vβ-encoding polynucleotide; (b) a Vβ CDR3-encoding polynucleotide according to SEQ ID NO:94, 100, 106, 112, or 118, and a Vα-encoding polynucleotide; or (c) a Vα CDR3-encoding polynucleotide according to SEQ ID NO:97, 103, 109, 115 or 121 and a Vβ CDR3-encoding polynucleotide according to SEQ ID NO:94, 100, 106, 112, or 118. In further embodiments, a Vβ-encoding polynucleotide is derived from a TRBV30 allele, a TRBV29 allele, or a TRBV9 allele. In some embodiments, a Vα-encoding polynucleotide is derived from a TRAV38-1 allele, a TRAV34 allele, a TRAV16 allele, or a TRAV5 allele.

Presently disclosed polynucleotides encoding binding proteins can, in some embodiments, comprise: (a) a V_α CDR3-encoding polynucleotide according to SEQ ID NO:97 and a V_β CDR3-encoding polynucleotide according to SEQ ID NO:94; (b) a V_α CDR3-encoding polynucleotide according to SEQ ID NO:103 and a V_β CDR3-encoding polynucleotide according to SEQ ID NO:100; (c) a V_α CDR3-encoding polynucleotide according to SEQ ID NO:109 and a V_β CDR3-encoding polynucleotide according to SEQ ID NO:106; (d) a V_α CDR3-encoding polynucleotide according to SEQ ID NO:115 and a V_β CDR3-encoding polynucleotide according to SEQ ID NO:112; or (e) a V_α CDR3-encoding polynucleotide according to SEQ ID NO:121 and a V_β CDR3-encoding polynucleotide according to SEQ ID NO:118. In certain embodiments, an isolated polynucleotide encoding a binding protein further comprises (a) a Vα CDR1-encoding polynucleotide according to SEQ ID NO:95, 101, 107, 113 or 119; (b) a Vα CDR2-encoding polynucleotide according to SEQ ID NO:96, 102, 108, 114 or 120; (c)a Vβ CDR1-encoding polynucleotide according to SEQ ID NO:92, 98, 104, 110 or 116; and/or (d) a Vβ CDR2-encoding polynucleotide according to SEQ ID NO:93, 99, 105, 111 or 117.

In particular embodiments, an isolated polynucleotide encoding a binding protein of the present disclosure comprises (a) a Vα CDR1-encoding polynucleotide according to SEQ ID NO:95, a Vα CDR2- encoding polynucleotide according to SEQ ID NO:96, a Vα CDR3-encoding polynucleotide according to SEQ ID NO:97, a Vβ CDR1-encoding polynucleotide according to SEQ ID NO:92, a Vβ CDR2-encoding polynucleotide according to SEQ ID NO:93, and Vβ CDR3-encoding polynucleotide according to SEQ ID NO:94; (b) a Vα CDR1-encoding polynucleotide according to SEQ ID NO:101, a Vα CDR2- encoding polynucleotide according to SEQ ID NO:102, a Vα CDR3-encoding polynucleotide according to SEQ ID NO:103, a Vβ CDR1-encoding polynucleotide according to SEQ ID NO:98, a Vβ CDR2-encoding polynucleotide according to SEQ ID NO:99, and Vβ CDR3-encoding polynucleotide according to SEQ ID NO:100; (c) a Vα CDR1-encoding polynucleotide according to SEQ ID NO:107, a Vα CDR2- encoding polynucleotide according to SEQ ID NO:108, a Vα CDR3-encoding polynucleotide according to SEQ ID NO:109, a Vβ CDR1-encoding polynucleotide according to SEQ ID NO:104, a Vβ CDR2-encoding

polynucleotide according to SEQ ID NO:105, and Vβ CDR3-encoding polynucleotide according to SEQ ID NO:106; (d) a Vα CDR1-encoding polynucleotide according to SEQ ID NO:113, a Vα CDR2- encoding polynucleotide according to SEQ ID NO:114, a Vα CDR3-encoding polynucleotide according to SEQ ID NO:115, a Vβ CDR1-encoding polynucleotide according to SEQ ID NO:110, a Vβ CDR2-encoding polynucleotide according to SEQ ID NO:111, and Vβ CDR3-encoding polynucleotide according to SEQ ID NO:112.; or (e) a Vα CDR1-encoding polynucleotide according to SEQ ID NO:119, a Vα CDR2- encoding polynucleotide according to SEQ ID NO:120, a Vα CDR3-encoding polynucleotide according to SEQ ID NO:121, a Vβ CDR1-encoding polynucleotide according to SEQ ID NO:116, a Vβ CDR2-encoding polynucleotide according to SEQ ID NO:117, and Vβ CDR3-encoding polynucleotide according to SEQ ID NO:118.

In some embodiments, the instant disclosure provides a polynucleotide encoding a binding protein, wherein a V_α-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity (*e.g.*, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identity) to SEQ ID NO:58, 66, 74, 82, or 90, and a V_β-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:56, 64, 72, 80, or 88. In further embodiments: (a) a Vα-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:58 and a Vβ-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:56; (b) a Vα-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:66 and a Vβ-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:64; (c) a Vα-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:74 and a Vβ-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:72; (d) a Vα-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:82 and a Vβ-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:80; or (e) a Vα-encoding polynucleotide comprises a nucleotide sequence having at

least 80% identity to SEQ ID NO:90 and a Vβ-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:88.

In particular embodiments, (a) a Vα-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:58 and a Vβ-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:56; (b) a Vα-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:66 and a Vβ-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:64; (c) a Vα-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:74 and a Vβ-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:72; (d) a Vα-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:82 and a Vβ-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:80; or (e) a Vα-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:90 and a Vβ-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:88.

Binding protein-encoding polynucleotides of the instant disclosure may, in certain embodiments, further comprise a polynucleotide that encodes a TCR α-chain constant domain, a polynucleotide that encodes a TCR β-chain constant domain, or both. In some embodiments, an isolated polynucleotide encoding a binding protein of the present disclosure further comprises: (a) a C_α-domain-encoding polynucleotide having at least 80% identity to SEQ ID NO:59, 67, 75, 83, or 91; and/or (b) a C_β-domain-encoding polynucleotide having at least 80% identity to SEQ ID NO:57, 65, 73, 81, or 89. In further embodiments, a C_α-domain-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:59, 67, 75, 83, or 91, and aC_β-domain-encoding polynucleotide comprises or consists of a nucleotide sequence according to SEQ ID NO:57, 65, 73, 81, or 89.

In particular embodiments, an isolated polynucleotide encoding a binding protein of the present disclosure comprises: (a) a V_α-encoding polynucleotide according to SEQ ID NO:58, a V_β-encoding polynucleotide according to SEQ ID NO:56, a C_α-domain-encoding polynucleotide according to SEQ ID NO:59, and a C_β-domain-

encoding polynucleotide according to SEQ ID NO:57; (b) a V_α-encoding polynucleotide according to SEQ ID NO:66, a V_β-encoding polynucleotide according to SEQ ID NO:64, a C_α-domain-encoding polynucleotide according to SEQ ID NO:67, and a C_β-domain-encoding polynucleotide according to SEQ ID NO:65; (c) a V_α-encoding polynucleotide according to SEQ ID NO:74, a V_β-encoding polynucleotide according to SEQ ID NO:72, a C_α-domain-encoding polynucleotide according to SEQ ID NO:75, and a C_β-domain-encoding polynucleotide according to SEQ ID NO:73; (d) a V_α-encoding polynucleotide according to SEQ ID NO:82, a V_β-encoding polynucleotide according to SEQ ID NO:80, a C_α-domain-encoding polynucleotide according to SEQ ID NO:83, and a C_β-domain-encoding polynucleotide according to SEQ ID NO:81; or (e) a V_α-encoding polynucleotide according to SEQ ID NO:90, a V_β-encoding polynucleotide according to SEQ ID NO:88, a C_α-domain-encoding polynucleotide according to SEQ ID NO:91, and a C_β-domain-encoding polynucleotide according to SEQ ID NO:89.

In further embodiments, two or more substituent gene products of a binding protein of this disclosure are expressed as a single peptide with the parts separated by a cleavable or removable segment. For instance, self-cleaving peptides useful for expression of separable polypeptides encoded by a single polynucleotide or vector are known in the art and include, for example, a Porcine teschovirus-1 2A (P2A) peptide, such as a peptide encoded by a polynucleotide having the nucleotide sequence shown in any one of SEQ ID NOS:128 or 129, a Thoseaasigna virus 2A (T2A) peptide, such as a peptide encoded by a polynucleotide having the nucleotide sequence shown in SEQ ID NO:132, an Equine rhinitis A virus (ERAV) 2A (E2A) peptide, such as a peptide encoded by a polynucleotide having the nucleotide sequence shown in SEQ ID NO:131, and a Foot-and-Mouth disease virus 2A (F2A) peptide, such as a peptide encoded by a polynucleotide having the nucleotide sequence shown in SEQ ID NO:130.

Accordingly, in certain embodiments, an isolated polynucleotide encoding a binding protein of the instant disclosure further comprises a polynucleotide encoding a self-cleaving peptide disposed between a TCR α-chain-encoding polynucleotide and a TCR β-chain-encoding polynucleotide, or disposed between a TCR Vβ domain-encoding polynucleotide and a TCR Vα-encoding polynucleotide, or disposed between

a TCR variable domain-encoding polynucleotide and a TCR constant domain-encoding polynucleotide, or any combination thereof. In particular embodiments, a polynucleotide encoding a self-cleaving peptide comprises or consists of a nucleotide sequence according to any one of SEQ ID NOS.:128-132. In further embodiments, a polynucleotide encodes a self-cleaving peptide comprising or consisting of an amino acid sequence according to any one of SEQ ID NOS.:124-127.

Also provided herein are vectors containing polynucleotides of the instant disclosure. Construction of an expression vector that is used for recombinantly producing a binding protein or high affinity engineered TCR specific for a MAGE-A1 peptide of interest can be accomplished by using any suitable molecular biology engineering techniques, including the use of restriction endonuclease digestion, ligation, transformation, plasmid purification, and DNA sequencing as described in, for example, Sambrook *et al.* (1989 and 2001 editions; *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY) and Ausubel *et al.* (Current Protocols in Molecular Biology, 2003). To obtain efficient transcription and translation, a polynucleotide in each recombinant expression construct includes at least one appropriate expression control sequence, such as a promoter operably (*i.e.*, operatively) linked to a nucleotide sequence encoding a binding protein. In addition, a polynucleotide encoding a binding protein of this disclosure may also include a sequence encoding a leader sequence at the amino-terminus of the binding protein (also referred to as a pre-binding protein), which leader sequence may be removed by the cell to produce a mature binding protein.

An exemplary vector may comprise a nucleic acid molecule capable of transporting another nucleic acid molecule to which it has been linked, or which is capable of replication in a host organism. Some examples of vectors include plasmids, viral vectors, cosmids, and others. Some vectors may be capable of autonomous replication in a host cell into which they are introduced (*e.g.* bacterial vectors having a bacterial origin of replication and episomal mammalian vectors), whereas other vectors may be integrated into the genome of a host cell or promote integration of the polynucleotide insert upon introduction into the host cell and thereby replicate along with the host genome (*e.g.*, lentiviral vector)). Additionally, some vectors are capable of directing the expression of genes to which they are operatively linked (these vectors

may be referred to as "expression vectors"). According to related embodiments, it is further understood that, if one or more agents (*e.g.*, polynucleotides encoding binding proteins or high affinity recombinant TCRs specific for MAGE-A1, or variants thereof, as described herein) is co-administered to a subject, that each agent may reside in separate or the same vectors, and multiple vectors (each containing a different agent the same agent) may be introduced to a cell or cell population or administered to a subject.

In certain embodiments, a polynucleotide encoding binding proteins or high affinity recombinant TCRs specific for MAGE-A1, may be operatively linked to certain elements of a vector. For example, polynucleotide sequences that are needed to effect the expression and processing of coding sequences to which they are ligated may be operatively linked. Expression control sequences may include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequences); sequences that enhance protein stability; and possibly sequences that enhance protein secretion. Expression control sequences may be operatively linked if they are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. In certain embodiments, polynucleotides encoding binding proteins of the instant disclosure are contained in an expression vector that is a viral vector, such as a lentiviral vector or a γ-retroviral vector.

Viral vectors include retrovirus, adenovirus, parvovirus (*e.g.*, adeno-associated viruses), coronavirus, negative strand RNA viruses such as ortho-myxovirus (*e.g.*, influenza virus), rhabdovirus (*e.g.*, rabies and vesicular stomatitis virus), paramyxovirus (*e.g.*, measles and Sendai), positive strand RNA viruses such as picornavirus and alphavirus, and double-stranded DNA viruses including adenovirus, herpesvirus (*e.g.*, Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (*e.g.*, vaccinia, fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include avian leukosis-sarcoma, mammalian C-type, B-type viruses, D type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin,

J. M., Retroviridae: The viruses and their replication, In Fundamental Virology, Third Edition, B. N. Fields *et al.*, Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

"Lentiviral vector," as used herein, means HIV-based lentiviral vectors for gene delivery, which can be integrative or non-integrative, have relatively large packaging capacity, and can transduce a range of different cell types. Lentiviral vectors are usually generated following transient transfection of three (packaging, envelope and transfer) or more plasmids into producer cells. Like HIV, lentiviral vectors enter the target cell through the interaction of viral surface glycoproteins with receptors on the cell surface. On entry, the viral RNA undergoes reverse transcription, which is mediated by the viral reverse transcriptase complex. The product of reverse transcription is a double-stranded linear viral DNA, which is the substrate for viral integration into the DNA of infected cells.

In particular embodiments, a recombinant or engineered expression vector is delivered to an appropriate cell (*i.e.*, is capable of delivering a binding protein-encoding polynucleotide of the present disclosure to a host cell), for example, a T cell or an antigen-presenting cell, *i.e.*, a cell that displays a peptide/MHC complex on its cell surface (*e.g.*, a dendritic cell) and lacks CD8. In certain embodiments, a host cell is a hematopoietic progenitor cell or a human immune system cell. For example, an immune system cell can be a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a natural killer cell, a dendritic cell, or any combination thereof.. In certain embodiments, wherein a T cell is the host, the T cell can be naïve, a central memory T cell, an effector memory T cell, or any combination thereof. Recombinant expression vectors of the present disclosure may therefore also include, for example, lymphoid tissue-specific transcriptional regulatory elements (TREs), such as a B lymphocyte, T lymphocyte, or dendritic cell specific TREs. Lymphoid tissue specific TREs are known in the art (*see, e.g.,* Thompson *et al.*, *Mol. Cell. Biol. 12*:1043, 1992); Todd *et al.*, *J. Exp. Med. 177*:1663, 1993); Penix *et al.*, *J. Exp. Med. 178*:1483, 1993).

In addition to vectors, certain embodiments relate to host cells that comprise the vectors that are presently disclosed. One of skill in the art readily understands that many suitable host cells are available in the art. A host cell may include any individual

cell or cell culture which may receive a vector or the incorporation of nucleic acids and/or proteins, as well as any progeny cells. The term also encompasses progeny of the host cell, whether genetically or phenotypically the same or different. Suitable host cells may depend on the vector and may include mammalian cells, animal cells, human cells, simian cells, insect cells, yeast cells, and bacterial cells. These cells may be induced to incorporate the vector or other material by use of a viral vector, transformation via calcium phosphate precipitation, DEAE-dextran, electroporation, microinjection, or other methods. *See*, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* 2d ed. (Cold Spring Harbor Laboratory, 1989).

Host Cells

Also provided are host cells (*i.e.*, modified cells) that include a heterologous polynucleotide encoding a binding protein of this disclosure. In certain embodiments, a host cell comprises a human immune cell such as, for example, a T cell, a NK cell, or a NK-T cell. In some embodiments, a host cell comprises a $CD4^+$T cell, a $CD8^+$ T cell, or both. Methods for transfecting/transducing T cells with desired nucleic acids have been described (*e.g.*, U.S. Patent Application Pub. No. US 2004/0087025) as have adoptive transfer procedures using T cells of desired target-specificity (*e.g.,* Schmitt *et al., Hum. Gen. 20*:1240, 2009; Dossett *et al., Mol. Ther. 17*:742, 2009; Till *et al., Blood 112*:2261, 2008; Wang *et al., Hum. Gene Ther. 18*:712, 2007; Kuball *et al., Blood 109*:2331, 2007; US 2011/0243972; US 2011/0189141; Leen *et al., Ann. Rev. Immunol. 25*:243, 2007), such that adaptation of these methodologies to the presently disclosed embodiments is contemplated, based on the teachings herein.

In certain embodiments, a modified cell comprises a heterologous polynucleotide encoding a binding protein, wherein the encoded binding protein comprises: (a) a T cell receptor (TCR) α-chain variable (V_α) domain having a CDR3 amino acid sequence according to any one of SEQ ID NOS.:26, 32, 38, 44, 50, or 51, and a TCR β-chain variable (V_β) domain; (b) a V_β domain having a CDR3 amino acid sequence according to any one of SEQ ID NOS.:23, 29, 35, 41, or 47, and a V_α domain; or (c) a V_α domain having a CDR3 amino acid sequence according to any one of SEQ ID NOS:26, 32, 38, 44, 50, or 51, and a V_β domain having a CDR3 amino acid sequence according to any one of SEQ ID NOs:23, 29, 35, 41, or 47; and wherein the

binding protein is capable of specifically binding to a MAGE-A1 peptide:HLA complex on a cell surface independent of CD8 or in the absence of CD8. In some embodiments, the encoded binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123):human leukocyte antigen (HLA) complex with a K_d less than or equal to about 10^{-8} M.

Any appropriate method can be used to transfect or transduce the cells, for example, the T cells, or to administer the polynucleotides or compositions of the present methods. Known methods for delivering polynucleotides to host cells include, for example, use of cationic polymers, lipid-like molecules, and certain commercial products such as, for example, IN-VIVO-JET PEI. Other methods include *ex vivo* transduction, injection, electroporation, DEAE-dextran, sonication loading, liposome-mediated transfection, receptor-mediated transduction, microprojectile bombardment, transposon-mediated transfer, and the like. Still further methods of transfecting or transducing host cells employ vectors, described in further detail herein.

In any of the foregoing embodiments, a host cell (*e.g.*, an immune cell) may be a "universal donor" cell that is modified to reduce or eliminate expression of one or more endogenous genes that encode a polypeptide involved in immune signaling or other related activities. Exemplary gene knockouts include those that encode PD-1, LAG-3, CTLA4, TIM3, an HLA molecule, a TCR molecule, or the like. Without wishing to be bound by theory, certain endogenously expressed immune cell proteins may be recognized as foreign by an allogeneic host receiving the host immune cells, which may result in elimination of the host immune cells (*e.g.*, an HLA allele), or may downregulate the immune activity of a modified cell (*e.g.*, PD-1, LAG-3, CTLA4), or may interfere with the binding activity of a heterologously expressed binding protein of the present disclosure (*e.g.*, an endogenous TCR that binds a non-MAGE-A1 antigen and thereby interferes with the modified cell binding a cell that expresses MAGE-A1 antigen). Accordingly, decreasing or eliminating expression or activity of such endogenous genes or proteins can improve the activity, tolerance, and persistence of modified cells within an allogeneic host, and allows for universal, "off-the-shelf" cells for administration (*e.g.*, to any recipient regardless of HLA type).

In certain embodiments, a host cell (*e.g.*, a modified immune cell) of this disclosure comprises a chromosomal gene knockout of one or more of a gene that encodes PD-1, LAG-3, CTLA4, TIM3, an HLA component (*e.g.*, a gene that encodes an α1 macroglobulin, an α2 macroglobulin, an α3 macroglobulin, a β1 microglobulin, or a β2 microglobulin), or a TCR component (*e.g.*, a gene that encodes a TCR variable region or a TCR constant region) (*see, e.g.,* Torikai *et al., Nature Sci. Rep. 6*:21757 (2016); Torikai *et al., Blood 119*(24):5697 (2012); and Torikai *et al., Blood 122*(8):1341 (2013), the gene editing techniques and compositions of which are herein incorporated by reference in their entirety). As used herein, the term "chromosomal gene knockout" refers to a genetic alteration in a modified cell that prevents production, by the modified cell, of a functionally active endogenous polypeptide product. Alterations resulting in a chromosomal gene knockout can include, for example, introduced nonsense mutations (including the formation of premature stop codons), missense mutations, gene deletion, and strand breaks, as well as the heterologous expression of inhibitory nucleic acid molecules that inhibit endogenous gene expression in the modified cell.

A chromosomal gene knockout may be introduced by chromosomal editing of the immune cell. In certain embodiments, the chromosomal gene knockout is made by chromosomal editing of the immune cell. Chromosomal editing can be performed using, for example, endonucleases. As used herein "endonuclease" refers to an enzyme capable of catalyzing cleavage of a phosphodiester bond within a polynucleotide chain. In certain embodiments, an endonuclease is capable of cleaving a targeted gene thereby inactivating or "knocking out" the targeted gene. An endonuclease may be a naturally occurring, recombinant, genetically modified, or fusion endonuclease. The nucleic acid strand breaks caused by the endonuclease are commonly repaired through the distinct mechanisms of homologous recombination or non-homologous end joining (NHEJ). During homologous recombination, a donor nucleic acid molecule may be used for gene "knock-in" to inactivate a target gene. NHEJ is an error-prone repair process that often results in changes to the DNA sequence at the site of the cleavage, *e.g.*, a substitution, deletion, or addition of at least one nucleotide. NHEJ may be used to "knock-out" a target gene. Methods of disrupting or knocking out genes or gene

expression in immune cells using endonucleases are known in the art and described, for example, in PCT Publication Nos. WO 2015/066262; WO 2013/074916; and WO 2014/059173; methods from each of which is incorporated by reference. Examples of endonucleases include zinc finger nucleases, TALE-nucleases, CRISPR-Cas nucleases, and meganucleases.

As used herein, a "zinc finger nuclease" (ZFN) refers to a fusion protein comprising a zinc finger DNA-binding domain fused to a non-specific DNA cleavage domain, such as a FokI endonuclease. Each zinc finger motif of about 30 amino acids binds to about 3 base pairs of DNA, and amino acids at certain residues can be changed to alter triplet sequence specificity (*see*, *e.g.*, Desjarlais *et al.*, *Proc. Natl. Acad. Sci. 90*:2256-2260, 1993; Wolfe *et al.*, *J. Mol. Biol. 285*:1917-1934, 1999). Multiple zinc finger motifs can be linked in tandem to create binding specificity to desired DNA sequences, such as regions having a length ranging from about 9 to about 18 base pairs. By way of background, ZFNs mediate genome editing by catalyzing the formation of a site-specific DNA double strand break (DSB) in the genome, and targeted integration of a transgene comprising flanking sequences homologous to the genome at the site of DSB is facilitated by homology directed repair. Alternatively, a DSB generated by a ZFN can result in knock out of target gene via repair by non-homologous end joining (NHEJ), which is an error-prone cellular repair pathway that results in the insertion or deletion of nucleotides at the cleavage site. In certain embodiments, a gene knockout comprises an insertion, a deletion, a mutation or a combination thereof, made using a ZFN molecule.

As used herein, a "transcription activator-like effector nuclease" (TALEN) refers to a fusion protein comprising a TALE DNA-binding domain and a DNA cleavage domain, such as a FokI endonuclease. A "TALE DNA binding domain" or "TALE" is composed of one or more TALE repeat domains/units, each generally having a highly conserved 33-35 amino acid sequence with divergent 12^{th} and 13^{th} amino acids. The TALE repeat domains are involved in binding of the TALE to a target DNA sequence. The divergent amino acid residues, referred to as the Repeat Variable Diresidue (RVD), correlate with specific nucleotide recognition. The natural (canonical) code for DNA recognition of these TALEs has been determined such that

an HD sequence at positions 12 and 13 leads to a binding to cytosine (C), NG binds to T, NI to A, NN binds to G or A, and NG binds to T and non-canonical (atypical) RVDs are also known (*see*, *e.g.*, U.S. Patent Publication No. US 2011/0301073, which atypical RVDs are incorporated by reference herein in its entirety). TALENs can be used to direct site-specific double-strand breaks (DSB) in the genome of T cells. Non-homologous end joining (NHEJ) ligates DNA from both sides of a double-strand break in which there is little or no sequence overlap for annealing, thereby introducing errors that knock out gene expression. Alternatively, homology directed repair can introduce a transgene at the site of DSB providing homologous flanking sequences are present in the transgene. In certain embodiments, a gene knockout comprises an insertion, a deletion, a mutation or a combination thereof, and made using a TALEN molecule.

As used herein, a "clustered regularly interspaced short palindromic repeats/Cas" (CRISPR/Cas) nuclease system refers to a system that employs a CRISPR RNA (crRNA)-guided Cas nuclease to recognize target sites within a genome (known as protospacers) via base-pairing complementarity and then to cleave the DNA if a short, conserved protospacer associated motif (PAM) immediately follows 3' of the complementary target sequence. CRISPR/Cas systems are classified into three types (*i.e.*, type I, type II, and type III) based on the sequence and structure of the Cas nucleases. The crRNA-guided surveillance complexes in types I and III need multiple Cas subunits. Type II system, the most studied, comprises at least three components: an RNA-guided Cas9 nuclease, a crRNA, and a *trans*-acting crRNA (tracrRNA). The tracrRNA comprises a duplex forming region. A crRNA and a tracrRNA form a duplex that is capable of interacting with a Cas9 nuclease and guiding the Cas9/crRNA:tracrRNA complex to a specific site on the target DNA via Watson-Crick base-pairing between the spacer on the crRNA and the protospacer on the target DNA upstream from a PAM. Cas9 nuclease cleaves a double-stranded break within a region defined by the crRNA spacer. Repair by NHEJ results in insertions and/or deletions which disrupt expression of the targeted locus. Alternatively, a transgene with homologous flanking sequences can be introduced at the site of DSB via homology directed repair. The crRNA and tracrRNA can be engineered into a single guide RNA (sgRNA or gRNA) (*see*, *e.g.*, Jinek *et al.*, *Science 337*:816-21, 2012). Further, the

region of the guide RNA complementary to the target site can be altered or programed to target a desired sequence (Xie *et al.*, PLOS One 9:e100448, 2014; U.S. Pat. Appl. Pub. No. US 2014/0068797, U.S. Pat. Appl. Pub. No. US 2014/0186843; U.S. Pat. No. 8,697,359, and PCT Publication No. WO 2015/071474; the techniques and compositions of each of which are incorporated by reference). In certain embodiments, a gene knockout comprises an insertion, a deletion, a mutation or a combination thereof, and made using a CRISPR/Cas nuclease system.

As used herein, a "meganuclease," also referred to as a "homing endonuclease," refers to an endodeoxyribonuclease characterized by a large recognition site (double stranded DNA sequences of about 12 to about 40 base pairs). Meganucleases can be divided into five families based on sequence and structure motifs: LAGLIDADG, GIY-YIG, HNH, His-Cys box and PD-(D/E)XK. Exemplary meganucleases include I-SceI, I-CeuI, PI-PspI, PI-Sce, I-SceIV, I-CsmI, I-PanI, I-SceII, I-PpoI, I-SceIII, I-CreI, I-TevI, I-TevII and I-TevIII, whose recognition sequences are known (*see*, *e.g.*, U.S. Patent Nos. 5,420,032 and 6,833,252; Belfort *et al.*, *Nucleic Acids Res. 25*:3379-3388, 1997; Dujon *et al.*, *Gene 82*:115-118, 1989; Perler *et al.*, *Nucleic Acids Res. 22*:1125-1127, 1994; Jasin, *Trends Genet. 12*:224-228, 1996; Gimble *et al.*, *J. Mol. Biol. 263*:163-180, 1996; Argast *et al.*, *J. Mol. Biol. 280*:345-353, 1998).

In certain embodiments, naturally-occurring meganucleases may be used to promote site-specific genome modification of a target selected from PD-1, LAG3, TIM3, CTLA4, an HLA-encoding gene, or a TCR component-encoding gene. In other embodiments, an engineered meganuclease having a novel binding specificity for a target gene is used for site-specific genome modification (*see*, *e.g.*, Porteus *et al.*, *Nat. Biotechnol. 23*:967-73, 2005; Sussman *et al.*, *J. Mol. Biol. 342*:31-41, 2004; Epinat *et al.*, *Nucleic Acids Res. 31*:2952-62, 2003; Chevalier *et al.*, *Molec. Cell 10*:895-905, 2002; Ashworth *et al.*, *Nature 441*:656-659, 2006; Paques *et al.*, *Curr. Gene Ther. 7*:49-66, 2007; U.S. Patent Publication Nos. US 2007/0117128; US 2006/0206949; US 2006/0153826; US 2006/0078552; and US 2004/0002092).

In certain embodiments, a chromosomal gene knockout comprises an inhibitory nucleic acid molecule that is introduced into a modified cell comprising a heterologous polynucleotide encoding an antigen-specific receptor that specifically binds to a tumor

associated antigen, wherein the inhibitory nucleic acid molecule encodes a target-specific inhibitor and wherein the encoded target-specific inhibitor inhibits endogenous gene expression (*i.e.*, of PD-1, TIM3, LAG3, CTLA4, an HLA component, a TCR component, or any combination thereof) in the modified cell.

A chromosomal gene knockout can be confirmed directly by DNA sequencing of the modified cell following use of the knockout procedure or agent. Chromosomal gene knockouts can also be inferred from the absence of gene expression (*e.g.,* the absence of an mRNA or polypeptide product encoded by the gene) following the knockout.

In some embodiments, a modified cell is a $CD4^+$ T cell that comprises a heterologous polynucleotide encoding a binding protein of the present disclosure (*e.g.*, a MAGE-A1-specific TCR from a $CD8^+$ T cell that is capable of specifically binding to a peptide antigen). In some embodiments, a heterologously encoded TCR of a modified $CD4^+$ T cell is a high-affinity TCR. In particular embodiments, a heterologously encoded TCR of a modified $CD4^+$ T cell is capable of specifically binding to a peptide:antigen HLA complex on a cell surface independent of CD8 or in the absence of CD8.

In further embodiments, a modified $CD4^+$ T cell further comprises a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor. As shown in the Examples, co-expression of a MAGE-A1-specific binding protein of the present disclosure and at least an extracellular portion of a CD8 co-receptor by a $CD4^+$ T cell can confer a new or improved functionality (*e.g.*, improved cytokine release, CTL response when bound to a MAGE-A1:HLA-expressing target cell) upon the $CD4^+$ T cell. An amino acid sequence of a CD8 co-receptor α-chain is provided in SEQ ID NO:143. Amino acid sequences of five different isoforms of CD8 co-receptor β-chain are provided in SEQ ID NOS:144-148, respectively. In some embodiments, a modified $CD4^+$ T cell of this disclosure further comprises a heterologous polynucleotide encoding a full-length CD8 co-receptor receptor β-chain, a heterologous polynucleotide encoding a full-length CD8 co-receptor α-chain, or both. A CD8-encoding polynucleotide may, in some embodiments, be

Also provided herein are methods for making a modified $CD4^+$ T cell, wherein the methods comprise transducing a CD4+ T cell with a heterologous polynucleotide encoding a TCR from a $CD8^+$ T cell that is capable of specifically binding a peptide antigen. In certain embodiments, a TCR-encoding polynucleotide used to modify a $CD4^+$ T cell is from a naturally occurring $CD8^+$ T cell (*i.e.*, the TCR is a naturally occurring TCR). Further embodiments of the methods may include transducing the $CD4^+$ T cell with a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor, which may in some embodiments comprise a CD8α and a CD8β from the $CD8^+$ T cell.

Compositions

Also provided herein are compositions (*e.g.*, pharmaceutical compositions) that comprise a modified cell as disclosed herein and a pharmaceutically acceptable carrier, diluent, or excipient. Suitable excipients include water, saline, dextrose, glycerol, or the like and combinations thereof. In embodiments, compositions comprising fusion proteins or host cells as disclosed herein further comprise a suitable infusion media. Suitable infusion media can be any isotonic medium formulation, typically normal saline, Normosol R (Abbott) or Plasma-Lyte A (Baxter), 5% dextrose in water, Ringer's lactate can be utilized. An infusion medium can be supplemented with human serum albumin or other human serum components. Compositions described herein may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers may be frozen to preserve the stability of the formulation until infusion into the patient.

An "effective amount" of a composition refers to an amount sufficient, at dosages and for periods of time needed, to achieve the desired clinical results or beneficial treatment, as described herein. An effective amount may be delivered in one or more administrations. If the administration is to a subject already known or confirmed to have a disease or disease-state, the term "therapeutic amount" may be used in reference to treatment, whereas "prophylactically effective amount" may be used to describe administrating an effective amount to a subject that is susceptible or at risk of developing a disease or disease-state (*e.g.*, recurrence) as a preventative course.

Compositions may be administered in a manner appropriate to the disease or condition to be treated (or prevented) as determined by persons skilled in the medical art. An appropriate dose and a suitable duration and frequency of administration of the compositions will be determined by such factors as the health condition of the patient, size of the patient (*i.e.*, weight, mass, or body area), the type and severity of the patient's condition, the particular form of the active ingredient, and the method of administration. In general, an appropriate dose and treatment regimen provide the composition(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (such as described herein, including an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival, or a lessening of symptom severity). For prophylactic use, a dose should be sufficient to prevent, delay the onset of, or diminish the severity of a disease associated with disease or disorder. Prophylactic benefit of the compositions administered according to the methods described herein can be determined by performing pre-clinical (including *in vitro* and *in vivo* studies) and clinical studies and analyzing data obtained therefrom by appropriate statistical, biological, and clinical methods and techniques.

A therapeutically effective dose is an amount of host cells (expressing a binding protein or high affinity recombinant TCR specific for human MAGE-A1) used in adoptive transfer that is capable of producing a clinically desirable result (*i.e.,* a sufficient amount to induce or enhance a specific T cell immune response against cells overexpressing MAGE-A1 (*e.g.*, a cytotoxic T cell response) in a statistically significant manner) in a treated human or non-human mammal. The dosage for any one patient depends upon many factors, including the patient's size, weight, body surface area, age, the particular therapy to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Doses will vary, but a preferred dose for administration of a host cell comprising a recombinant expression vector as described herein is about 10^7 cells/m^2, about 5 x 10^7 cells/m^2, about 10^8 cells/m^2, about 5 x 10^8 cells/m^2, about 10^9 cells/m^2, about 5 x 10^9 cells/m^2, about 10^{10} cells/m^2, about 5 x 10^{10} cells/m^2, or about 10^{11} cells/m^2. In certain embodiments, a unit dose comprises a modified cell as described herein at a dose of about 10^7 cells/m^2 to about 10^{11} cells/m^2.

In certain embodiments, a unit dose comprises (i) a composition comprising at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% engineered $CD4^+$ T cells, combined with (ii) a composition comprising at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% engineered $CD8^+$ T cells, in about a 1:1 ratio. In further embodiments, a unit dose contains a reduced amount or substantially no naïve T cells (*i.e.*, has less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, less than about 5%, or less than about 1% the population of naïve T cells present in a unit dose as compared to a patient sample having a comparable number of PBMCs).

In some embodiments, a unit dose comprises (i) a composition comprising at least about 50% engineered $CD4^+$ T cells, combined with (ii) a composition comprising at least about 50% engineered $CD8^+$ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In further embodiments, a unit dose comprises (i) a composition comprising at least about 60% modified $CD4^+$ T cells, combined with (ii) a composition comprising at least about 60% modified $CD8^+$ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In still further embodiments, a unit dose comprises (i) a composition comprising at least about 70% modified $CD4^+$ T cells, combined with (ii) a composition comprising at least about 70% modified $CD8^+$ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In some embodiments, a unit dose comprises (i) a composition comprising at least about 80% modified $CD4^+$ T cells, combined with (ii) a composition comprising at least about 80% modified $CD8^+$ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In some embodiments, a unit dose comprises (i) a composition comprising at least about 85% modified $CD4^+$ T cells, combined with (ii) a composition comprising at least about 85% modified $CD8^+$ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In some embodiments, a unit dose comprises (i) a composition

comprising at least about 90% modified $CD4^+$ T cells, combined with (ii) a composition comprising at least about 90% modified $CD8^+$ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells.

In any of the embodiments described herein, a unit dose comprises equal, or approximately equal numbers, of modified $CD45RA^-$ $CD3^+$ $CD8^+$ and modified $CD45RA^-$ $CD3^+$ $CD4^+$ T_M cells.

The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including, *e.g.*, parenteral or intravenous administration or formulation. If the subject composition is administered parenterally, the composition may also include sterile aqueous or oleaginous solution or suspension. Suitable non-toxic parenterally acceptable diluents or solvents include water, Ringer's solution, isotonic salt solution, 1,3-butanediol, ethanol, propylene glycol or polythethylene glycols in mixtures with water. Aqueous solutions or suspensions may further comprise one or more buffering agents, such as sodium acetate, sodium citrate, sodium borate or sodium tartrate. Of course, any material used in preparing any dosage unit formulation should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations. Dosage unit form, as used herein, refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit may contain a predetermined quantity of modified cells or active compound calculated to produce the desired effect in association with an appropriate pharmaceutical carrier.

As used herein, administration of a composition refers to delivering the same to a subject, regardless of the route or mode of delivery. Administration may be effected continuously or intermittently, and parenterally. Administration may be for treating a subject already confirmed as having a recognized condition, disease or disease state, or for treating a subject susceptible to or at risk of developing such a condition, disease or disease state. Co-administration with an adjunctive therapy may include simultaneous and/or sequential delivery of multiple agents in any order and on any dosing schedule (*e.g.*, modified cells with one or more cytokines; immunosuppressive therapy such as calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a

mycophenolic acid prodrug, HDAC inhibitors, DNA hypomethylation agents, or any combination thereof).

In certain embodiments, a plurality of doses of a modified cell described herein is administered to the subject, which may be administered at intervals between administrations of about two to about four weeks.

Methods of Treatment

In certain aspects, the instant disclosure is directed to methods for treating a hyperproliferative disorder or a condition characterized by MAGE-A1 expression (*e.g.*, aberrant MAGE-A1 expression) by administering to human subject in need thereof a modified cell, composition, or unit dose as disclosed herein (or any combination thereof).

A condition associated with MAGE-A1 expression includes any disorder or condition in which underactivity, over-activity or improper activity of a MAGE-A1 cellular or molecular event is present, and may be the result of unusually high (with statistical significance) levels of MAGE-A1 expression or inappropriate (*i.e.*, not occurring in healthy cells of the given cell type) expression in afflicted cells (*e.g.*, myeloma cells), relative to normal cells. A subject having such a disorder or condition would benefit from treatment with a composition or method of the presently described embodiments. Some conditions associated with aberrant MAGE-A1 expression thus may include acute as well as chronic disorders and diseases, such as those pathological conditions that predispose the subject to a particular disorder.

Some examples of conditions associated with MAGE-A1 expression include proliferative disorders or hyperproliferative disorders, which refer to states of activated and/or proliferating cells (which may also be transcriptionally overactive) in a subject including tumors, neoplasms, cancer, malignancy, etc. In addition to activated or proliferating cells, the hyperproliferative disorder may also include an aberration or dysregulation of cell death processes, whether by necrosis or apoptosis. Such aberration of cell death processes may be associated with a variety of conditions, including cancer (including primary, secondary malignancies as well as metastasis), or other conditions.

The presence of a hyperproliferative disorder or malignant condition in a subject refers to the presence of dysplastic, cancerous and/or transformed cells in the subject, including, for example neoplastic, tumor, non-contact inhibited or oncogenically transformed cells, or the like (*e.g.,* solid cancers; hematologic cancers including lymphomas and leukemias, such as acute myeloid leukemia, chronic myeloid leukemia, etc.), which are known in the art and for which criteria for diagnosis and classification are established (*e.g.,* Hanahan and Weinberg, *Cell* 144:646, 2011; Hanahan and Weinberg, *Cell* 100:57, 2000; Cavallo *et al.*, *Canc. Immunol. Immunother.* 60:319, 2011; Kyrigideis *et al.*, *J. Carcinog.* 9:3, 2010). In certain embodiments, such cancer cells may be cells of acute myeloid leukemia, B-cell lymphoblastic leukemia, T-cell lymphoblastic leukemia, or myeloma, including cancer stem cells that are capable of initiating and serially transplanting any of these types of cancer (*see*, *e.g.,* Park *et al.*, *Molec. Therap.* 17:219, 2009).

In certain embodiments, there are provided methods for treating a hyperproliferative disorder, such as a hematological malignancy or a solid cancer, wherein the method comprises administering to a human subject in need thereof a modified cell, composition, or unit dose of the present disclosure. Exemplary hematological malignancies include acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM).

In further embodiments, there are provided methods for treating a hyperproliferative disorder, such as a solid cancer is selected from non-small cell lung cancer (NSCLC), triple negative breast cancer (TNBC), ovarian cancer, malignant melanoma, colon cancer, colorectal adenocarcinoma, colorectal cancer, biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, mesothelioma, osteosarcoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma,

rhabdomyosarcoma, skin cancer, soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, or uterine cancer.

As understood by a person skilled in the medical art, the terms, "treat" and "treatment," refer to medical management of a disease, disorder, or condition of a subject (*i.e.,* patient, host, who may be a human or non-human animal) (*see, e.g.,* Stedman's Medical Dictionary). In general, an appropriate dose and treatment regimen provide one or more of a binding protein or high affinity recombinant TCR specific for human MAGE-A1 or a host cell expressing the same, and optionally an adjunctive therapy (*e.g.*, a cytokine such as IL-2, IL-15, IL-21 or any combination thereof), in an amount sufficient to provide therapeutic or prophylactic benefit. Therapeutic or prophylactic benefit resulting from therapeutic treatment or prophylactic or preventative methods include, for example an improved clinical outcome, wherein the object is to prevent or retard or otherwise reduce (*e.g.,* decrease in a statistically significant manner relative to an untreated control) an undesired physiological change or disorder, or to prevent, retard or otherwise reduce the expansion or severity of such a disease or disorder. Beneficial or desired clinical results from treating a subject include abatement, lessening, or alleviation of symptoms that result from or are associated the disease or disorder to be treated; decreased occurrence of symptoms; improved quality of life; longer disease-free status (*i.e.,* decreasing the likelihood or the propensity that a subject will present symptoms on the basis of which a diagnosis of a disease is made); diminishment of extent of disease; stabilized (*i.e.,* not worsening) state of disease; delay or slowing of disease progression; amelioration or palliation of the disease state; and remission (whether partial or total), whether detectable or undetectable; or overall survival.

"Treatment" can also mean prolonging survival when compared to expected survival if a subject were not receiving treatment. Subjects in need of the methods and compositions described herein include those who already have the disease or disorder, as well as subjects prone to have or at risk of developing the disease or disorder. Subjects in need of prophylactic treatment include subjects in whom the disease, condition, or disorder is to be prevented (*i.e.,* decreasing the likelihood of occurrence or recurrence of the disease or disorder). The clinical benefit provided by the

compositions (and preparations comprising the compositions) and methods described herein can be evaluated by design and execution of *in vitro* assays, preclinical studies, and clinical studies in subjects to whom administration of the compositions is intended to benefit, as described in the examples.

In certain embodiments of the presently disclosed methods, a modified cell is capable of promoting an antigen-specific T cell response against a MAGE-A1 in a class I HLA-restricted manner. In some embodiments, a class I HLA-restricted response is transporter-associated with antigen processing (TAP) independent. In some embodiments, an antigen-specific T cell response promoted by a modified cell administered according ot the presently disclosed methods comprises at least one of a CD4+ helper T lymphocyte (Th) response and a CD8+ cytotoxic T lymphocyte (CTL) response. In particular embodiments, a CTL response elicited according to the instantly disclosed methods is directed against a cell having aberrant MAGE-A1 expression (*e.g.*, a MAGE-A1+ tumor cell). The level of a CTL immune response may be determined by any one of numerous immunological methods described herein and routinely practiced in the art. The level of a CTL immune response may be determined prior to and following administration of any one of the herein described MAGE-A1-specific binding proteins expressed by, for example, a T cell. Cytotoxicity assays for determining CTL activity may be performed using any one of several techniques and methods routinely practiced in the art (*see, e.g.,* Henkart *et al.*, "Cytotoxic T-Lymphocytes" *in Fundamental Immunology*, Paul (ed.) (2003 Lippincott Williams & Wilkins, Philadelphia, PA), pages 1127-50, and references cited therein).

Antigen-specific T cell responses are typically determined by comparisons of observed T cell responses according to any of the herein described T cell functional parameters (*e.g.,* proliferation, cytokine release, CTL activity, altered cell surface marker phenotype, etc.) that may be made between T cells that are exposed to a cognate antigen in an appropriate context (*e.g.*, the antigen used to prime or activate the T cells, when presented by immunocompatible antigen-presenting cells) and T cells from the same source population that are exposed instead to a structurally distinct or irrelevant control antigen. A response to the cognate antigen that is greater, with statistical significance, than the response to the control antigen signifies antigen-specificity.

A biological sample may be obtained from a subject for determining the presence and level of an immune response to a MAGE-A1-derived antigen peptide as described herein. A "biological sample" as used herein may be a blood sample (from which serum or plasma may be prepared), biopsy specimen, body fluids (*e.g.,* lung lavage, ascites, mucosal washings, synovial fluid), bone marrow, lymph nodes, tissue explant, organ culture, or any other tissue or cell preparation from the subject or a biological source. Biological samples may also be obtained from the subject prior to receiving any immunogenic composition, which biological sample is useful as a control for establishing baseline (*i.e.,* pre-immunization) data.

Modified cells of this disclosure are useful, in certain embodiments, in adoptive cell therapies. For example, in some embodiments, a modified cell is modified (*e.g.*, transduced with a recombinant expression vector or polynucleotide of the present disclosure) *ex vivo*, and then administered to a subject in need thereof. In certain embodiments, modified cell is an allogeneic cell, a syngeneic cell, or an autologous cell (*i.e.*, relative to the subject administered the modified cell). In any of the presently disclosed methods, a modified cell comprises a modified human immune cell selected from a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a natural killer cell, a dendritic cell, or any combination thereof. In certain embodiments, a modified cell is a T cell, *e.g.,* is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

In particular embodiments, a modified cell used in the presently disclosed methods is a CD4+ T cell. In some such embodiments, a modified CD4+ T cell further comprises a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor, and optionally encodes a complete CD8 α-chain, a complete CD8 β-chain, or both. Such methods may, in certain embodiments, further comprise administering to the subject a CD8+ T cell that is capable of specifically binding to a MAGE-A1 peptide:HLA complex on a cell surface, such as a CD8+ modified T cell according to the present disclosure.

Presently disclosed treatment or prevention methods may include any appropriate method of administering or dosing a modified cell, or a combination therapy. For example, in certain embodiments, a plurality of doses of a modified cell as

described herein is administered to the subject, which may be administered at intervals between administrations of about two to about four weeks. In addition, treatment or prevention methods of this disclosure may be administered to a subject as part of a treatment course or regimen, which may comprise additional treatments prior to, or after, administration of the instantly disclosed unit doses, cells, or compositions. In further embodiments, a cytokine is administered sequentially, provided that the subject was administered the recombinant host cell at least three or four times before cytokine administration. In certain embodiments, the cytokine is administered subcutaneously (*e.g.*, IL-2, IL-15, IL-21). In still further embodiments, the subject being treated is further receiving immunosuppressive therapy, such as calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof. In yet further embodiments, the subject being treated has received a non-myeloablative or a myeloablative hematopoietic cell transplant, wherein the treatment may be administered at least two to at least three months after the non-myeloablative hematopoietic cell transplant. In some embodiments, subject has been administered one or more of a DNA hypomethylation agent and a HDAC inhibitor, either or both of which may enhance MAGE-A1 expression (*see* Weon, J.L. and P.R. Potts, *Curr Opin Cell Biol*, 2015. 37: p. 1-8) and thereby enhance an adoptive cell therapy targeting MAGE-A1.

Methods according to the instant disclosure may, in certain embodiments, further include administering one or more additional agents to treat the disease or disorder in a combination therapy. For example, in certain embodiments, a combination therapy comprises administering a modified cell with (concurrently, simultaneously, or sequentially) an immune checkpoint inhibitor. In some embodiments, a combination therapy comprises administering a modified cell with an agonist of a stimulatory immune checkpoint agent. In further embodiments, a combination therapy comprises administering a modified cell with a secondary therapy, such as chemotherapeutic agent, a radiation therapy, a surgery, an antibody, or any combination thereof.

As used herein, the term "immune suppression agent" or "immunosuppression agent" refers to one or more cells, proteins, molecules, compounds or complexes

providing inhibitory signals to assist in controlling or suppressing an immune response. For example, immune suppression agents include those molecules that partially or totally block immune stimulation; decrease, prevent or delay immune activation; or increase, activate, or up regulate immune suppression. Exemplary immunosuppression agents to target (*e.g.*, with an immune checkpoint inhibitor) include PD-1, PD-L1, PD-L2, LAG3, CTLA4, B7-H3, B7-H4, CD244/2B4, HVEM, BTLA, CD160, TIM3, GAL9, KIR, PVR1G (CD112R), PVRL2, adenosine, A2aR, immunosuppressive cytokines (*e.g.*, IL-10, IL-4, IL-1RA, IL-35), IDO, arginase, VISTA, TIGIT, LAIR1, CEACAM-1, CEACAM-3, CEACAM-5, Treg cells, or any combination thereof.

An immune suppression agent inhibitor (also referred to as an immune checkpoint inhibitor) may be a compound, an antibody, an antibody fragment or fusion polypeptide (*e.g.,* Fc fusion, such as CTLA4-Fc or LAG3-Fc), an antisense molecule, a ribozyme or RNAi molecule, or a low molecular weight organic molecule. In any of the embodiments disclosed herein, a method may comprise a modified cell with one or more inhibitor of any one of the following immune suppression components, singly or in any combination.

In certain embodiments, a modified cell cell is used in combination with a PD-1 inhibitor, for example a PD-1-specific antibody or binding fragment thereof, such as pidilizumab, nivolumab, pembrolizumab, MEDI0680 (formerly AMP-514), AMP-224, BMS-936558 or any combination thereof. In further embodiments, a modified cell of the present disclosure is used in combination with a PD-L1 specific antibody or binding fragment thereof, such as BMS-936559, durvalumab (MEDI4736), atezolizumab (RG7446), avelumab (MSB0010718C), MPDL3280A, or any combination thereof.

In certain embodiments, a modified cell of the present disclosure is used in combination with a LAG3 inhibitor, such as LAG525, IMP321, IMP701, 9H12, BMS-986016, or any combination thereof.

In certain embodiments, a modified cell is used in combination with an inhibitor of CTLA4. In particular embodiments, a modified cell is used in combination with a CTLA4 specific antibody or binding fragment thereof, such as ipilimumab, tremelimumab, CTLA4-Ig fusion proteins (e.g., abatacept, belatacept), or any combination thereof.

In certain embodiments, a modified cell is used in combination with a B7-H3 specific antibody or binding fragment thereof, such as enoblituzumab (MGA271), 376.96, or both. A B7-H4 antibody binding fragment may be a scFv or fusion protein thereof, as described in, for example, *Dangaj et al., Cancer Res. 73*:4820, 2013, as well as those described in U.S. Patent No. 9,574,000 and PCT Patent Publication Nos. WO /201640724A1 and WO 2013/025779A1.

In certain embodiments, a modified cell is used in combination with an inhibitor of CD244.

In certain embodiments, a modified cell is used in combination with an inhibitor of BLTA, HVEM, CD160, or any combination thereof. Anti CD-160 antibodies are described in, for example, PCT Publication No. WO 2010/084158.

In certain embodiments, a modified cell is used in combination with an inhibitor of TIM3.

In certain embodiments, a modified cell is used in combination with an inhibitor of Gal9.

In certain embodiments, a modified cell is used in combination with an inhibitor of adenosine signaling, such as a decoy adenosine receptor.

In certain embodiments, a modified cell is used in combination with an inhibitor of A2aR.

In certain embodiments, a modified cell is used in combination with an inhibitor of KIR, such as lirilumab (BMS-986015).

In certain embodiments, a modified cell is used in combination with an inhibitor of an inhibitory cytokine (typically, a cytokine other than TGFβ) or Treg development or activity.

In certain embodiments a modified cell is used in combination with an IDO inhibitor, such as levo-1-methyl tryptophan, epacadostat (INCB024360; Liu *et al.*, *Blood 115*:3520-30, 2010), ebselen (Terentis *et al.* , *Biochem. 49*:591-600, 2010), indoximod, NLG919 (Mautino *et al.*, American Association for Cancer Research 104th Annual Meeting 2013; Apr 6-10, 2013), 1-methyl-tryptophan (1-MT)-tira-pazamine, or any combination thereof.

In certain embodiments, a modified cell is used in combination with an arginase inhibitor, such as N(omega)-Nitro-L-arginine methyl ester (L-NAME), N-omega-hydroxy-nor-l-arginine (nor-NOHA), L-NOHA, 2(S)-amino-6-boronohexanoic acid (ABH), S-(2-boronoethyl)-L-cysteine (BEC), or any combination thereof.

In certain embodiments, a modified cell is used in combination with an inhibitor of VISTA, such as CA-170 (Curis, Lexington, Mass.).

In certain embodiments, a modified cell is used in combination with an inhibitor of TIGIT such as, for example, COM902 (Compugen, Toronto, Ontario Canada), an inhibitor of CD155, such as, for example, COM701 (Compugen), or both.

In certain embodiments, a modified cell is used in combination with an inhibitor of PVRIG, PVRL2, or both. Anti-PVRIG antibodies are described in, for example, PCT Publication No. WO 2016/134333. Anti-PVRL2 antibodies are described in, for example, PCT Publication No. WO 2017/021526.

In certain embodiments, a modified cell is used in combination with a LAIR1 inhibitor.

In certain embodiments, a modified cell is used in combination with an inhibitor of CEACAM-1, CEACAM-3, CEACAM-5, or any combination thereof.

In certain embodiments, a modified cell is used in combination with an agent that increases the activity (*i.e.*, is an agonist) of a stimulatory immune checkpoint molecule. For example, a modified cell can be used in combination with a CD137 (4-1BB) agonist (such as, for example, urelumab), a CD134 (OX-40) agonist (such as, for example, MEDI6469, MEDI6383, or MEDI0562), lenalidomide, pomalidomide, a CD27 agonist (such as, for example, CDX-1127), a CD28 agonist (such as, for example, TGN1412, CD80, or CD86), a CD40 agonist (such as, for example, CP-870,893, rhuCD40L, or SGN-40), a CD122 agonist (such as, for example, IL-2) an agonist of GITR (such as, for example, humanized monoclonal antibodies described in PCT Patent Publication No. WO 2016/054638), an agonist of ICOS (CD278) (such as, for example, GSK3359609, mAb 88.2, JTX-2011, Icos 145-1, Icos 314-8, or any combination thereof). In any of the embodiments disclosed herein, a method may comprise administering a modified cell with one or more agonist of a stimulatory

immune checkpoint molecule, including any of the foregoing, singly or in any combination.

In certain embodiments, a combination therapy comprises a modified cell and a secondary therapy comprising one or more of: an antibody or antigen binding-fragment thereof that is specific for a cancer antigen expressed by the non-inflamed solid tumor, a radiation treatment, a surgery, a chemotherapeutic agent, a cytokine, RNAi, or any combination thereof.

In certain embodiments, a combination therapy method comprises administering a modified cell and further administering a radiation treatment or a surgery. Radiation therapy is well-known in the art and includes X-ray therapies, such as gamma-irradiation, and radiopharmaceutical therapies. Surgeries and surgical techniques appropriate to treating a given cancer in a subject are well-known to those of ordinary skill in the art.

In certain embodiments, a combination therapy method comprises administering an a modified cell and further administering a chemotherapeutic agent. A chemotherapeutic agent includes, but is not limited to, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as an intercalating agent), and a DNA repair inhibitor. Illustrative chemotherapeutic agents include, without limitation, the following groups: anti-metabolites/anti-cancer agents, such as pyrimidine analogs (5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine) and purine analogs, folate antagonists and related inhibitors (mercaptopurine, thioguanine, pentostatin and 2- chlorodeoxyadenosine (cladribine)); antiproliferative/antimitotic agents including natural products such as vinca alkaloids (vinblastine, vincristine, and vinorelbine), microtubule disruptors such as taxane (paclitaxel, docetaxel), vincristin, vinblastin, nocodazole, epothilones and navelbine, epidipodophyllotoxins (etoposide, teniposide), DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, Cytoxan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylmelamineoxaliplatin, iphosphamide,

melphalan, merchlorehtamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, temozolamide, teniposide, triethylenethiophosphoramide and etoposide (VP 16)); antibiotics such as dactinomycin (actinomycin D), daunorubicin, doxorubicin (adriamycin), idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin; enzymes (L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates -busulfan, nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes— dacarbazinine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate); platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, nilutamide) and aromatase inhibitors (letrozole, anastrozole); anticoagulants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine, clopidogrel, abciximab; antimigratory agents; antisecretory agents (breveldin); immunosuppressives (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); anti-angiogenic compounds (TNP470, genistein) and growth factor inhibitors (vascular endothelial growth factor (VEGF) inhibitors, fibroblast growth factor (FGF) inhibitors); angiotensin receptor blocker; nitric oxide donors; anti-sense oligonucleotides; antibodies (trastuzumab, rituximab); chimeric antigen receptors; cell cycle inhibitors and differentiation inducers (tretinoin); mTOR inhibitors, topoisomerase inhibitors (doxorubicin (adriamycin), amsacrine, camptothecin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin, irinotecan (CPT-11) and mitoxantrone, topotecan, irinotecan), corticosteroids (cortisone, dexamethasone, hydrocortisone, methylpednisolone, prednisone, and prenisolone); growth factor signal transduction kinase inhibitors; mitochondrial dysfunction inducers, toxins such as Cholera toxin, ricin, Pseudomonas exotoxin, Bordetella pertussis

adenylate cyclase toxin, or diphtheria toxin, and caspase activators; and chromatin disruptors.

Cytokines are increasingly used to manipulate host immune response towards anticancer activity. *See, e.g.*, Floros & Tarhini, *Semin. Oncol. 42*(4):539-548, 2015. Cytokines useful for promoting immune anticancer or antitumor response include, for example, IFN-α, IL-2, IL-3, IL-4, IL-10, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, IL-21, IL-24, and GM-CSF, singly or in any combination with a modified cell of this disclosure.

EXAMPLES

EXAMPLE 1

GENERATION OF HIGH-AFFINITY TCRS SPECIFIC FOR CANCER EPITOPES

Generation of high-affinity TCRs for use in adoptive cell therapies is difficult due to thymic selection, wherein TCRs with high-affinity for self-antigens (*e.g.*, MART1 and MAGE-A1) are removed and, therefore, relatively rare as compared to TCRs specific for foreign antigens (*see, e.g.*, Figures 1A and 1B). As shown in Figures 2A and 2B, a new screening and enrichment process was developed to identify high-affinity TCRs specific for MAGE-A1. Briefly, CD8+ T cells from peripheral blood mononuclear cells (PBMCs) of 12 healthy donors were stimulated once with peptide-pulsed autologous DCs and twice with peptide-pulsed autologous PBMCs, in the presence of IL-2, IL-7, IL-15 and IL-21, to obtain polyclonal MAGE-A1-specific CD8+ T cell lines. The stimulated cell lines from all donors were pooled and sorted several times using limited concentrations MAGE-A1 peptide:MHC multimers, which produced enriched populations of high-affinity T cell clones. TCRβ genes from the populations were sequenced to the frequency of TCRs in pooled and individual pMHC sorts.

Figure 3 shows exemplary data from a series of pMHC sorts that enriched for T cells expressing TCRβ CDR3 specific for the MAGE-A1 antigen. High-affinity clones

identified from the pool strongly bound MAGE-A1:MHC, correlating with lower EC_{50} (Figures 4A, 4B).

EXAMPLE 2

IN VITRO FUNCTIONALITY OF A MAGE-A1-SPECIFIC TCR

A high-affinity MAGE-A1-specific $CD8^+$ T cell clone "MA2" generated using the method of Example 1 (Figure 5A) was selected for further testing. As shown in Figure 5B, $MA2^+$ $CD8^+$ T cells selectively produced cytokines when co-cultured with MAGE-A1-expressing HAL-A*0201^+ U266 multiple myeloma cells (effector to target (E:T) ratio of 10:1, 4 hrs). In a standard 4 hr. Cr^{51}-release assay, $MA2^+$ T cells were capable of killing target cells in the presence or absence of exogenous IFN-γ and MAGE-A1 peptide (Figure 5C).

EXAMPLE 3

MAGE-A1-SPECIFIC CD8 TCR BINDS TETRAMER INDEPENDENT OF CD8

$CD8^+$ TCRs recognize antigens presented by class I HLA molecules, while $CD4^+$ TCRs recognize antigens presented in the context of class II HLA. To test whether the high-affinity MA2 TCR could bind MAGE-A1:HLA I independent of CD8, $CD4^+$ T cells were transduced with MA2 TCR (*see, e.g.,* schematic diagrams of Figures 6A and 6B). As shown in Figures 7A and 7B, $CD4^+$ T cells transduced with MA2 TCR bound MAGE-A1:HLA tetramers with an affinity that was comparable (~5-fold difference in B_{max}) to MA2 CD8+ T cells. However, as shown in Figure 7C, the transformed $CD4^+$ T cells did not kill target cells *in vitro*.

EXAMPLE 4

FUNCTIONAL TESTING OF AN ENGINEERED $CD4^+$ T CELL EXPRESSING A MAGE-A1-SPECIFIC CD8 TCR AND CD8 CO-RECEPTOR

Next, the ability of a $CD8^+$ co-receptor to improve functionality of high-affinity CD8-TCR-expressing $CD4^+$ T cells was investigated (*see, e.g.,* Figure 6A). As

illustrated in the diagram of Figure 8A, $CD4^+$ T cells were transduced with both a high-affinity Class-I-restricted MAGE-A1-specific TCR and a CD8 co-receptor. Figure 8B shows that a greater proportion of $CD4^+$ T cells transduced with both exogenous CD8 TCR and CD8 co-receptor produced cytokines in response to antigen, as compared to $CD4^+$ T cells transduced with the exogenous CD8 TCR alone. Figure 8C shows that the dually transduced $CD4^+$ T cells surprisingly exhibited cytolytic activity against MEL526 target cells, at rates comparable to $CD8^+$ T cells expressing the same high-affinity TCR. As shown in Figure 8D, the dually transduced $CD4^+$ T cells also proliferated more robustly following stimulation with antigen than $MA2^+$ $CD4^+$ cells without CD8.

These data show that high-affinity MAGE-A1-specific TCRs of the present disclosure, and $CD8^+$ and $CD4^+$ T cells expressing the same, are useful for targeting and killing MAGE-A1-expressing cancer cells and have use in cellular immunotherapies against MAGE-A1-expressing diseases.

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, if any, including U.S. Provisional Patent Application No. 62/471,956, filed March 15, 2017, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. An isolated modified cell comprising a heterologous polynucleotide encoding a binding protein, wherein the encoded binding protein comprises:

a T cell receptor (TCR) α-chain variable (V_α) domain having CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:48-50, respectively, and a TCR β-chain variable (V_β) domain having CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:45-47, respectively,

wherein the binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123):human leukocyte antigen (HLA)-A*0201 complex on a cell surface independent of CD8 or in the absence of CD8.

2. The isolated modified cell according to claim 1, wherein the encoded binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123):HLA-A*0201 complex with a K_d less than or equal to about 10^{-8} M.

3. The isolated modified cell of claim 1 or 2, wherein (a) the V_β domain of the encoded binding protein is derived from a TRBV30 allele, a TRBV29 allele, or a TRBV9 allele; and/or (b) the V_α domain of the encoded binding protein is derived from a TRAV38-1 allele, a TRAV34 allele, a TRAV16 allele, or a TRAV5 allele.

4. The isolated modified cell according to any one of claims 1-3, wherein the encoded V_α domain comprises an amino acid sequence that is at least about 90% identical to an amino acid sequence according to any one of SEQ ID NOS.:3, 7, 11, 15, and 19, and the encoded V_β domain comprises an amino acid sequence that is at least about 90% identical to the amino acid sequence according to any one of SEQ ID NOS.:1, 5, 9, 13, 17.

5. The isolated modified cell according to any one of claims 1-4, wherein the encoded Vα domain comprises an amino acid sequence that is at least about 90% identical to an amino acid sequence according to SEQ ID NO.:19, and the encoded Vβ domain comprises an

amino acid sequence that is at least about 90% identical to the amino acid sequence according to SEQ ID NO.:17.

6. The isolated modified cell according to any one of claims 1-5, wherein: (a) the encoded binding protein is a pre-binding protein and wherein the encoded V_α domain comprises or consists of an amino acid sequence according to SEQ ID NO.:19; and/or (b) the encoded binding protein is a pre-binding protein and wherein the encoded V_β domain comprises or consists of an amino acid sequence according to SEQ ID NO.:17.

7. The isolated modified cell according to claim 6, wherein: (a) the encoded binding protein is a pre-binding protein and wherein the encoded V_α domain comprises or consists of an amino acid sequence according to SEQ ID NO.:19; and (b) the encoded V_β domain comprises or consists of an amino acid sequence according to SEQ ID NO.:17.

8. The isolated modified cell according to any one of claims 1-7, further comprising: (a) a heterologous polynucleotide encoding a TCR α-chain constant (C_α) domain having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO.:4, 8, 12, 16, or 20; and/or (b) a heterologous polynucleotide encoding a TCR β-chain constant (C_β) domain comprising an amino acid sequence with at least 90% sequence identity to the amino acid sequence according to SEQ ID NO.:2, 6, 10, 14, or 18.

9. The isolated modified cell according to any one of claims 1-8, wherein the encoded binding protein is a pre-binding protein and the modified cell comprises a polynucleotide encoding (1) a TCR α-chain pre-protein comprising (1)(a) a Vα domain comprising or consisting of SEQ ID NO.: 19 and (1)(b) a Cα domain comprising or consisting of SEQ ID NO.: 20, and a polynucleotide encoding (2) a TCR β-chain pre-protein comprising (2)(a) a Vβ domain comprising or consisting of SEQ ID NO.: 17 and (2)(b) a Cβ domain comprising or consisting of SEQ ID NO.: 18.

10. The isolated modified cell according to any one of claims 1-9, wherein the encoded binding protein comprises a T cell receptor (TCR), an antigen-binding fragment of a TCR, or a chimeric antigen receptor, wherein, optionally:(i) the TCR, the chimeric antigen receptor, or the antigen-binding fragment of the TCR is chimeric, humanized or human;

(ii) the antigen-binding fragment of the TCR comprises a single chain TCR (scTCR); and/or

(iii) the binding protein is a chimeric antigen receptor, further optionally a TCR-CAR.

11. The isolated modified cell according to claim 10, wherein the binding protein is a TCR.

12. The isolated modified cell according to any one of claims 1-11, wherein the modified cell is a human immune cell.

13. The isolated modified cell according to claim 12, wherein the immune cell is a T cell, a NK cell, or a NK-T cell, wherein, optionally, the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

14. The isolated modified cell according to claim 13, wherein the immune cell is a CD4+ T cell, a CD8+ T cell, or both.

15. The isolated modified cell according to any one of claims 12-14, wherein the modified cell comprises a chromosomal gene knockout of a PD-1 gene; a LAG3 gene; a TIM3 gene; a CTLA4 gene; an HLA component gene; a TCR component gene, or any combination thereof; wherein, optionally, the chromosomal gene knockout comprises a knockout of an HLA component gene selected from an α1 macroglobulin gene, an α2 macroglobulin gene, an α3 macroglobulin gene, a β1 microglobulin gene, or a β2 microglobulin gene, wherein, further optionally, the chromosomal gene knockout comprises a knockout of a TCR component gene

selected from a TCR α variable region gene, a TCR β variable region gene, a TCR constant region gene, or a combination thereof.

16. The isolated modified cell according to any one of claims 14-15, wherein the modified cell is a CD4+ T cell and further comprises a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor.

17. The isolated modified cell according to claim 16, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises:

(i) the CD8 co-receptor α-chain amino acid sequence of SEQ ID NO.:143; and/or

(ii) the CD8 co-receptor β-chain amino acid sequence of any one of SEQ ID NOs.:144-148.

18. The isolated modified cell according to claim 17, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises:

(i) the CD8 co-receptor α-chain amino acid sequence of SEQ ID NO.:143; and

(ii) the CD8 co-receptor β-chain amino acid sequence of SEQ ID NO.:144 or 145.

19. The isolated modified cell according to claim 17 or 18, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises:

(i) the CD8 co-receptor α-chain amino acid sequence of SEQ ID NO.:143; and

(ii) the CD8 co-receptor β-chain amino acid sequence of SEQ ID NO.:144.

20. A composition comprising a modified cell according to any one of claims 1-19 and a pharmaceutically acceptable carrier, diluent, or excipient, wherein, optionally, the composition comprises modified CD4+ T cells, modified CD8+ T cells, or modified CD4+ T cells and modified CD8+ T cells, according to any one of claims 14-19.

21. A unit dose, comprising an effective amount of (i) the modified cell according to any one of claims 1-19 or (ii) a composition according to claim 20, wherein, optionally, (a) the

unit dose comprises (i) at least about 30% modified CD4+ T cells, combined with (ii) a composition comprising at least about 30% modified CD8+ T cells, in about a 1:1 ratio; and/or (b) the unit dose comprises (i) at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% engineered CD4+ T cells, combined with (ii) at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% engineered CD8+ T cells, in about a 1:1 ratio, wherein, further optionally, the unit dose comprises:

(1) at least about 30% modified CD4+ T cells, combined with a composition comprising at least about 30% modified CD8+ T cells, in about a 1:1 ratio;

(2) at least about 40% modified CD4+ T cells, combined with a composition comprising at least about 40% modified CD8+ T cells, in about a 1:1 ratio;

(3) at least about 50% modified CD4+ T cells, combined with a composition comprising at least about 50% modified CD8+ T cells, in about a 1:1 ratio;

(4) at least about 60% modified CD4+ T cells, combined with a composition comprising at least about 60% modified CD8+ T cells, in about a 1:1 ratio;

(5) at least about 70% modified CD4+ T cells, combined with a composition comprising at least about 70% modified CD8+ T cells, in about a 1:1 ratio;

(6) at least about 70% modified CD4+ T cells, combined with a composition comprising at least about 70% modified CD8+ T cells, in about a 1:1 ratio;

(7) at least about 80% modified CD4+ T cells, combined with a composition comprising at least about 80% modified CD8+ T cells, in about a 1:1 ratio;

(8) at least about 85% modified CD4+ T cells, combined with a composition comprising at least about 85% modified CD8+ T cells, in about a 1:1 ratio;

(9) at least about 90% modified CD4+ T cells, combined with a composition comprising at least about 90% modified CD8+ T cells, in about a 1:1 ratio; or

(10) at least about 95% modified CD4+ T cells, combined with a composition comprising at least about 95% modified CD8+ T cells, in about a 1:1 ratio; and/or (c) the unit dose contains substantially no naïve T cells; and/or (d) the unit dose comprises equal, or

approximately equal numbers, of modified CD45RA$^-$ CD3$^+$ CD8$^+$ and modified CD45RA$^-$ CD3$^+$ CD4$^+$ TM cells.

22. An isolated polynucleotide encoding:

(1) a binding protein having a TCR V_α domain and a TCR V_β domain, wherein:

(i) the encoded V_α domain comprises CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:48-50, respectively, and the encoded V_β domain comprises CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:45-47, respectively; and

(ii) the encoded binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123): human leukocyte antigen (HLA)-A*0201 complex on a cell surface independent of or in the absence of CD8; and

(2) at least an extracellular portion of a CD8 co-receptor.

23. The isolated polynucleotide of claim 22, wherein the encoded V_α domain comprises or consists of the amino acid sequence of SEQ ID NO.: 19 and the encoded V_β domain comprises or consists of the amino acid sequence of SEQ ID NO.: 17.

24. The isolated polynucleotide of claim 22 or 23, wherein the encoded binding protein further comprises a TCR α-chain constant (C_α) domain, a TCR β-chain constant (C_β) domain, or both, wherein, optionally:(a) the encoded binding protein comprises a TCR α-chain constant (C_α) domain having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO.:4, 8, 12, 16, and/or a TCR β-chain constant (C_β) domain comprising an amino acid sequence with at least 90% sequence identity to the amino acid sequence according to SEQ ID NO.:2, 6, 10, 14, or 18; wherein, further optionally, the encoded binding protein is a pre-binding protein comprising (1) a TCR α-chain pre-protein comprising (1)(a) a Vα domain comprising or consisting of SEQ ID NO.: 19 and (1)(b) a Cα domain comprising or consisting of SEQ ID NO.: 20, and (2) a TCR β-chain pre-protein comprising (2)(a) a Vβ domain comprising or consisting of SEQ ID NO.: 17 and (2)(b) a Cβ domain comprising or consisting of SEQ ID NO.: 18; and/or

(b) the V_{α}-encoding polynucleotide comprises or consists of a nucleotide sequence having at least 80% identity to SEQ ID NO.:90, and the V_{β}-encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO.:88.

25. The isolated polynucleotide of any one of claims 22-24, wherein: (1) the encoded at least an extracellular portion of a CD8 co-receptor comprises:

(i) the CD8 co-receptor α-chain amino acid sequence of SEQ ID NO.:143; and

(ii) the CD8 co-receptor β-chain amino acid sequence of SEQ ID NO.:144; and/or (2) the encoded binding protein is a TCR comprising a TCR α-chain and a TCR β-chain, wherein the polynucleotide further comprises a polynucleotide encoding a self-cleaving peptide disposed between the TCR α-chain- encoding polynucleotide and the TCR β-chain-encoding polynucleotide, wherein, optionally, the polynucleotide encodes a self-cleaving peptide comprising or consisting of an amino acid sequence according to any one of SEQ ID NOS.:124-127.

26. An expression vector, comprising a polynucleotide according to any one of claims 22-25 operably linked to an expression control sequence, wherein, optionally, (1) the vector is capable of delivering the polynucleotide to a host cell, wherein, further optionally, the host cell is a hematopoietic progenitor cell or a human immune system cell, wherein, even further optionally, the human immune system cell is a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a natural killer cell, a dendritic cell, or any combination thereof, wherein, even further optionally, the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof, and/or

(2) the vector is a viral vector, wherein, further optionally,

the viral vector is a lentiviral vector or a γ-retroviral vector.

27. A method for treating a hyperproliferative disorder associated with MAGE-A1 expression, comprising administering to human subject in need thereof a modified cell according to any one of claims 1-19, a composition according to claim 20, or a unit dose according to claim 21.

28. Use of a modified cell according to any one of claims 1-19, a composition according to claim 20, or a unit dose according to claim 21, in the preparation of a medicament for treating a hyperproliferative disorder associated with MAGE-A1 expression.

29. The method according to claim 27, or the use according to claim 28, wherein the hyperproliferative disorder is a hematological malignancy or a solid cancer.

30. The method or use according to claim 29, wherein the hematological malignancy is selected from acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM); and wherein the solid cancer is selected from non-small cell lung cancer (NSCLC), triple negative breast cancer (TNBC), ovarian cancer, malignant melanoma, colon cancer, colorectal adenocarcinoma, colorectal cancer, biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, mesothelioma, osteosarcoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, or uterine cancer.

31. The method according to any one of claims 27 or 29 or 30, or the use according to any one of claims 28-30, wherein: (a) the modified cell is an allogeneic cell, a syngeneic cell, or an autologous cell, relative to the subject; and/or (b) the modified cell is administered to the

subject at a dose of about 10^7 cells/m^2 to about 10^{11} cells/m^2; and/or (c) the modified cell, composition, or unit dose is used in combination with a PD-1-specific antibody or antigen-binding fragment thereof and/or a PD-L1-specific antibody or antigen-binding fragment thereof, wherein, optionally, the PD-L1-specific antibody comprises atezolizumab.

32. A method for making a modified cell of any one of claims 1-19, comprising introducing into the cell or a cell culture a polynucleotide encoding the binding protein, wherein, optionally, (1) the polynucleotide is comprised in a vector and/or (2) the polynucleotide is according to any one of claims 22-25.

Fred Hutchinson Cancer Center

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

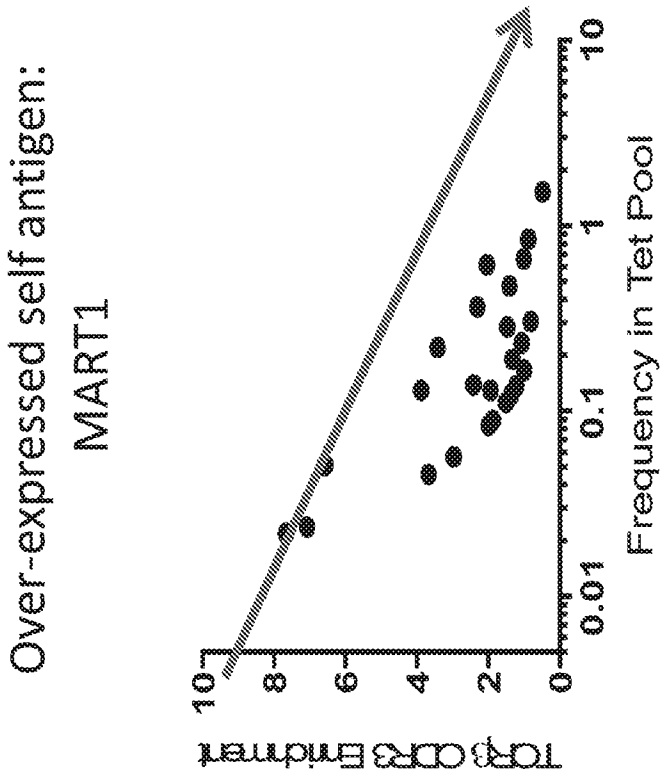
Over-expressed self antigen:
MART1
TCRb CDR3 Enrichment
0
2
4
6
8
10
0.01
0.1
1
10
Frequency in Tet Pool

FIG. 1B

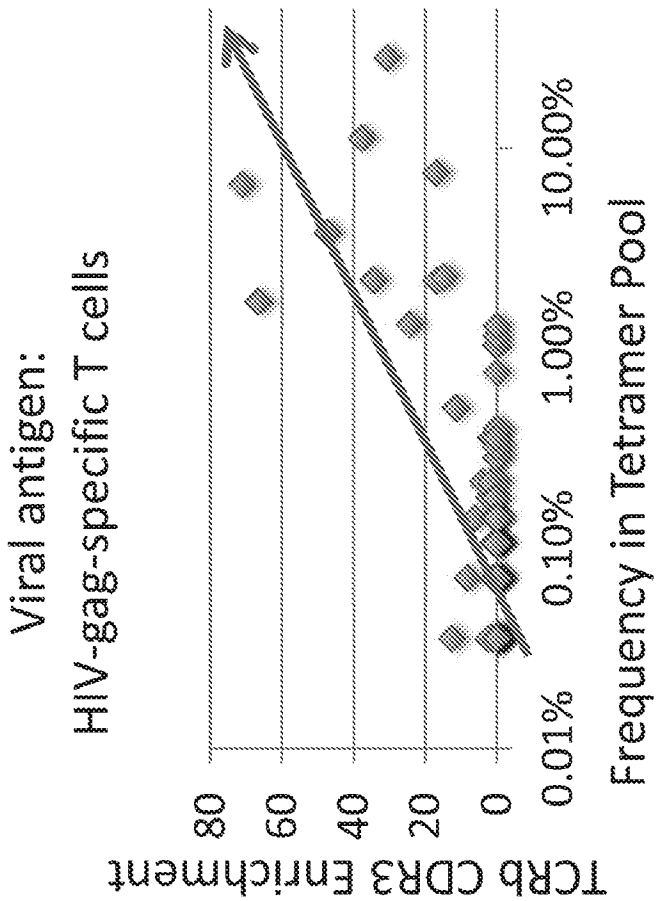
Viral antigen:
HIV-gag-specific T cells
TCRb CDR3 Enrichment
0
20
40
60
80
0.01%
0.10%
1.00%
10.00%
Frequency in Tetramer Pool

FIG. 1A

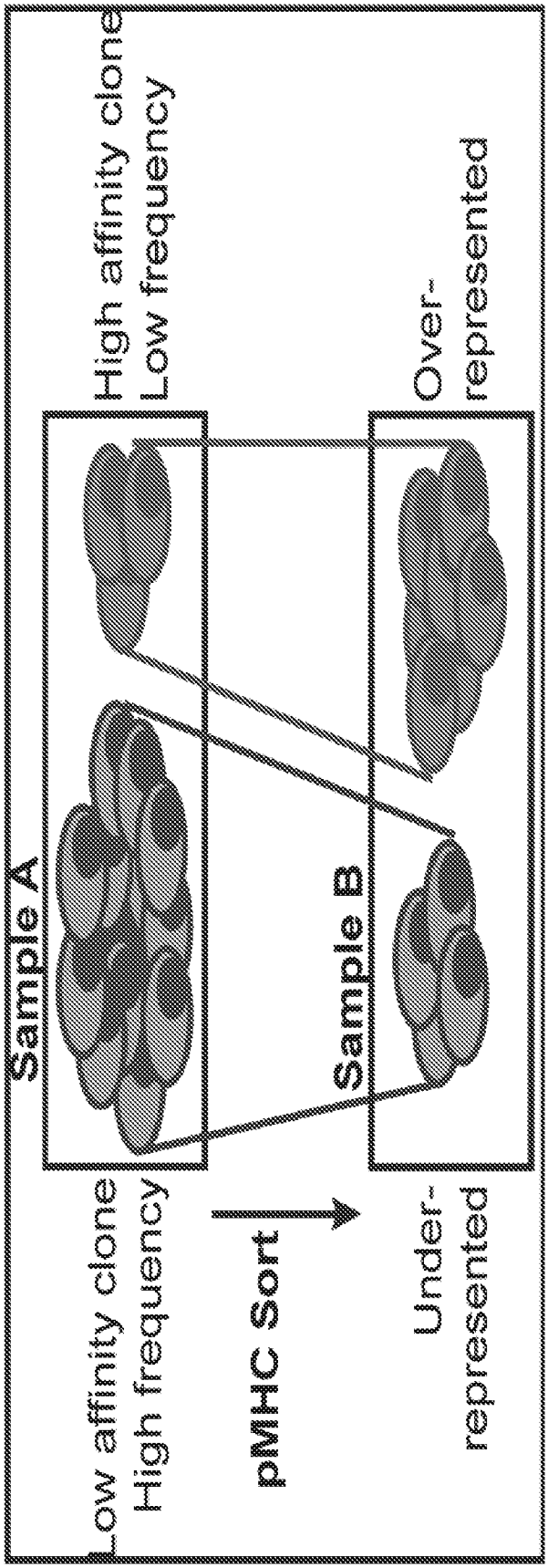
Sample A
High affinity clone
Low frequency
Low affinity clone
High frequency
pMHC Sort
Sample B
Over-
represented
Under-
represented

FIG. 2A

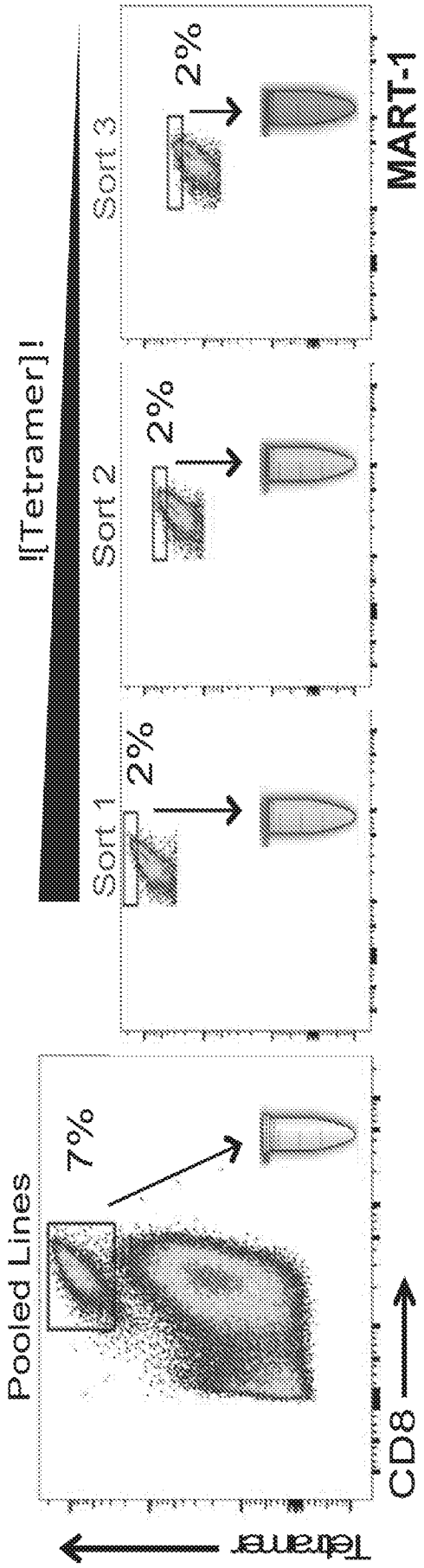
Pooled Lines
7%
Tetramer
CD8
[Tetramer]
Sort 1
2%
Sort 2
2%
Sort 3
2%
MART-1

FIG. 2B

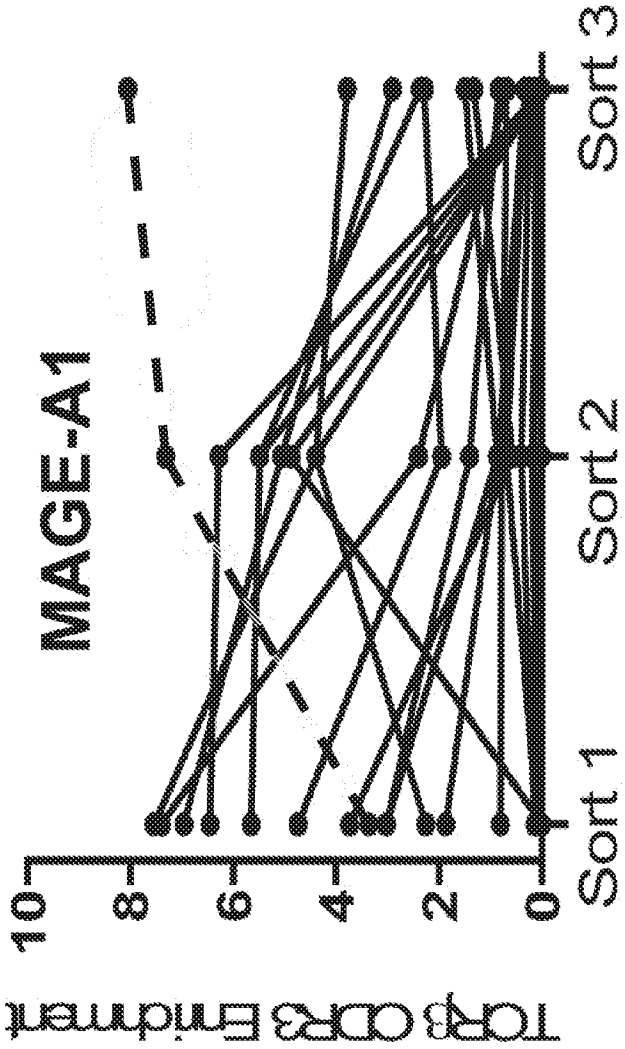

FIG. 3

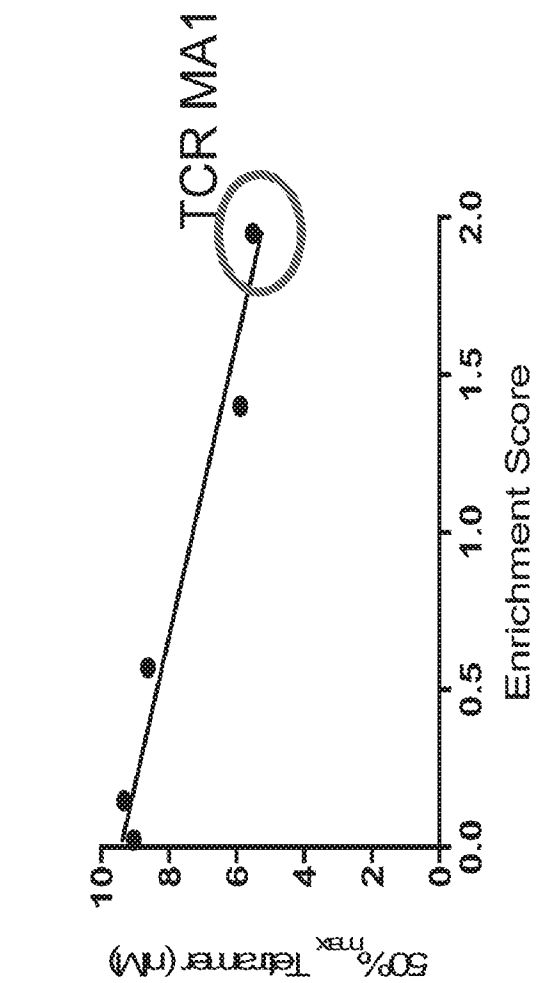
TCR MA1
10
8
6
4
2
0
0.0
0.5
1.0
1.5
2.0
Enrichment Score
50%max Tetramer (nM)

FIG. 4B

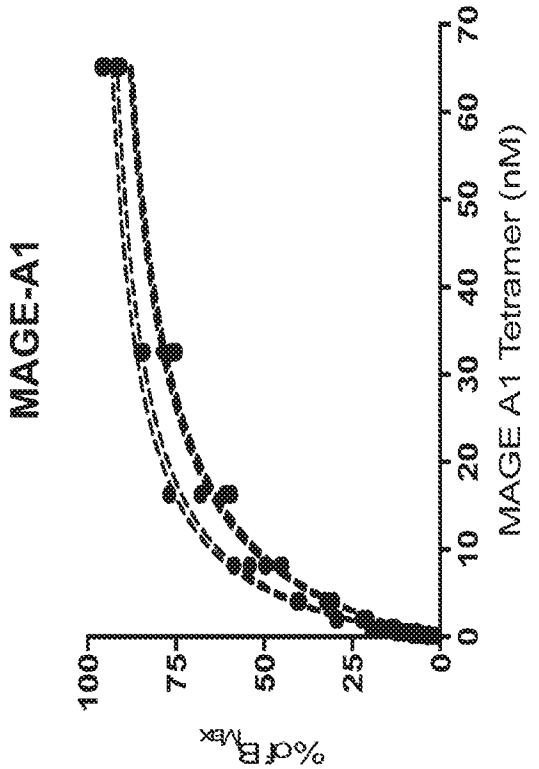
MAGE-A1
100
75
50
25
0
0
10
20
30
40
50
60
70
MAGE A1 Tetramer (nM)
%of Bmax

FIG. 4A

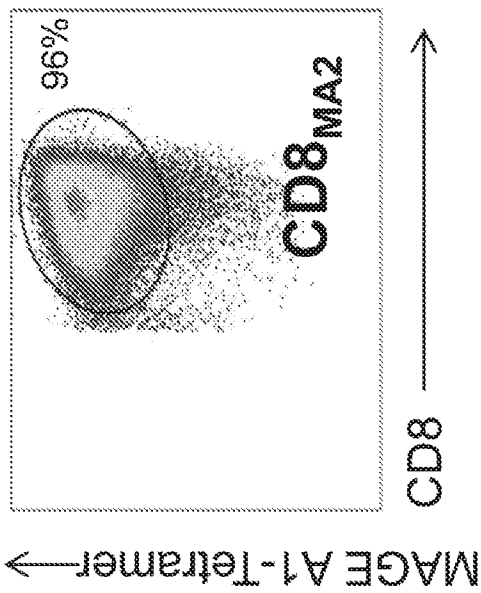

FIG. 5A

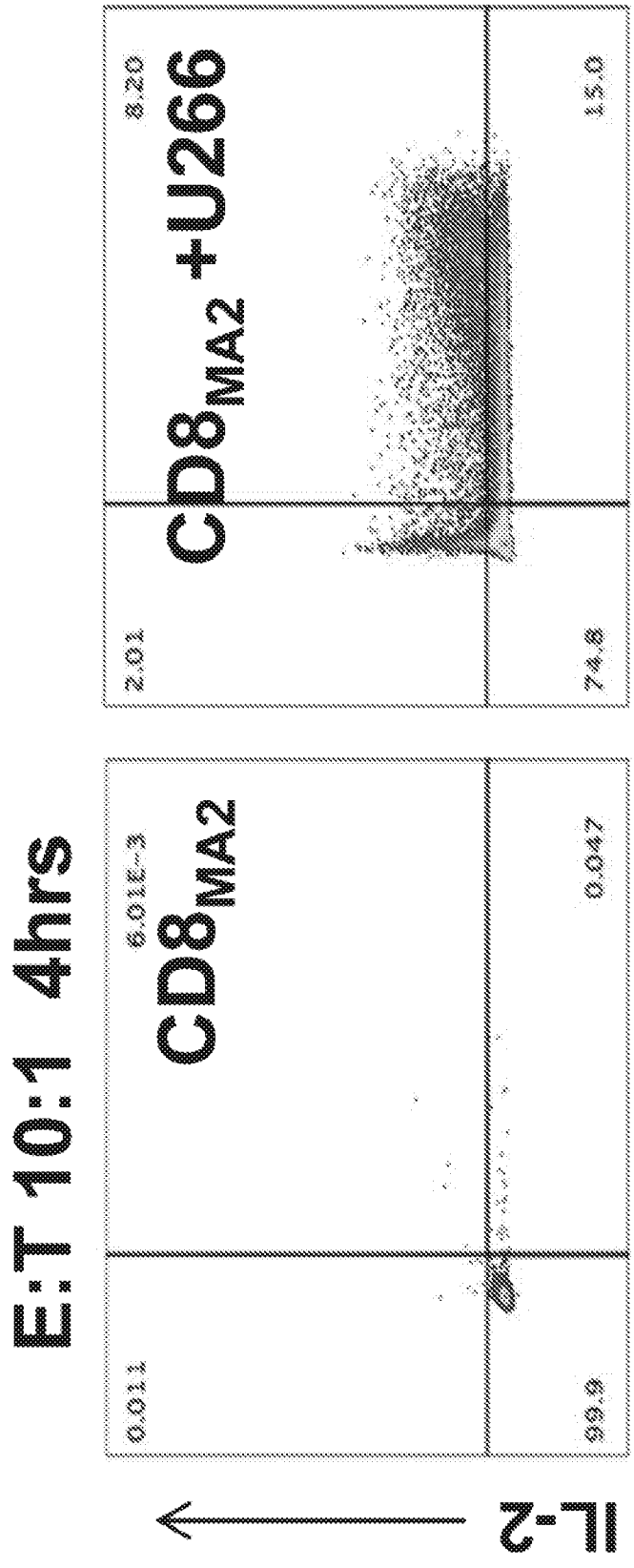
E:T 10:1 4hrs
CD8MA2
6.01E-3
0.011
0.047
99.9
CD8MA2 +U266
8.20
2.01
13.0
74.8
IL-2
IFNγ

FIG. 5B

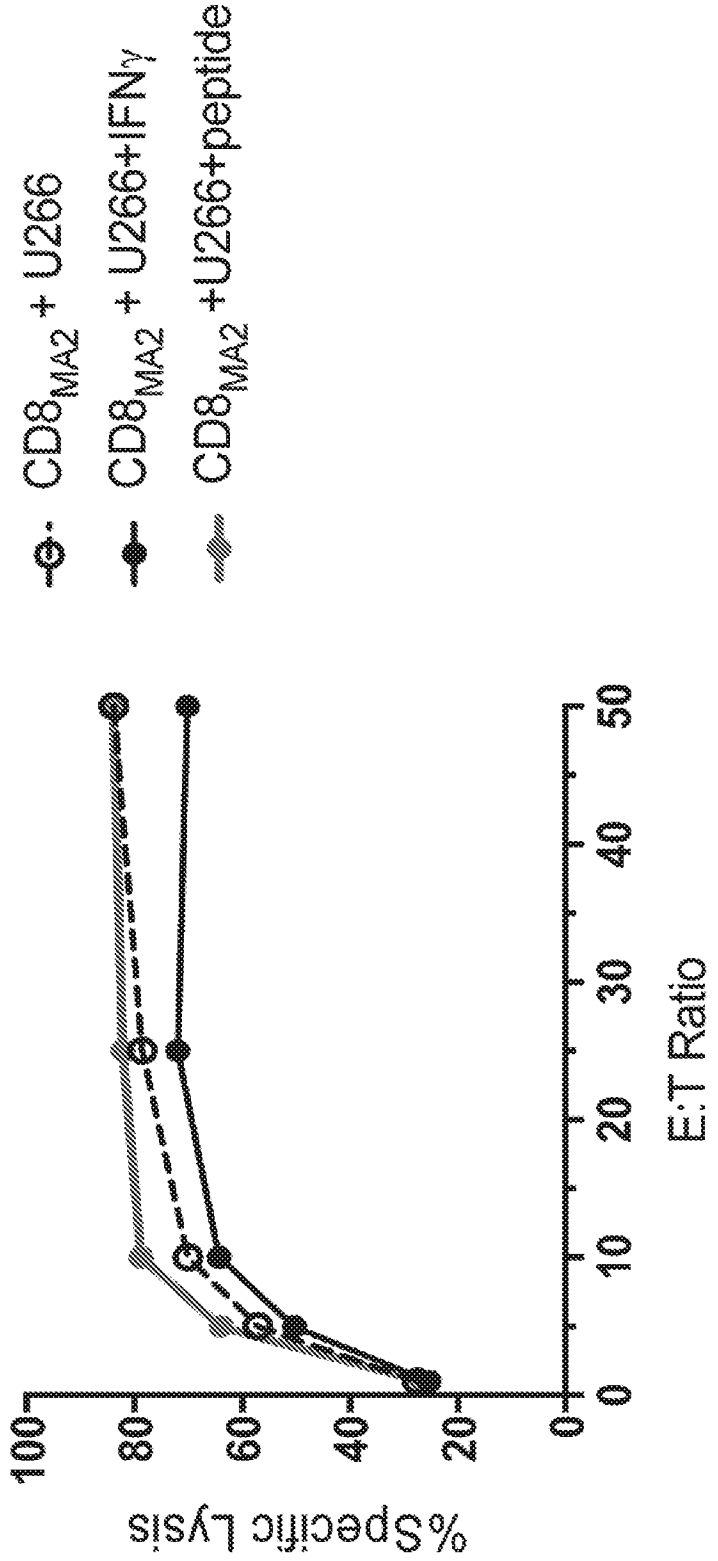
CD8MA2 + U266
CD8MA2 + U266+IFNγ
CD8MA2 +U266+peptide
%Specific Lysis
100
80
60
40
20
0
0
10
20
30
40
50
E:T Ratio

FIG. 5C

Transduce CD8+ T cells with Ag-specific TCR.
Enhance Ag-specific tumor cell killing.
Co-transduce CD4+ T cells with Ag-specific TCR plus CD8 co-receptor.
IL2, help
CD8+ T cell
activation
transduced TCR
MHC, class I
CD8
CD4+ T cell
activation
CD4
tumor cell

FIG. 6A

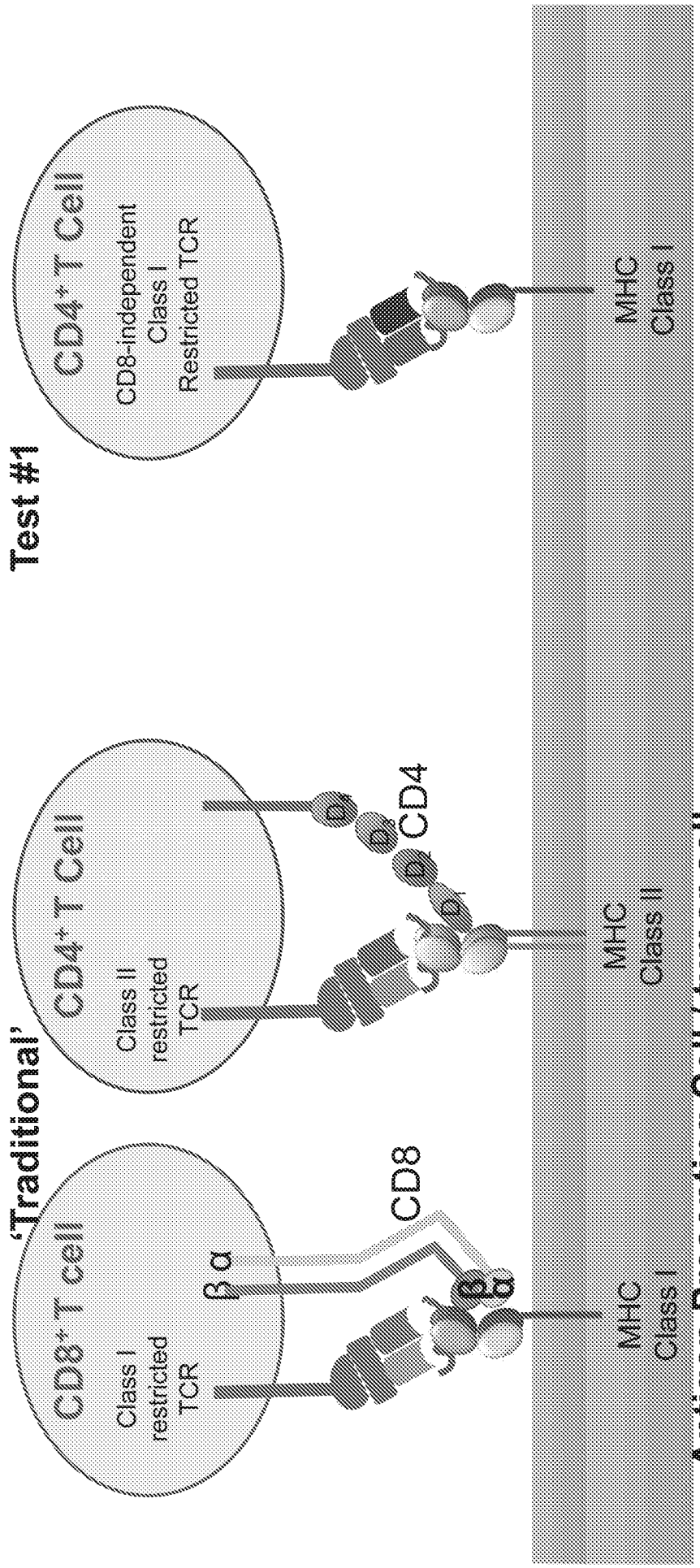
'Traditional'
Test #1
CD8+ T cell
Class I restricted TCR
CD8
CD4+ T Cell
Class II restricted TCR
CD4
CD4+ T Cell
CD8-independent Class I Restricted TCR
MHC Class I
MHC Class II
MHC Class I
Antigen Presenting Cell / tumor cell

FIG. 6B

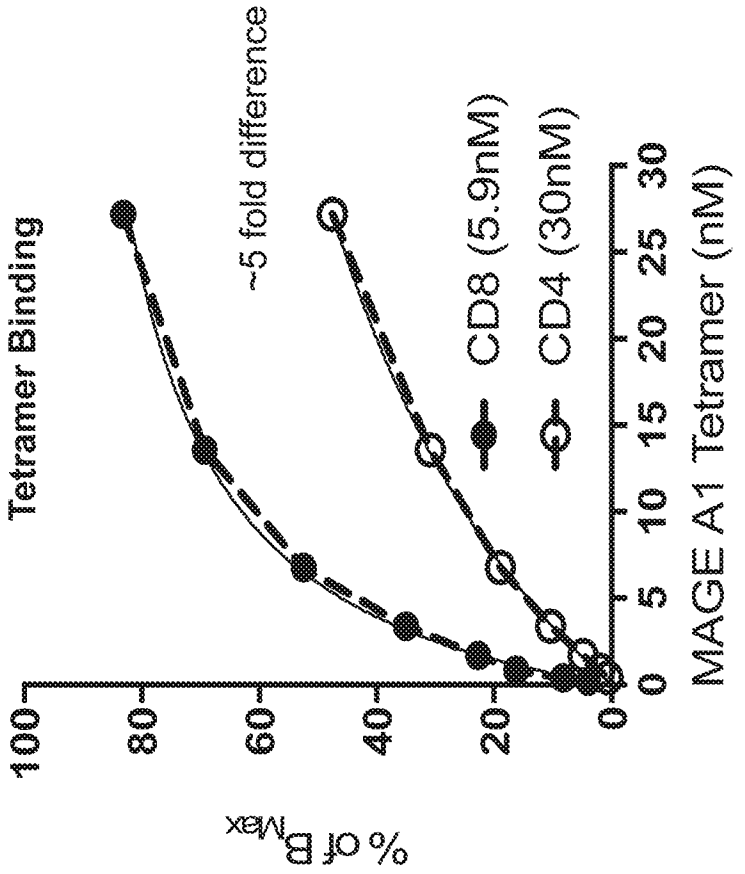
Tetramer Binding
~5 fold difference
CD8 (5.9nM)
CD4 (30nM)
% of B_{Max}
100
80
60
40
20
0
0
5
10
15
20
25
30
MAGE A1 Tetramer (nM)

FIG. 7B

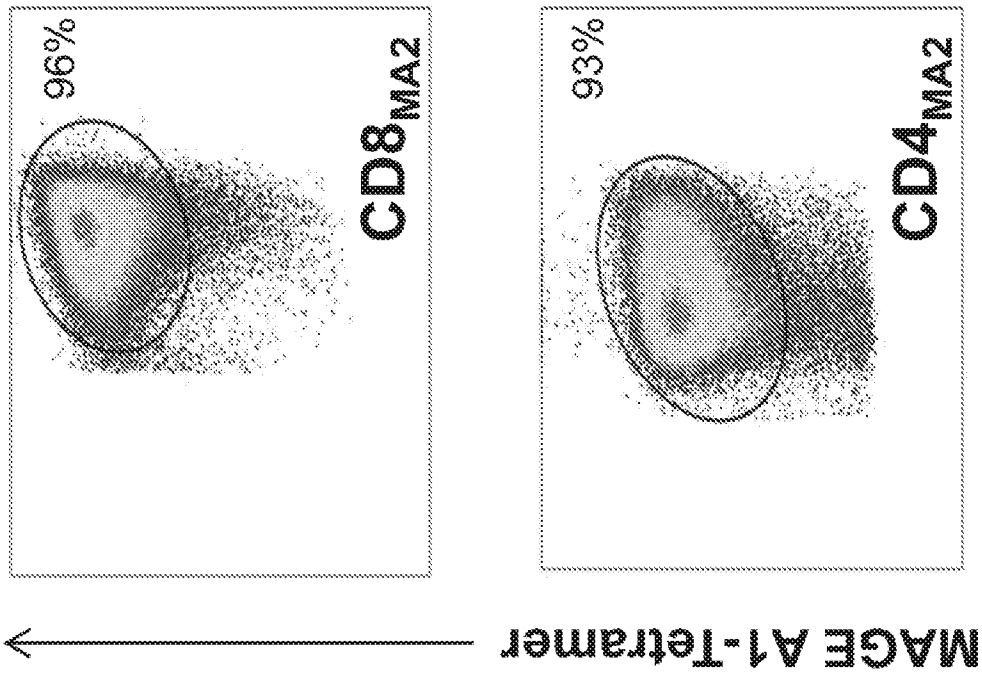
MAGE A1-Tetramer
96%
$CD8_{MA2}$
93%
$CD4_{MA2}$

FIG. 7A

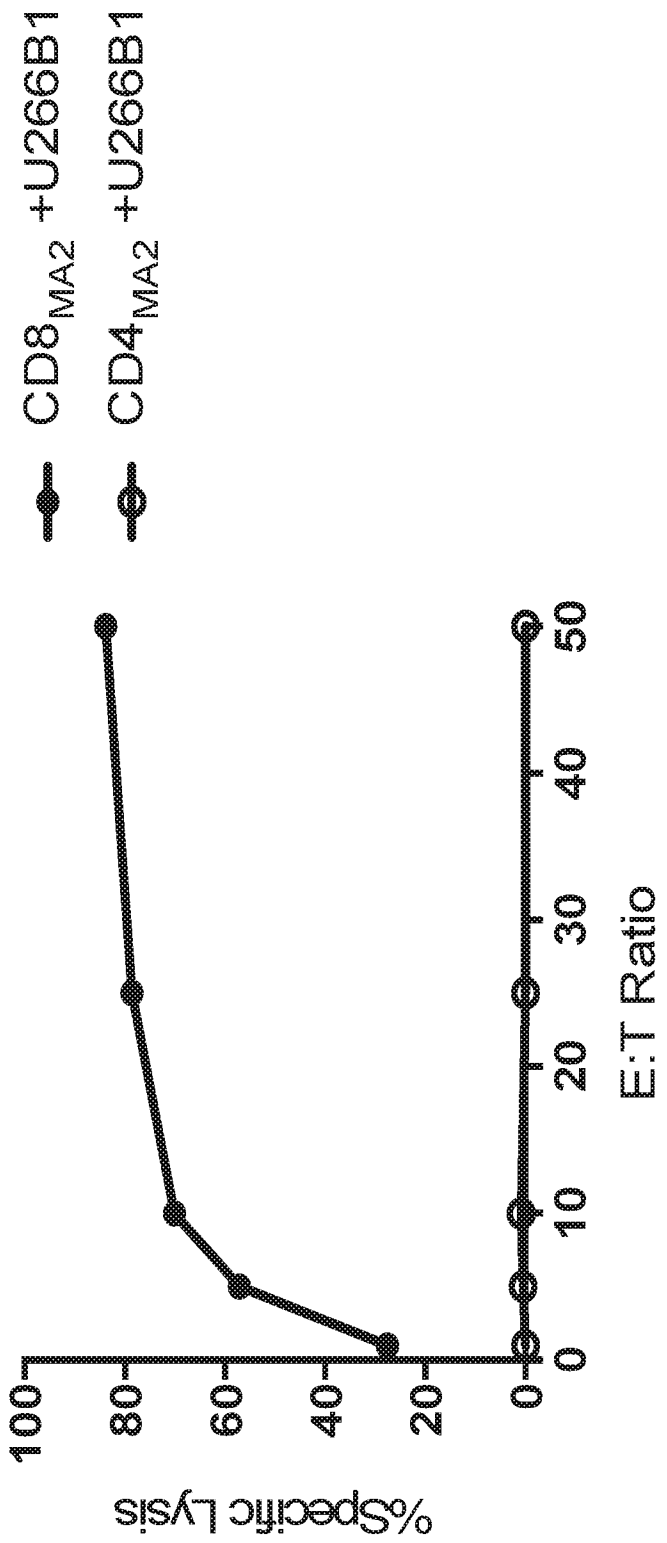
CD8MA2 +U266B1
CD4MA2 +U266B1
%Specific Lysis
100
80
60
40
20
0
0
10
20
30
40
50
E:T Ratio

FIG. 7C

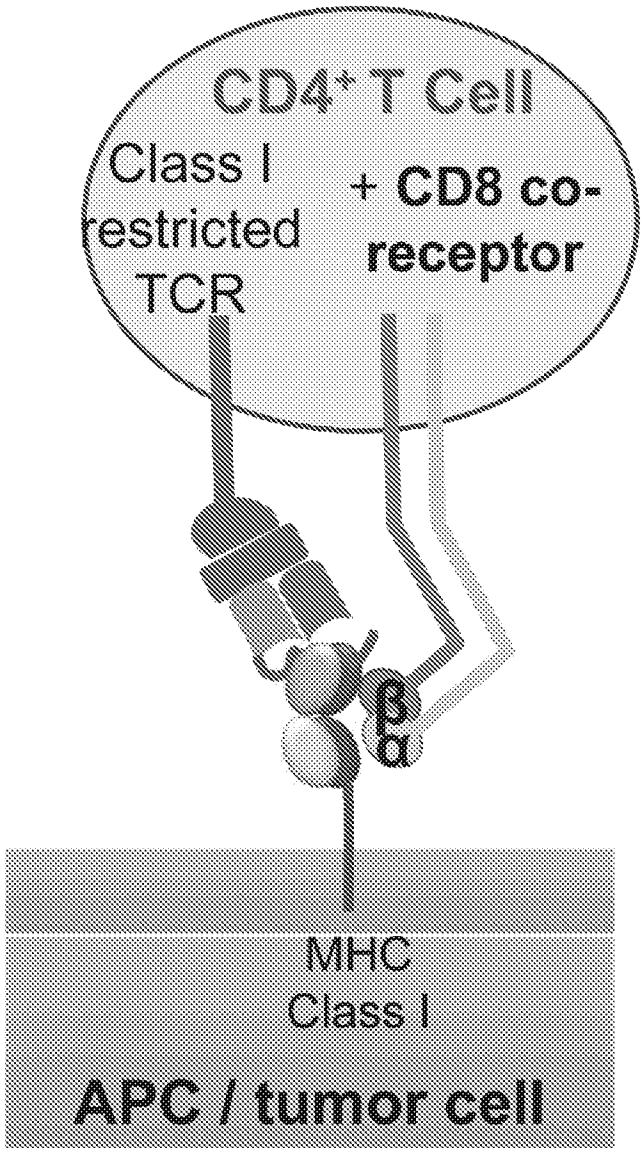
CD4+ T Cell
Class I restricted TCR
+ CD8 co-receptor
β
α
MHC Class I
APC / tumor cell

FIG. 8A

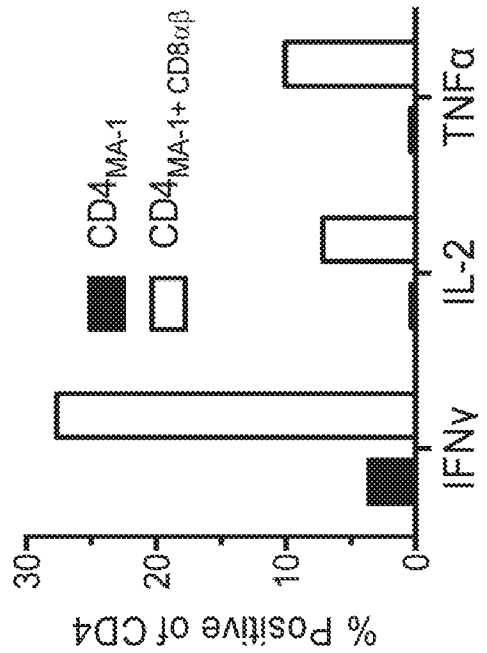
% Positive of CD4
30
20
10
0
IFNγ
IL-2
TNFα
$CD4_{MA-1}$
$CD4_{MA-1+}$ $CD8\alpha\beta$

FIG. 8B

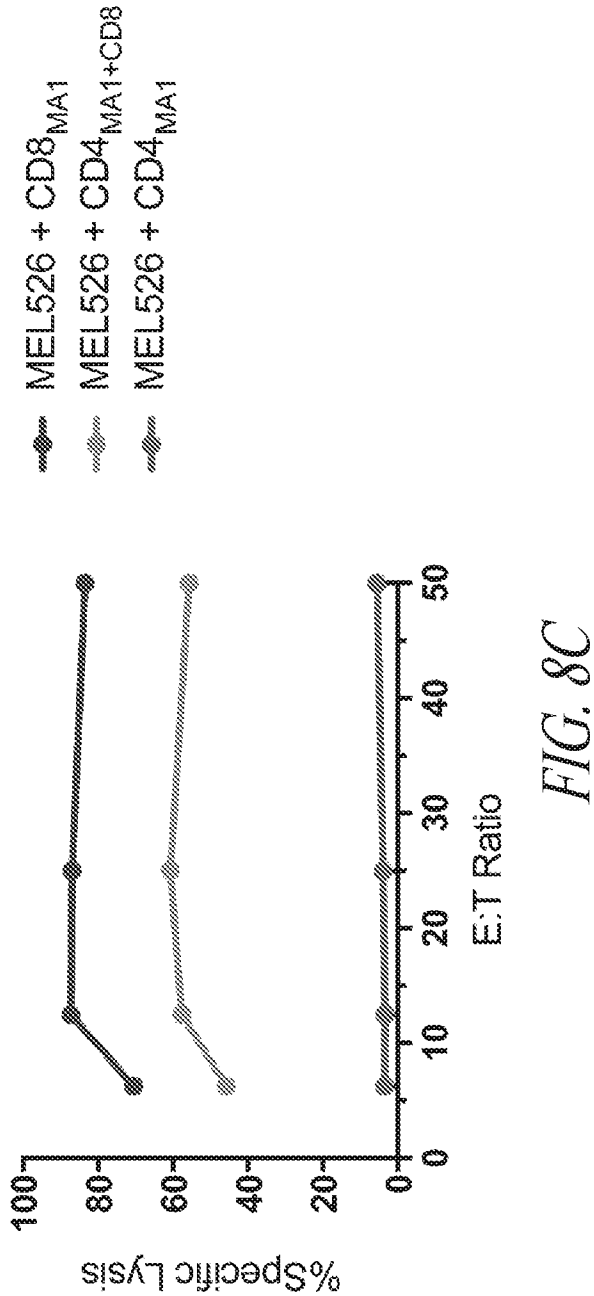
%Specific Lysis
100
80
60
40
20
0
0
10
20
30
40
50
E:T Ratio
MEL526 + $CD8_{MA1}$
MEL526 + $CD4_{MA1+CD8}$
MEL526 + $CD4_{MA1}$

FIG. 8C

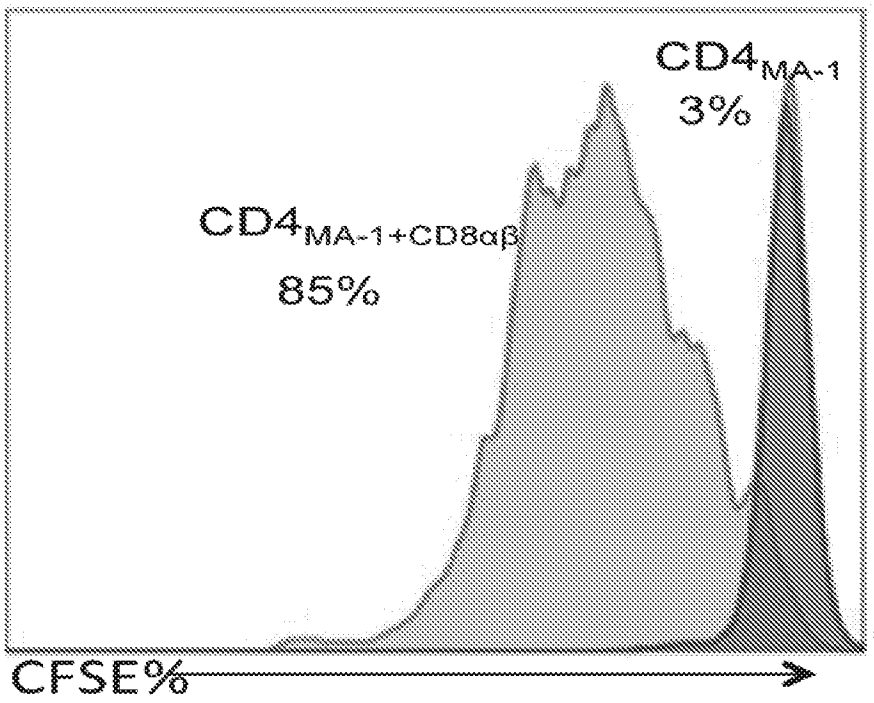

FIG. 8D

SEQUENCE LISTING

<110> Fred Hutchinson Cancer Research Center
Chapuis,Aude
Schmitt, Thomas
McAfee, Megan

<120> HIGH AFFINITY MAGE-A1 SPECIFIC TCRS AND
USES THEREOF

<130> 360056.446WO

<140> PCT
<141> 2018-03-15

<150> US 62/471,956
<151> 2017-03-15

<160> 148

<170> FastSEQ for Windows Version 4.0

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(amino acid)

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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
Asn Asn Arg Asp Ser Tyr Asn Ser Pro Leu His Phe Gly Asn Gly Thr

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        115                 120                 125
Arg Leu Thr Val Thr
    130

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      (amino acid)

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 1               5                  10                  15
Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu
            20                  25                  30
Ala Thr Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
        35                  40                  45
Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys
    50                  55                  60
Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu
65                  70                  75                  80
Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys
                85                  90                  95
Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp
            100                 105                 110
Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg
        115                 120                 125
Ala Asp Cys Gly Phe Thr Ser Val Ser Tyr Gln Gln Gly Val Leu Ser
    130                 135                 140
Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala
145                 150                 155                 160
Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp
                165                 170                 175
Phe


<210> 3
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      (amino acid)
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 1   5   10   15
Gly Thr Arg Ala Gln Arg Val Thr Gln Pro Glu Lys Leu Leu Ser Val
            20   25   30
Phe Lys Gly Ala Pro Val Glu Leu Lys Cys Asn Tyr Ser Tyr Ser Gly
        35   40   45
Ser Pro Glu Leu Phe Trp Tyr Val Gln Tyr Ser Arg Gln Arg Leu Gln
    50   55   60
Leu Leu Leu Arg His Ile Ser Arg Glu Ser Ile Lys Gly Phe Thr Ala
65   70   75   80
Asp Leu Asn Lys Gly Glu Thr Ser Phe His Leu Lys Lys Pro Phe Ala
                85   90   95
Gln Glu Glu Asp Ser Ala Met Tyr Tyr Cys Ala Leu Arg Ser Gly Gly
            100   105   110
Tyr Gln Lys Val Thr Phe Gly Thr Gly Thr Lys Leu Gln Val Ile Pro
        115   120   125

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      (amino acid)

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Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
            20   25   30
Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys
        35   40   45
Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
    50   55   60
Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
65   70   75   80
Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp
                85   90   95
Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe
            100   105   110
Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala
        115   120   125
Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser
    130   135   140

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(amino acid)

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 1   5   10   15
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            20   25   30
Ala Thr Gly Gln Arg Val Thr Leu Arg Cys Ser Pro Arg Ser Gly Asp
        35   40   45
Leu Ser Val Tyr Trp Tyr Gln Gln Ser Leu Asp Gln Gly Leu Gln Phe
    50   55   60
Leu Ile Gln Tyr Tyr Asn Gly Glu Glu Arg Ala Lys Gly Asn Ile Leu
65   70   75   80
Glu Arg Phe Ser Ala Gln Gln Phe Pro Asp Leu His Ser Glu Leu Asn
                85   90   95
Leu Ser Ser Leu Glu Leu Gly Asp Ser Ala Leu Tyr Phe Cys Ala Ser
            100   105   110
Ser Gln Gly Asp Glu Lys Leu Phe Phe Gly Ser Gly Thr Gln Leu Ser
        115   120   125
Val Leu
    130

<210> 6
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<220>
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(amino acid)

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 1   5   10   15
Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu
            20   25   30
Ala Thr Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
        35   40   45
Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys
    50   55   60
Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu
65   70   75   80

```
Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys
                85                  90                  95
Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp
            100                 105                 110
Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg
        115                 120                 125
Ala Asp Cys Gly Phe Thr Ser Val Ser Tyr Gln Gln Gly Val Leu Ser
    130                 135                 140
Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala
145                 150                 155                 160
Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp
                165                 170                 175
Phe


<210> 7
<211> 130
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence alpha chain variable domain
      (amino acid)

<400> 7
Met Lys Thr Phe Ala Gly Phe Ser Phe Leu Phe Leu Trp Leu Gln Leu
 1               5                  10                  15
Asp Cys Met Ser Arg Gly Glu Asp Val Glu Gln Ser Leu Phe Leu Ser
            20                  25                  30
Val Arg Glu Gly Asp Ser Ser Val Ile Asn Cys Thr Tyr Thr Asp Ser
        35                  40                  45
Ser Ser Thr Tyr Leu Tyr Trp Tyr Lys Gln Glu Pro Gly Ala Gly Leu
    50                  55                  60
Gln Leu Leu Thr Tyr Ile Phe Ser Asn Met Asp Met Lys Gln Asp Gln
65                  70                  75                  80
Arg Leu Thr Val Leu Leu Asn Lys Lys Asp Lys His Leu Ser Leu Arg
                85                  90                  95
Ile Ala Asp Thr Gln Thr Gly Asp Ser Ala Ile Tyr Phe Cys Ala Glu
            100                 105                 110
Ser Ile Asp Ala Arg Leu Met Phe Gly Asp Gly Thr Gln Leu Val Val
        115                 120                 125
Lys Pro
    130

<210> 8
<211> 141
<212> PRT
<213> Artificial Sequence
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<220>
<223> Synthetic sequence alpha chain constant domain
(amino acid)

<400> 8
Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
 1   5   10   15
Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
            20   25   30
Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys
        35   40   45
Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
    50   55   60
Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
65   70   75   80
Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp
                85   90   95
Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe
            100   105   110
Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala
        115   120   125
Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser
    130   135   140

<210> 9
<211> 127
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence beta chain variable domain
(amino acid)

<400> 9
Met Leu Cys Ser Leu Leu Ala Leu Leu Leu Gly Thr Phe Phe Gly Val
 1   5   10   15
Arg Ser Gln Thr Ile His Gln Trp Pro Ala Thr Leu Val Gln Pro Val
            20   25   30
Gly Ser Pro Leu Ser Leu Glu Cys Thr Val Glu Gly Thr Ser Asn Pro
        35   40   45
Asn Leu Tyr Trp Tyr Arg Gln Ala Ala Gly Arg Gly Leu Gln Leu Leu
    50   55   60
Phe Tyr Ser Val Gly Ile Gly Gln Ile Ser Ser Glu Val Pro Gln Asn
65   70   75   80
Leu Ser Ala Ser Arg Pro Gln Asp Arg Gln Phe Ile Leu Ser Ser Lys
                85   90   95
Lys Leu Leu Leu Ser Asp Ser Gly Phe Tyr Leu Cys Ala Leu Ser Thr

100 105 110
Ser Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr Val Thr
115 120 125

<210> 10
<211> 178
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence beta chain constant domain
(amino acid)

<400> 10
Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser
1 5 10 15
Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu Ala
20 25 30
Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn Gly
35 40 45
Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys Glu
50 55 60
Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu Arg
65 70 75 80
Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys Gln
85 90 95
Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp Arg
100 105 110
Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg Ala
115 120 125
Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly Val Leu Ser Ala
130 135 140
Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val
145 150 155 160
Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp Ser
165 170 175
Arg Gly

<210> 11
<211> 133
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence slpha chain variable domain
(amino acid)

<400> 11
Met Thr Arg Val Ser Leu Leu Trp Ala Val Val Val Ser Thr Cys Leu
 1   5   10   15
Glu Ser Gly Met Ala Gln Thr Val Thr Gln Ser Gln Pro Glu Met Ser
            20   25   30
Val Gln Glu Ala Glu Thr Val Thr Leu Ser Cys Thr Tyr Asp Thr Ser
        35   40   45
Glu Asn Asn Tyr Tyr Leu Phe Trp Tyr Lys Gln Pro Pro Ser Arg Gln
    50   55   60
Met Ile Leu Val Ile Arg Gln Glu Ala Tyr Lys Gln Gln Asn Ala Thr
65   70   75   80
Glu Asn Arg Phe Ser Val Asn Phe Gln Lys Ala Ala Lys Ser Phe Ser
                85   90   95
Leu Lys Ile Ser Asp Ser Gln Leu Gly Asp Thr Ala Met Tyr Phe Cys
            100   105   110
Ala Phe Met Lys Ser His Ser Gly Tyr Ile Phe Gly Thr Gly Thr Arg
        115   120   125
Leu Lys Val Leu Ala
    130

<210> 12
<211> 141
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence alpha chain constant domain
      (amino acid)

<400> 12
Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
 1   5   10   15
Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
            20   25   30
Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys
        35   40   45
Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
    50   55   60
Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
65   70   75   80
Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp
                85   90   95
Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe
            100   105   110
Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala
        115   120   125
Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser
    130   135   140

<210> 13
<211> 129
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence beta chain variable domain
(amino acid)

<400> 13
Met Leu Cys Ser Leu Leu Ala Leu Leu Leu Gly Thr Phe Phe Gly Val
 1   5   10   15
Arg Ser Gln Thr Ile His Gln Trp Pro Ala Thr Leu Val Gln Pro Val
            20   25   30
Gly Ser Pro Leu Ser Leu Glu Cys Thr Val Glu Gly Thr Ser Asn Pro
        35   40   45
Asn Leu Tyr Trp Tyr Arg Gln Ala Ala Gly Arg Gly Leu Gln Leu Leu
    50   55   60
Phe Tyr Ser Val Gly Ile Gly Gln Ile Ser Ser Glu Val Pro Gln Asn
65   70   75   80
Leu Ser Ala Ser Arg Pro Gln Asp Arg Gln Phe Ile Leu Ser Ser Lys
                85   90   95
Lys Leu Leu Leu Ser Asp Ser Gly Phe Tyr Leu Cys Ala Trp Ser Val
            100   105   110
Ala Val Asn Thr Glu Ala Phe Phe Gly Gln Gly Thr Arg Leu Thr Val
        115   120   125
Val

<210> 14
<211> 177
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence beta chain constant domain
(amino acid)

<400> 14
Glu Asp Leu Asn Lys Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro
 1   5   10   15
Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu
            20   25   30
Ala Thr Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp Val Asn
        35   40   45
Gly Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys
    50   55   60

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Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu
65                  70                  75                  80
Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys
                85                  90                  95
Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp
            100                 105                 110
Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg
        115                 120                 125
Ala Asp Cys Gly Phe Thr Ser Val Ser Tyr Gln Gln Gly Val Leu Ser
    130                 135                 140
Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala
145                 150                 155                 160
Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp
                165                 170                 175
Phe


<210> 15
<211> 132
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence alpha chain variable domain
      (amino acid)

<400> 15
Met Thr Arg Val Ser Leu Leu Trp Ala Val Val Val Ser Thr Cys Leu
 1               5                  10                  15
Glu Ser Gly Met Ala Gln Thr Val Thr Gln Ser Gln Pro Glu Met Ser
            20                  25                  30
Val Gln Glu Ala Glu Thr Val Thr Leu Ser Cys Thr Tyr Asp Thr Ser
        35                  40                  45
Glu Asn Asn Tyr Tyr Leu Phe Trp Tyr Lys Gln Pro Pro Ser Arg Gln
    50                  55                  60
Met Ile Leu Val Ile Arg Gln Glu Ala Tyr Lys Gln Gln Asn Ala Thr
65                  70                  75                  80
Glu Asn Arg Phe Ser Val Asn Phe Gln Lys Ala Ala Lys Ser Phe Ser
                85                  90                  95
Leu Lys Ile Ser Asp Ser Gln Leu Gly Asp Thr Ala Met Tyr Phe Cys
            100                 105                 110
Ala Phe Gly Glu Gly Ala Arg Leu Met Phe Gly Asp Gly Thr Gln Leu
        115                 120                 125
Val Val Lys Pro
    130

<210> 16
<211> 141
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<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence alpha chain constant domain
(amino acid)

<400> 16
Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
1 5 10 15
Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
20 25 30
Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys
35 40 45
Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
50 55 60
Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
65 70 75 80
Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp
85 90 95
Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe
100 105 110
Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala
115 120 125
Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser
130 135 140

<210> 17
<211> 129
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence beta chain variable domain
(amino acid)

<400> 17
Met Leu Cys Ser Leu Leu Ala Leu Leu Leu Gly Thr Phe Phe Gly Val
1 5 10 15
Arg Ser Gln Thr Ile His Gln Trp Pro Ala Thr Leu Val Gln Pro Val
20 25 30
Gly Ser Pro Leu Ser Leu Glu Cys Thr Val Glu Gly Thr Ser Asn Pro
35 40 45
Asn Leu Tyr Trp Tyr Arg Gln Ala Ala Gly Arg Gly Leu Gln Leu Leu
50 55 60
Phe Tyr Ser Ile Gly Ile Asp Gln Ile Ser Ser Glu Val Pro Gln Asn
65 70 75 80
Leu Ser Ala Ser Arg Pro Gln Asp Arg Gln Phe Ile Leu Ser Ser Lys

```
                85                  90                  95
Lys Leu Leu Leu Ser Asp Ser Gly Phe Tyr Leu Cys Ala Trp Ser Val
            100                 105                 110
Thr Arg His Asn Glu Gln Phe Phe Gly Pro Gly Thr Arg Leu Thr Val
        115                 120                 125
Leu


<210> 18
<211> 178
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence beta chain constant domain
      (amino acid)

<400> 18
Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser
 1               5                  10                  15
Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu Ala
            20                  25                  30
Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn Gly
        35                  40                  45
Lys Glu Val His Ser Gly Val Cys Thr Asp Pro Gln Pro Leu Lys Glu
    50                  55                  60
Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu Arg
65                  70                  75                  80
Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys Gln
                85                  90                  95
Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp Arg
            100                 105                 110
Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg Ala
        115                 120                 125
Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly Val Leu Ser Ala
    130                 135                 140
Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val
145                 150                 155                 160
Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp Ser
                165                 170                 175
Arg Gly


<210> 19
<211> 135
<212> PRT
<213> Artificial Sequence
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<220>
<223> Synthetic sequence alpha chain variable domain
(amino acid)

<400> 19
Met Leu Cys Ser Leu Leu Ala Leu Leu Leu Gly Thr Phe Phe Glu Pro
 1   5   10   15
Arg Thr Ser Gln Glu Leu Glu Gln Ser Pro Gln Ser Leu Ile Val Gln
            20   25   30
Glu Gly Lys Asn Leu Thr Ile Asn Cys Thr Ser Ser Lys Thr Leu Tyr
        35   40   45
Gly Leu Tyr Trp Tyr Lys Gln Lys Tyr Gly Glu Gly Leu Ile Phe Leu
    50   55   60
Met Met Leu Gln Lys Gly Gly Glu Glu Lys Ser His Glu Lys Ile Thr
65   70   75   80
Ala Lys Leu Asp Glu Lys Lys Gln Gln Ser Ser Leu His Ile Thr Ala
                85   90   95
Ser Gln Pro Ser His Ala Gly Ile Tyr Leu Cys Gly Ala Ala Pro Thr
            100   105   110
Tyr Ser Asn Tyr Gly Gly Ser Gln Gly Asn Leu Ile Phe Gly Lys Gly
        115   120   125
Thr Lys Leu Ser Val Lys Pro
    130   135

<210> 20
<211> 141
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence alpha chain constant domain
(amino acid)

<400> 20
Asp Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys
 1   5   10   15
Ser Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr
            20   25   30
Asn Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Cys
        35   40   45
Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala
    50   55   60
Trp Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser
65   70   75   80
Ile Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp
                85   90   95
Val Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe
            100   105   110

Gln Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala
        115   120   125
Gly Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser
    130   135   140

<210> 21
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain CDR1 domain
      (amino acid)

<400> 21
Met Asp His Glu Asn
 1   5

<210> 22
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain CDR2 domain
      (amino acid)

<400> 22
Ser Tyr Asp Val Lys Met
 1   5

<210> 23
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain CDR3 domain
      (amino acid)

<400> 23
Cys Ala Ser Asn Asn Arg Asp Ser Tyr Asn Ser Pro Leu His Phe
 1   5   10   15

<210> 24
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain CDR1 domain
(amino acid)

<400> 24
Tyr Ser Gly Ser Pro Glu
1 5

<210> 25
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain CDR2 domain
(amino acid)

<400> 25
His Ile Ser Arg
1

<210> 26
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain CDR3 domain
(amino acid)

<400> 26
Cys Ala Leu Arg Ser Gly Gly Tyr Gln Lys Val Thr Phe
1 5 10

<210> 27
<211> 5
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic sequence 1388.2b beta chain CDR1 domain
(amino acid)

<400> 27
Ser Gly Asp Leu Ser
1 5

<210> 28
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b beta chain CDR2 domain
(amino acid)

<400> 28
Tyr Tyr Asn Gly Glu Glu
1 5

<210> 29
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b beta chain CDR3 domain
(amino acid)

<400> 29
Cys Ala Ser Ser Gln Gly Asp Glu Lys Leu Phe Phe
1 5 10

<210> 30
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain CDR1 domain
(amino acid)

<400> 30
Asp Ser Ser Ser Thr Tyr

 1   5

<210> 31
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain CDR2 domain
      (amino acid)

<400> 31
Ile Phe Ser Asn Met Asp Met
 1   5

<210> 32
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain CDR3 domain
      (amino acid)

<400> 32
Cys Ala Glu Ser Ile Asp Ala Arg Leu Met Phe
 1   5   10

<210> 33
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain CDR1 domain
      (amino acid)

<400> 33
Gly Thr Ser Asn Pro Asn
 1   5

<210> 34
<211> 5
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic sequence 1388.3 beta chain CDR2 domain
(amino acid)

<400> 34
Ser Val Gly Ile Gly
1 5

<210> 35
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain CDR3 domain
(amino acid)

<400> 35
Cys Ala Leu Ser Thr Ser Tyr Glu Gln Tyr Phe
1 5 10

<210> 36
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain CDR1 domain
(amino acid)

<400> 36
Thr Ser Glu Asn Asn Tyr Tyr
1 5

<210> 37
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain CDR2 domain
(amino acid)

<400> 37
Gln Glu Ala Tyr Lys Gln Gln Asn

 1   5

<210> 38
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain CDR3 domain
      (amino acid)

<400> 38
Cys Ala Phe Met Lys Ser His Ser Gly Tyr Ile Phe
 1   5   10

<210> 39
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b beta chain CDR1 domain
      (amino acid)

<400> 39
Gly Thr Ser Asn Pro Asn
 1   5

<210> 40
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b beta chain CDR2 domain
      (amino acid)

<400> 40
Ser Val Gly Ile Gly
 1   5

<210> 41
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b beta chain CDR3
domain (amino acid)

<400> 41
Cys Ala Trp Ser Val Ala Val Asn Thr Glu Ala Phe Phe
 1   5   10

<210> 42
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b alpha chain CDR1
domain (amino acid)

<400> 42
Thr Ser Glu Asn Asn Tyr Tyr
 1   5

<210> 43
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b alpha chain CDR2
domain (amino acid)

<400> 43
Gln Glu Ala Tyr Lys Gln Gln Asn
 1   5

<210> 44
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b alpha chain CDR3
domain (amino acid)

<400> 44

Cys Ala Phe Gly Glu Gly Ala Arg Leu Met Phe
 1   5   10

<210> 45
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 beta chain CDR1 domain
      (amino acid)

<400> 45
Gly Thr Ser Asn Pro Asn
 1   5

<210> 46
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 beta chain CDR2 domain
      (amino acid)

<400> 46
Ser Ile Gly Ile Asp
 1   5

<210> 47
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 beta chain CDR3 domain
      (amino acid)

<400> 47
Cys Ala Trp Ser Val Thr Arg His Asn Glu Gln Phe Phe
 1   5   10

<210> 48
<211> 5
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 alpha chain CDR1 domain
(amino acid)

<400> 48
Lys Thr Leu Tyr Gly
1 5

<210> 49
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 alpha chain CDR2 domain
(amino acid)

<400> 49
Leu Gln Lys Gly Gly Glu Glu
1 5

<210> 50
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 alpha chain CDR3 domain
(amino acid)

<400> 50
Cys Gly Ala Ala Pro Thr Tyr Ser Asn Tyr Gly Gly Ser Gln Gly Asn
1 5 10 15
Leu Ile Phe

<210> 51
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence 18648.2 alpha chain CDR3 domain
(amino acid)

<400> 51
Cys Ala Leu Arg Gly Leu Asn Tyr Gly Gln Asn Phe Val Phe
 1   5   10

<210> 52
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain variable
      domain

<400> 52
atgggaatca ggctcctctg tcgtgtggcc ttttgtttcc tggctgtagg cctcgtagat 60
gtgaaagtaa cccagagctc gagatatcta gtcaaaagga cgggagagaa agtttttctg 120
gaatgtgtcc aggatatgga ccatgaaaat atgttctggt atcgacaaga cccaggtctg 180
gggctacggc tgatctattt ctcatatgat gttaaaatga aagaaaaagg agatattcct 240
gaggggtaca gtgtctctag agagaagaag gagcgcttct ccctgattct ggagtccgcc 300
agcaccaacc agacatctat gtacctctgc gccagcaaca acagagacag ctacaacagc 360
cccctccact ttgggaacgg gaccaggctc actgtgacg   399

<210> 53
<211> 531
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain constant
      domain (Native NA)

<400> 53
gaggacctga acaaagtgtt ccccccagag gtggccgtgt tcgagccttc tgaggccgag 60
atcagccaca cccagaaagc caccctcgtg tgcctggcca ccggcttttt ccccgaccac 120
gtggaactgt cttggtgggt caacggcaaa gaggtgcact ccggcgtgtg caccgatccc 180
cagcctctga aagaacagcc cgccctgaac gacagccggt actgcctgtc cagcagactg 240
agagtgtccg ccaccttctg gcagaacccc cggaaccact tcagatgcca ggtgcagttc 300
tacggcctga gcgagaacga cgagtggacc caggacagag ccaagcccgt gacacagatc 360
gtgtctgccg aagcctgggg cagagccgat tgcggcttta cctccgtgtc ctatcagcag 420
ggcgtgctga gcgccaccat cctgtacgag atcctgctgg gcaaggccac actgtacgcc 480
gtgctggtgt ctgccctggt gctgatggcc atggtcaagc ggaaggactt c   531

<210> 54
<211> 384
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain variable
domain (Native NA)

<400> 54
atgaagccca ccctcatctc agtgcttgtg ataatattta tactcagagg aacaagagcc 60
cagagagtga ctcagcccga gaagctcctc tctgtcttta aaggggcccc agtggagctg 120
aagtgcaact attcctattc tgggagtcct gaactcttct ggtatgtcca gtactccaga 180
caacgcctcc agttactctt gagacacatc tctagagaga gcatcaaagg cttcactgct 240
gaccttaaca aaggcgagac atctttccac ctgaagaaac catttgctca agaggaagac 300
tcagccatgt attactgcgc cctgagaagc ggcggctacc agaaggtgac ctttggaact 360
ggaacaaagc tccaagtcat ccca 384

<210> 55
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain constant
domain (Native NA)

<400> 55
gatatccaga accctgaccc tgccgtgtac cagctgagag actctaaatc cagtgacaag 60
tctgtctgcc tattcaccga ttttgattct caaacaaatg tgtcacaaag taaggattct 120
gatgtgtata tcacagacaa atgtgtgcta gacatgaggt ctatggactt caagagcaac 180
agtgctgtgg cctggagcaa caaatctgac tttgcatgtg caaacgcctt caacaacagc 240
attattccag aagacacctt cttccccagc ccagaaagtt cctgtgatgt caagctggtc 300
gagaaaagct ttgaaacaga tacgaaccta aactttcaaa acctgtcagt gattgggttc 360
cgaatcctcc tcctgaaagt ggccgggttt aatctgctca tgacgctgcg gctgtggtcc 420
agctga 426

<210> 56
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain variable
domain (Codon-optimized NA)

<400> 56
atgggaatta gactgctgtg ccgggtggcc ttctgcttcc tggctgtggg actggtggac 60
gtgaaagtga cccagagcag cagatacctc gtgaagcgga ccggcgagaa ggtgttcctg 120
gaatgcgtgc aggacatgga ccacgagaat atgttctggt acagacagga ccccggcctg 180
ggcctgcggc tgatctactt cagctacgac gtgaagatga aggaaaaggg cgacatcccc 240
gagggctaca gcgtgtccag agagaagaaa gagcggttca gcctgatcct ggaaagcgcc 300
agcaccaacc agaccagcat gtacctgtgc gcctccaaca accgggacag ctacaacagc 360

cccctgcact tcggcaacgg caccagactg accgtgacc 399

<210> 57
<211> 531
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain constant
domain (Codon-optimized NA)

<400> 57
gaggacctga acaaagtgtt ccccccagag gtggccgtgt tcgagccttc tgaggccgag 60
atcagccaca cccagaaagc caccctcgtg tgcctggcca ccggcttttt ccccgaccac 120
gtggaactgt cttggtgggt caacggcaaa gaggtgcact ccggcgtgtg caccgatccc 180
cagcctctga aagaacagcc cgccctgaac gacagccggt actgcctgtc cagcagactg 240
agagtgtccg ccaccttctg gcagaacccc cggaaccact tcagatgcca ggtgcagttc 300
tacggcctga gcgagaacga cgagtggacc caggacagag ccaagcccgt gacacagatc 360
gtgtctgccg aagcctgggg cagagccgat tgcggcttta cctccgtgtc ctatcagcag 420
ggcgtgctga gcgccaccat cctgtacgag atcctgctgg gcaaggccac actgtacgcc 480
gtgctggtgt ctgccctggt gctgatggcc atggtcaagc ggaaggactt c 531

<210> 58
<211> 390
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain variable
domain (Native NA)

<400> 58
atgaagacat ttgctggatt ttcgttcctg tttttgtggc tgcagctgga ctgtatgagt 60
agaggagagg atgtggagca gagtcttttc ctgagtgtcc gagagggaga cagctccgtt 120
ataaactgca cttacacaga cagctcctcc acctacttat actggtataa gcaagaacct 180
ggagcaggtc tccagttgct gacgtatatt ttttcaaata tggacatgaa acaagaccaa 240
agactcactg ttctattgaa taaaaaggat aaacatctgt ctctgcgcat tgcagacacc 300
cagactgggg actcagctat ctacttctgt gcagagagta tcgatgccag actcatgttt 360
ggagatggaa ctcagctggt ggtgaagccc 390

<210> 59
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain constant

domain (Codon-optimized NA)

<400> 59
gacatccaga accccgaccc tgcagtgtac cagctgcggg acagcaagag cagcgacaag 60
agcgtgtgcc tgttcaccga cttcgacagc cagaccaacg tgtcccagag caaggacagc 120
gacgtgtaca tcaccgataa gtgcgtgctg gacatgcgga gcatggactt caagagcaac 180
agcgccgtgg cctggtccaa caagagcgac ttcgcctgcg ccaacgcctt caacaacagc 240
attatccccg aggacacatt cttcccaagc cccgagagca gctgcgacgt gaagctggtg 300
gaaaagagct tcgagacaga caccaacctg aacttccaga acctcagcgt gatcggcttc 360
cggatcctgc tgctgaaggt ggccggcttc aacctgctga tgaccctgcg gctgtggtcc 420
agctga 426

<210> 60
<211> 390
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b beta chain variable
domain (Native NA)

<400> 60
atgggcttca ggctcctctg ctgtgtggcc ttttgtctcc tgggagcagg cccagtggat 60
tctggagtca cacaaacccc aaagcacctg atcacagcaa ctggacagcg agtgacgctg 120
agatgctccc ctaggtctgg agacctctct gtgtactggt accaacagag cctggaccag 180
ggcctccagt tcctcattca gtattataat ggagaagaga gagcaaaagg aaacattctt 240
gaacgattct ccgcacaaca gttccctgac ttgcactctg aactaaacct gagctctctg 300
gagctggggg actcagcttt gtatttctgt gccagcagcc agggggatga aaaactgttt 360
tttggcagtg gaacccagct ctctgtcttg 390

<210> 61
<211> 531
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b beta chain constant
domain (Native NA)

<400> 61
gaggacctga acaaagtgtt ccccccagag gtggccgtgt tcgagccttc tgaggccgag 60
atcagccaca cccagaaagc caccctcgtg tgcctggcca ccggcttttt ccccgaccac 120
gtggaactgt cttggtgggt caacggcaaa gaggtgcact ccggcgtgtg caccgatccc 180
cagcctctga aagaacagcc cgccctgaac gacagccggt actgcctgtc cagcagactg 240
agagtgtccg ccaccttctg gcagaacccc cggaaccact tcagatgcca ggtgcagttc 300
tacggcctga gcgagaacga cgagtggacc caggacagag ccaagcccgt gacacagatc 360
gtgtctgccg aagcctgggg cagagccgat tgcggcttta cctccgtgtc ctatcagcag 420
ggcgtgctga gcgccaccat cctgtacgag atcctgctgg gcaaggccac actgtacgcc 480

gtgctggtgt ctgccctggt gctgatggcc atggtcaagc ggaaggactt c 531

<210> 62
<211> 390
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain variable
domain (Native NA)

<400> 62
atgaagacat ttgctggatt ttcgttcctg tttttgtggc tgcagctgga ctgtatgagt 60
agaggagagg atgtggagca gagtcttttc ctgagtgtcc gagagggaga cagctccgtt 120
ataaactgca cttacacaga cagctcctcc acctacttat actggtataa gcaagaacct 180
ggagcaggtc tccagttgct gacgtatatt ttttcaaata tggacatgaa acaagaccaa 240
agactcactg ttctattgaa taaaaaggat aaacatctgt ctctgcgcat tgcagacacc 300
cagactgggg actcagctat ctacttctgt gcagagagta tcgatgccag actcatgttt 360
ggagatggaa ctcagctggt ggtgaagccc 390

<210> 63
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain constant
domain (Native NA)

<400> 63
gatatccaga accctgaccc tgccgtgtac cagctgagag actctaaatc cagtgacaag 60
tctgtctgcc tattcaccga ttttgattct caaacaaatg tgtcacaaag taaggattct 120
gatgtgtata tcacagacaa atgtgtgcta gacatgaggt ctatggactt caagagcaac 180
agtgctgtgg cctggagcaa caaatctgac tttgcatgtg caaacgcctt caacaacagc 240
attattccag aagacacctt cttccccagc ccagaaagtt cctgtgatgt caagctggtc 300
gagaaaagct ttgaaacaga tacgaaccta aactttcaaa acctgtcagt gattgggttc 360
cgaatcctcc tcctgaaagt ggccgggttt aatctgctca tgacgctgcg gctgtggtcc 420
agctga 426

<210> 64
<211> 390
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b beta chain variable
domain(Codon-optimized NA)

<400> 64
atgggcttca gactgctgtg ctgcgtggcc ttctgtctgc tgggagccgg ccctgtggat 60
agcggcgtga cacagacacc caagcacctg atcaccgcca ccggccagcg cgtgacactg 120
agatgtagcc ctagaagcgg cgacctgagc gtgtactggt atcagcagag cctggaccag 180
ggcctgcagt tcctgatcca gtactacaac ggcgaggaac gggccaaggg caacatcctg 240
gaacggttca gcgcccagca gttccccgat ctgcacagcg agctgaacct gagcagcctg 300
gaactgggcg acagcgccct gtacttctgt gccagttctc agggcgacga gaagctgttc 360
ttcggcagcg gcacacagct gagcgtgctg 390

<210> 65
<211> 531
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b beta chain constant
domain (Codon-optimized NA)

<400> 65
gaagatctga acaaggtgtt ccccccagag gtggccgtgt tcgagccttc tgaggccgag 60
atcagccaca cccagaaagc caccctcgtg tgcctggcca ccggcttttt ccccgaccac 120
gtggaactgt cttggtgggt caacggcaaa gaggtgcact ccggcgtgtg caccgatccc 180
cagcctctga aagaacagcc cgccctgaac gacagccggt actgcctgtc cagcagactg 240
agagtgtccg ccaccttctg gcagaacccc cggaaccact tcagatgcca ggtgcagttc 300
tacggcctga gcgagaacga cgagtggacc caggacagag ccaagcccgt gacccagatc 360
gtgtctgccg aagcctgggg cagagccgat tgcggcttta ccagcgtgtc ctatcagcag 420
ggcgtgctga gcgccaccat cctgtacgag atcctgctgg gcaaggccac cctgtacgcc 480
gtgctggtgt ctgccctggt gctgatggcc atggtcaagc ggaaggactt c 531

<210> 66
<211> 390
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain variable
domain (Codon-optimized NA)

<400> 66
atgaagacct tcgccggctt cagcttcctg ttcctgtggc tgcagctgga ctgcatgagc 60
agaggcgagg acgtggaaca gagcctgttt ctgtccgtgc gcgagggcga ctccagcgtg 120
atcaattgca cctacaccga cagcagcagc acctacctgt attggtacaa gcaggaaccc 180
ggcgctggcc tgcagctgct gacctacatc ttcagcaaca tggacatgaa gcaggaccag 240
cggctgaccg tgctgctgaa caagaaggat aagcacctgt ccctgcggat cgccgatacc 300
cagacaggcg actccgccat ctacttttgc gccgagagca tcgacgcccg gctgatgttt 360
ggagatggca cccagctggt cgtgaagccc 390

<210> 67

<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain constant
domain (Codon-optimized NA)

<400> 67
gacatccaga accccgaccc tgcagtgtac cagctgcggg acagcaagag cagcgacaag 60
agcgtgtgcc tgttcaccga cttcgacagc cagaccaacg tgtcccagag caaggacagc 120
gacgtgtaca tcaccgataa gtgcgtgctg gacatgcgga gcatggactt caagagcaac 180
agcgccgtgg cctggtccaa caagagcgac ttcgcctgcg ccaacgcctt caacaacagc 240
attatccccg aggacacatt cttcccaagc cccgagagca gctgcgacgt gaagctggtg 300
gaaaagagct tcgagacaga caccaacctg aacttccaga acctcagcgt gatcggcttc 360
cggatcctgc tgctgaaggt ggccggcttc aacctgctga tgaccctgcg gctgtggtcc 420
agctga 426

<210> 68
<211> 381
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain variable
domain (Native NA)

<400> 68
atgctctgct ctctccttgc ccttctcctg ggcactttct ttggggtcag atctcagact 60
attcatcaat ggccagcgac cctggtgcag cctgtgggca gcccgctctc tctggagtgc 120
actgtggagg gaacatcaaa ccccaaccta tactggtacc gacaggctgc aggcaggggc 180
ctccagctgc tcttctactc cgttggtatt ggccagatca gctctgaggt gccccagaat 240
ctctcagcct ccagacccca ggaccggcag ttcatcctga gttctaagaa gctccttctc 300
agtgactctg gcttctatct ctgcgccctg agcaccagct acgagcagta cttcgggccg 360
ggcaccaggc tcacggtcac a 381

<210> 69
<211> 537
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain constant
domain (Native NA)

<400> 69
gacctgaaaa acgtgttccc acccgaggtc gctgtgtttg agccatcaga agcagagatc 60
tcccacaccc aaaaggccac actggtgtgc ctggccacag gcttctaccc cgaccacgtg 120

gagctgagct ggtgggtgaa tgggaaggag gtgcacagtg gggtctgcac agacccgcag 180
cccctcaagg agcagcccgc cctcaatgac tccagatact gcctgagcag ccgcctgagg 240
gtctcggcca ccttctggca gaacccccgc aaccacttcc gctgtcaagt ccagttctac 300
gggctctcgg agaatgacga gtggacccag gatagggcca aacctgtcac ccagatcgtc 360
agcgccgagg cctggggtag agcagactgt ggcttcacct ccgagtctta ccagcaaggg 420
gtcctgtctg ccaccatcct ctatgagatc ttgctaggga aggccacctt gtatgccgtg 480
ctggtcagtg ccctcgtgct gatggccatg gtcaagagaa aggattccag aggctag 537

<210> 70
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain variable
domain (Native NA)

<400> 70
atgacacgag ttagcttgct gtgggcagtc gtggtctcca cctgtcttga atccggcatg 60
gcccagacag tcactcagtc tcaaccagag atgtctgtgc aggaggcaga gactgtgacc 120
ctgagttgca catatgacac cagtgagaat aattattatt tgttctggta caagcagcct 180
cccagcaggc agatgattct cgttattcgc caagaagctt ataagcaaca gaatgcaacg 240
gagaatcgtt tctctgtgaa cttccagaaa gcagccaaat ccttcagtct caagatctca 300
gactcacagc tgggggacac tgcgatgtat ttctgtgctt tcatgaagtc ccactccgga 360
tacatctttg gaacaggcac caggctgaag gttttagca 399

<210> 71
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain constant
domain (Native NA)

<400> 71
gatatccaga accctgaccc tgccgtgtac agctgagag actctaaatc cagtgacaag 60
tctgtctgcc tattcaccga ttttgattct caaacaaatg tgtcacaaag taaggattct 120
gatgtgtata tcacagacaa atgtgtgcta gacatgaggt ctatggactt caagagcaac 180
agtgctgtgg cctggagcaa caaatctgac tttgcatgtg caaacgcctt caacaacagc 240
attattccag aagacacctt cttccccagc ccagaaagtt cctgtgatgt caagctggtc 300
gagaaaagct ttgaaacaga tacgaaccta aactttcaaa acctgtcagt gattgggttc 360
cgaatcctcc tcctgaaagt ggccgggttt aatctgctca tgacgctgcg gctgtggtcc 420
agctga 426

<210> 72
<211> 381
<212> DNA

<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain variable
domain(Codon-optimized NA)

<400> 72
atgctgtgtt ctctgctggc cctgctgctg ggcaccttct ttggagtgcg gagccagacc 60
atccaccagt ggcctgctac actggtgcag cctgtgggca gccctctgag cctggaatgt 120
accgtggaag gcaccagcaa ccccaacctg tactggtaca gacaggccgc tggcagaggc 180
ctgcagctgc tgttttacag cgtgggcatc ggccagatca gcagcgaggt gccccagaat 240
ctgagcgcca gcagacccca ggaccggcag tttatcctga gcagcaagaa gctgctgctg 300
agcgacagcg gcttctacct gtgtgccctg agcaccagct acgagcagta cttcggccca 360
ggcaccagac tgaccgtgac c 381

<210> 73
<211> 534
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain constant
domain (Codon-optimized NA)

<400> 73
gacctgaaga acgtgttccc cccagaggtg gccgtgttcg agccttctga ggccgagatc 60
agccacaccc agaaagccac cctcgtgtgt ctggccaccg gcttttaccc cgaccacgtg 120
gaactgtctt ggtgggtcaa cggcaaagag gtgcactccg gcgtgtgcac cgatccccag 180
cctctgaaag aacagcccgc cctgaacgac agccggtact gcctgtccag cagactgaga 240
gtgtccgcca ccttctggca gaacccccgg aaccacttca gatgccaggt gcagttctac 300
ggcctgagcg agaacgacga gtggacccag gacagagcca agcccgtgac ccagatcgtg 360
tctgccgaag cctggggcag agccgattgc ggctttacca gcgagagcta ccagcagggc 420
gtgctgtctg ccaccatcct gtacgagatc ctgctgggaa aggccaccct gtacgccgtg 480
ctggtgtctg ccctggtgct gatggccatg gtcaagcgga aggacagcag aggc 534

<210> 74
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain variable
domain (Codon-optimized NA)

<400> 74
atgacccggg tgtcactgct gtgggctgtg gtggtgtcca cctgtctgga aagcggcatg 60
gcccagaccg tgacacagtc ccagcctgag atgagcgtgc aggaagccga gacagtgacc 120
ctgagctgca cctacgacac ctccgagaac aactactacc tgttttggta caagcagccc 180

cccagccggc agatgatcct cgtgatcaga caggaagcct ataagcagca gaacgccacc 240
gagaacagat tcagcgtgaa cttccagaag gccgccaaga gctttagcct gaagatcagc 300
gacagccagc tgggcgacac cgccatgtac ttttgcgcct ttatgaagtc ccacagcggc 360
tacatcttcg gcaccggcac acggctgaaa gtgctggct 399

<210> 75
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain constant
domain (Codon-optimized NA)

<400> 75
gacatccaga accccgaccc tgcagtgtac cagctgcggg acagcaagag cagcgacaag 60
agcgtgtgcc tgttcaccga cttcgacagc cagaccaacg tgtcccagag caaggacagc 120
gacgtgtaca tcaccgataa gtgcgtgctg gacatgcgga gcatggactt caagagcaac 180
agcgccgtgg cctggtccaa caagagcgac ttcgcctgcg ccaacgcctt caacaacagc 240
attatccccg aggacacatt cttcccaagc cccgagagca gctgcgacgt gaagctggtg 300
gaaaagagct tcgagacaga caccaacctg aacttccaga acctcagcgt gatcggcttc 360
cggatcctgc tgctgaaggt ggccggcttc aacctgctga tgaccctgcg gctgtggtcc 420
agctga 426

<210> 76
<211> 387
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b beta chain variable
domain (Native NA)

<400> 76
atgctctgct ctctccttgc ccttctcctg ggcactttct ttggggtcag atctcagact 60
attcatcaat ggccagcgac cctggtgcag cctgtgggca gcccgctctc tctggagtgc 120
actgtggagg gaacatcaaa ccccaaccta tactggtacc gacaggctgc aggcaggggc 180
ctccagctgc tcttctactc cgttggtatt ggccagatca gctctgaggt gccccagaat 240
ctctcagcct ccagacccca ggaccggcag ttcatcctga gttctaagaa gctccttctc 300
agtgactctg gcttctatct ctgtgcctgg agtgttgcgg tgaacactga agctttcttt 360
ggacaaggca ccagactcac agttgta 387

<210> 77
<211> 531
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic sequence 17804.1b beta chain constant
domain (Native NA)

<400> 77
gaggacctga acaaagtgtt ccccccagag gtggccgtgt tcgagccttc tgaggccgag 60
atcagccaca cccagaaagc caccctcgtg tgcctggcca ccggcttttt ccccgaccac 120
gtggaactgt cttggtgggt caacggcaaa gaggtgcact ccggcgtgtg caccgatccc 180
cagcctctga aagaacagcc cgccctgaac gacagccggt actgcctgtc cagcagactg 240
agagtgtccg ccaccttctg gcagaacccc cggaaccact tcagatgcca ggtgcagttc 300
tacggcctga gcgagaacga cgagtggacc caggacagag ccaagcccgt gacacagatc 360
gtgtctgccg aagcctgggg cagagccgat tgcggcttta cctccgtgtc ctatcagcag 420
ggcgtgctga gcgccaccat cctgtacgag atcctgctgg gcaaggccac actgtacgcc 480
gtgctggtgt ctgccctggt gctgatggcc atggtcaagc ggaaggactt c 531

<210> 78
<211> 396
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b alpha chain variable
domain (Native NA)

<400> 78
atgacacgag ttagcttgct gtgggcagtc gtggtctcca cctgtcttga atccggcatg 60
gcccagacag tcactcagtc tcaaccagag atgtctgtgc aggaggcaga gactgtgacc 120
ctgagttgca catatgacac cagtgagaat aattattatt tgttctggta caagcagcct 180
cccagcaggc agatgattct cgttattcgc caagaagctt ataagcaaca gaatgcaacg 240
gagaatcgtt tctctgtgaa cttccagaaa gcagccaaat ccttcagtct caagatctca 300
gactcacagc tgggggacac tgcgatgtat ttctgcgcct tcggcgaggg cgccagactc 360
atgtttggag atggaactca gctggtggtg aagccc 396

<210> 79
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b alpha chain constant
domain (Native NA)

<400> 79
gatatccaga accctgaccc tgccgtgtac cagctgagag actctaaatc cagtgacaag 60
tctgtctgcc tattcaccga ttttgattct caaacaaatg tgtcacaaag taaggattct 120
gatgtgtata tcacagacaa atgtgtgcta gacatgaggt ctatggactt caagagcaac 180
agtgctgtgg cctggagcaa caaatctgac tttgcatgtg caaacgcctt caacaacagc 240
attattccag aagacacctt cttccccagc ccagaaagtt cctgtgatgt caagctggtc 300
gagaaaagct ttgaaacaga tacgaaccta aactttcaaa acctgtcagt gattgggttc 360

cgaatcctcc tcctgaaagt ggccgggttt aatctgctca tgacgctgcg gctgtggtcc 420
agctga 426

<210> 80
<211> 387
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b beta chain variable
domain (Codon-optimized NA)

<400> 80
atgctgtgtt ctctgctggc cctgctgctg ggcaccttct ttggagtgcg gagccagacc 60
atccaccagt ggcctgctac actggtgcag cctgtgggca gccctctgag cctggaatgt 120
accgtggaag gcaccagcaa ccccaacctg tactggtaca gacaggccgc tggcagaggc 180
ctgcagctgc tgttttacag cgtgggcatc ggccagatca gcagcgaggt gccccagaat 240
ctgagcgcca gcagacccca ggaccggcag tttatcctga gcagcaagaa gctgctgctg 300
agcgacagcg gcttctacct gtgcgcttgg agcgtggccg tgaacaccga ggcattcttt 360
gggcagggca cccggctgac cgtggtg 387

<210> 81
<211> 531
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b beta chain constant
domain (Codon-optimized NA)

<400> 81
gaagatctga acaaggtgtt ccccccagag gtggccgtgt tcgagccttc tgaggccgag 60
atcagccaca cccagaaagc caccctcgtg tgcctggcca ccggcttttt ccccgaccac 120
gtggaactgt cttggtgggt caacggcaaa gaggtgcact ccggcgtgtg caccgatccc 180
cagcctctga aagaacagcc cgccctgaac gacagccggt actgcctgtc cagcagactg 240
agagtgtccg ccaccttctg gcagaacccc cggaaccact tcagatgcca ggtgcagttc 300
tacggcctga gcgagaacga cgagtggacc caggacagag ccaagcccgt gacccagatc 360
gtgtctgccg aagcctgggg cagagccgat tgcggcttta ccagcgtgtc ctatcagcag 420
ggcgtgctgt ctgccaccat cctgtacgag atcctgctgg gaaaggccac cctgtacgcc 480
gtgctggtgt ctgccctggt gctgatggcc atggtcaagc ggaaggactt c 531

<210> 82
<211> 396
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b alpha chain variable

domain (Codon-optimized NA)

<400> 82
atgaccagag tgtctctgct gtgggctgtg gtggtgtcca cctgtctgga aagcggcatg 60
gcccagaccg tgacacagtc ccagcctgag atgagcgtgc aggaagccga gacagtgacc 120
ctgagctgca cctacgacac cagcgagaac aactactacc tgttttggta caagcagccc 180
cccagccggc agatgatcct cgtgatcaga caggaagcct ataagcagca gaacgccacc 240
gagaacagat tcagcgtgaa cttccagaag gccgccaaga gcttcagcct gaagatcagc 300
gacagccagc tgggcgacac cgccatgtac ttttgcgcct ttggcgaggg cgccagactg 360
atgtttggcg acggaaccca gctggtcgtg aagccc 396

<210> 83
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.1b alpha chain constant
domain (Codon-optimized NA)

<400> 83
gacatccaga accccgaccc tgcagtgtac cagctgcggg acagcaagag cagcgacaag 60
agcgtgtgcc tgttcaccga cttcgacagc cagaccaacg tgtcccagag caaggacagc 120
gacgtgtaca tcaccgataa gtgcgtgctg gacatgcgga gcatggactt caagagcaac 180
agcgccgtgg cctggtccaa caagagcgac ttcgcctgcg ccaacgcctt caacaacagc 240
attatccccg aggacacatt cttcccaagc cccgagagca gctgcgacgt gaagctggtg 300
gaaaagagct tcgagacaga caccaacctg aacttccaga acctcagcgt gatcggcttc 360
cggatcctgc tgctgaaggt ggccggcttc aacctgctga tgaccctgcg gctgtggtcc 420
agctga 426

<210> 84
<211> 387
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 beta chain variable
domain (Native NA)

<400> 84
atgctctgct ctctccttgc ccttctcctg ggcactttct ttggggtcag atctcagact 60
attcatcaat ggccagcgac cctggtgcag cctgtgggca gcccgctctc tctggagtgc 120
actgtggagg gaacatcaaa ccccaaccta tactggtacc gacaggctgc aggcaggggc 180
ctccagctgc tcttctactc cattggtatt gaccagatca gctctgaggt gccccagaat 240
ctctcagcct ccagacccca ggaccggcag ttcattctga gttctaagaa gctcctcctc 300
agtgactctg gcttctatct ctgtgcctgg agtgtaacca ggcacaatga gcagttcttc 360
gggccaggga cacggctcac cgtgcta 387

<210> 85
<211> 537
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 beta chain constant
domain (Native NA)

<400> 85
gacctgaaaa acgtgttccc acccgaggtc gctgtgtttg agccatcaga agcagagatc 60
tcccacaccc aaaaggccac actggtgtgc ctggccacag gcttctaccc cgaccacgtg 120
gagctgagct ggtgggtgaa tgggaaggag gtgcacagtg gggtctgcac agacccgcag 180
cccctcaagg agcagcccgc cctcaatgac tccagatact gcctgagcag ccgcctgagg 240
gtctcggcca ccttctggca gaacccccgc aaccacttcc gctgtcaagt ccagttctac 300
gggctctcgg agaatgacga gtggacccag gatagggcca aacctgtcac ccagatcgtc 360
agcgccgagg cctggggtag agcagactgt ggcttcacct ccgagtctta ccagcaaggg 420
gtcctgtctg ccaccatcct ctatgagatc ttgctaggga aggccacctt gtatgccgtg 480
ctggtcagtg ccctcgtgct gatggccatg gtcaagagaa aggattccag aggctag 537

<210> 86
<211> 405
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 alpha chain variable
domain (Native NA)

<400> 86
atgctgtgca gcctgctggc cctgctgctg ggcaccttct tcgagcccag aaccagccaa 60
gaactggagc agagtcctca gtccttgatc gtccaagagg gaaagaatct caccataaac 120
tgcacgtcat caaagacgtt atatggctta tactggtata agcaaaagta tggtgaaggt 180
cttatcttct tgatgatgct acagaaaggt ggggaagaga aaagtcatga aaagataact 240
gccaagttgg atgagaaaaa gcagcaaagt tccctgcata tcacagcctc ccagcccagc 300
catgcaggca tctacctctg tggagcagcc cctacatact cgaattatgg aggaagccaa 360
ggaaatctca tctttggaaa aggcactaaa ctctctgtta aacca 405

<210> 87
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 alpha chain constant
domain (Native NA)

<400> 87

gatatccaga accctgaccc tgccgtgtac cagctgagag actctaaatc cagtgacaag 60
tctgtctgcc tattcaccga ttttgattct caaacaaatg tgtcacaaag taaggattct 120
gatgtgtata tcacagacaa atgtgtgcta gacatgaggt ctatggactt caagagcaac 180
agtgctgtgg cctggagcaa caaatctgac tttgcatgtg caaacgcctt caacaacagc 240
attattccag aagacacctt cttccccagc ccagaaagtt cctgtgatgt caagctggtc 300
gagaaaagct ttgaaacaga tacgaaccta aactttcaaa acctgtcagt gattgggttc 360
cgaatcctcc tcctgaaagt ggccgggttt aatctgctca tgacgctgcg gctgtggtcc 420
agctga 426

<210> 88
<211> 387
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 beta chain variable
domain(Codon-optimized NA)

<400> 88
atgctgtgtt ctctgctggc tctgctgctg ggcaccttct ttggagtgcg gagccagacc 60
atccaccagt ggcctgctac actggtgcag cctgtgggca gccctctgag cctggaatgt 120
accgtggaag gcaccagcaa ccccaacctg tactggtaca gacaggccgc tggcagaggc 180
ctgcagctgc tgttttacag catcggcatc gaccagatca gcagcgaggt gccccagaac 240
ctgagcgcca gcagacccca ggaccggcag tttatcctga gcagcaagaa gctgctgctg 300
agcgacagcg gcttctacct gtgcgcttgg agcgtgaccc ggcacaacga gcagttcttt 360
ggccctggca cccggctgac cgtgctg 387

<210> 89
<211> 534
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 beta chain constant
domain (Codon-optimized NA)

<400> 89
gacctgaaga acgtgttccc cccagaggtg gccgtgttcg agccttctga ggccgagatc 60
agccacaccc agaaagccac cctcgtgtgt ctggccaccg gcttttaccc cgaccacgtg 120
gaactgtctt ggtgggtcaa cggcaaagag gtgcactccg gcgtgtgcac cgatccccag 180
cctctgaaag aacagcccgc cctgaacgac agccggtact gcctgtccag cagactgaga 240
gtgtccgcca ccttctggca gaacccccgg aaccacttca gatgccaggt gcagttctac 300
ggcctgagcg agaacgacga gtggacccag gacagagcca agcccgtgac ccagatcgtg 360
tctgccgaag cctggggcag agccgattgc ggctttacca gcgagagcta ccagcagggc 420
gtgctgtctg ccaccatcct gtacgagatc ctgctgggaa aggccaccct gtacgccgtg 480
ctggtgtctg ccctggtgct gatggccatg gtcaagcgga aggacagcag aggc 534

<210> 90

<211> 405
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 alpha chain variable
domain (Codon-optimized NA)

<400> 90
atgctgtgca gcctgctggc cctgctgctg ggaacatttt tcgagccccg gaccagccag 60
gaactggaac agagcccaca gagcctgatc gtgcaggaag gcaagaacct gaccatcaac 120
tgcaccagct ccaagacact gtacggcctg tattggtata agcagaagta cggcgagggc 180
ctgatcttcc tgatgatgct gcagaagggc ggcgaggaaa agagccacga gaagatcacc 240
gccaagctgg acgagaagaa gcagcagtcc agcctgcaca tcaccgcctc ccagccttct 300
cacgccggca tctatctgtg tggcgccgct cccacctaca gcaactatgg cggcagccag 360
ggcaatctga tcttcggcaa gggcaccaag ctgagcgtga agccc 405

<210> 91
<211> 426
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17804.2 alpha chain constant
domain (Codon-optimized NA)

<400> 91
gacatccaga accccgaccc tgcagtgtac cagctgcggg acagcaagag cagcgacaag 60
agcgtgtgcc tgttcaccga cttcgacagc cagaccaacg tgtcccagag caaggacagc 120
gacgtgtaca tcaccgataa gtgcgtgctg gacatgcgga gcatggactt caagagcaac 180
agcgccgtgg cctggtccaa caagagcgac ttcgcctgcg ccaacgcctt caacaacagc 240
attatccccg aggacacatt cttcccaagc cccgagagca gctgcgacgt gaagctggtg 300
gaaaagagct tcgagacaga caccaacctg aacttccaga acctcagcgt gatcggcttc 360
cggatcctgc tgctgaaggt ggccggcttc aacctgctga tgaccctgcg gctgtggtcc 420
agctga 426

<210> 92
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain CDR1 domain
(Codon-optimized NA)

<400> 92
atggaccacg agaat 15

<210> 93
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain CDR2 domain
(Codon-optimized NA)

<400> 93
agctacgacg tgaagatg 18

<210> 94
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 beta chain CDR3 domain
(Codon-optimized NA)

<400> 94
tgcgcctcca acaaccggga cagctacaac agccccctgc acttc 45

<210> 95
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain CDR1 domain
(Codon-optimized NA)

<400> 95
tacagcggca gccccgag 18

<210> 96
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain CDR2 domain
(Codon-optimized NA)

<400> 96

cacatcagca ga 12

<210> 97
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.1 alpha chain CDR3 domain
(Codon-optimized NA)

<400> 97
tgcgccctga gatccggcgg ctaccagaaa gtgacattt 39

<210> 98
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b beta chain CDR1 domain
(Codon-optimized NA)

<400> 98
agcggcgacc tgagc 15

<210> 99
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic sequence 1388.2b beta chain CDR2 domain
(Codon-optimized NA)

<400> 99
tactacaacg gcgaggaa 18

<210> 100
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b beta chain CDR3 domain
(Codon-optimized NA)

<400> 100
tactacaacg gcgaggaa 18

<210> 101
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain CDR1
domain (Codon-optimized NA)

<400> 101
gacagcagca gcacctac 18

<210> 102
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain CDR2
domain (Codon-optimized NA)

<400> 102
atcttcagca acatggacat g 21

<210> 103
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.2b alpha chain CDR3
domain (Codon-optimized NA)

<400> 103
tgcgccgaga gcatcgacgc ccggctgatg ttt 33

<210> 104
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain CDR1 domain

(Codon-optimized NA)

<400> 104
ggcaccagca accccaac 18

<210> 105
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain CDR2 domain
(Codon-optimized NA)

<400> 105
agcgtgggca tcggc 15

<210> 106
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 beta chain CDR3 domain
(Codon-optimized NA)

<400> 106
tgtgccctga gcaccagcta cgagcagtac ttc 33

<210> 107
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain CDR1 domain
(Codon-optimized NA)

<400> 107
acctccgaga acaactacta c 21

<210> 108
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain CDR2 domain

(Codon-optimized NA)

<400> 108
caggaagcct ataagcagca gaac 24

<210> 109
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 1388.3 alpha chain CDR3 domain
(Codon-optimized NA)

<400> 109
tgcgccttta tgaagtccca cagcggctac atcttcggc 39

<210> 110
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.1b beta chain CDR1 domain
(Codon-optimized NA)

<400> 110
ggcaccagca accccaac 18

<210> 111
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.1b beta chain CDR2 domain
(Codon-optimized NA)

<400> 111
agcgtgggca tcggc 15

<210> 112
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.1b beta chain CDR3 domain

(Codon-optimized NA)

<400> 112
tgcgcttgga gcgtggccgt gaacaccgag gcattcttt 39

<210> 113
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.1b alpha chain CDR1 domain (Codon-optimized NA)

<400> 113
accagcgaga acaactacta c 21

<210> 114
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.1b alpha chain CDR2 domain (Codon-optimized NA)

<400> 114
caggaagcct ataagcagca gaac 24

<210> 115
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.1b alpha chain CDR3 domain (Codon-optimized NA)

<400> 115
tgcgcctttg gcgagggcgc cagactgatg ttt 33

<210> 116
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.2 beta chain CDR1 domain

(Codon-optimized NA)

<400> 116
ggcaccagca accccaac 18

<210> 117
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.2 beta chain CDR2 domain
(Codon-optimized NA)

<400> 117
agcatcggca tcgac 15

<210> 118
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.2 beta chain CDR3 domain
(Codon-optimized NA)

<400> 118
tgcgcttgga gcgtgacccg gcacaacgag cagttcttt 39

<210> 119
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.2 alpha chain CDR1
domain (Codon-optimized NA)

<400> 119
aagacactgt acggc 15

<210> 120
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.2 alpha chain CDR2

domain (Codon-optimized NA)

<400> 120
ctgcagaagg gcggcgagga a 21

<210> 121
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence 17084.2 alpha chain CDR3
domain (Codon-optimized NA)

<400> 121
tgtggcgccg ctcccaccta cagcaactat ggcggcagcc agggcaatct gatcttc 57

<210> 122
<211> 309
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Human MAGE-A1 (amino acid)

<400> 122
Met Ser Leu Glu Gln Arg Ser Leu His Cys Lys Pro Glu Glu Ala Leu
1 5 10 15
Glu Ala Gln Gln Glu Ala Leu Gly Leu Val Cys Val Gln Ala Ala Thr
20 25 30
Ser Ser Ser Ser Pro Leu Val Leu Gly Thr Leu Glu Glu Val Pro Thr
35 40 45
Ala Gly Ser Thr Asp Pro Pro Gln Ser Pro Gln Gly Ala Ser Ala Phe
50 55 60
Pro Thr Thr Ile Asn Phe Thr Arg Gln Arg Gln Pro Ser Glu Gly Ser
65 70 75 80
Ser Ser Arg Glu Glu Glu Gly Pro Ser Thr Ser Cys Ile Leu Glu Ser
85 90 95
Leu Phe Arg Ala Val Ile Thr Lys Lys Val Ala Asp Leu Val Gly Phe
100 105 110
Leu Leu Leu Lys Tyr Arg Ala Arg Glu Pro Val Thr Lys Ala Glu Met
115 120 125
Leu Glu Ser Val Ile Lys Asn Tyr Lys His Cys Phe Pro Glu Ile Phe
130 135 140
Gly Lys Ala Ser Glu Ser Leu Gln Leu Val Phe Gly Ile Asp Val Lys
145 150 155 160
Glu Ala Asp Pro Thr Gly His Ser Tyr Val Leu Val Thr Cys Leu Gly

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                165                 170                 175
Leu Ser Tyr Asp Gly Leu Leu Gly Asp Asn Gln Ile Met Pro Lys Thr
            180                 185                 190
Gly Phe Leu Ile Ile Val Leu Val Met Ile Ala Met Glu Gly Gly His
        195                 200                 205
Ala Pro Glu Glu Glu Ile Trp Glu Glu Leu Ser Val Met Glu Val Tyr
    210                 215                 220
Asp Gly Arg Glu His Ser Ala Tyr Gly Glu Pro Arg Lys Leu Leu Thr
225                 230                 235                 240
Gln Asp Leu Val Gln Glu Lys Tyr Leu Glu Tyr Arg Gln Val Pro Asp
                245                 250                 255
Ser Asp Pro Ala Arg Tyr Glu Phe Leu Trp Gly Pro Arg Ala Leu Ala
            260                 265                 270
Glu Thr Ser Tyr Val Lys Val Leu Glu Tyr Val Ile Lys Val Ser Ala
        275                 280                 285
Arg Val Arg Phe Phe Phe Pro Ser Leu Arg Glu Ala Ala Leu Arg Glu
    290                 295                 300
Glu Glu Glu Gly Val
305

<210> 123
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Human MAGE-A1 278-286


<400> 123
Lys Val Leu Glu Tyr Val Ile Lys Val
 1               5

<210> 124
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Porcine teschovirus-1 2A (P2A)
      peptide

<400> 124
Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val
 1               5                  10                  15
Glu Glu Asn Pro Gly Pro
            20
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<210> 125
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Foot-and-Mouth disease virus 2A
(F2A) peptide

<400> 125
Gly Ser Gly Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala
 1   5   10   15
Gly Asp Val Glu Ser Asn Pro Gly Pro
            20   25

<210> 126
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Equine rhinitis A virus (ERAV)
2A (E2A) peptide

<400> 126
Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala Gly Asp Val Glu Ser
 1   5   10   15
Asn Pro Gly Pro
            20

<210> 127
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Thoseaasigna virus 2A (T2A)
peptide

<400> 127
Leu Glu Gly Gly Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp
 1   5   10   15
Val Glu Glu Asn Pro Gly Pro Arg
            20

<210> 128
<211> 66
<212> DNA

<213> Artificial Sequence

<220>
<223> Synthetic sequence Porcine teschovirus-1 2A (P2A)
peptide (NA)

<400> 128
ggaagcggag ctactaactt cagcctgctg aagcaggctg gagacgtgga ggagaaccct 60
ggacct 66

<210> 129
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence Porcine teschovirus-1 2A (P2A)
peptide (CO-NA)

<400> 129
ggttccggag ccacgaactt ctctctgtta aagcaagcag gagacgtgga agaaaacccc 60
ggtccc 66

<210> 130
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence Foot-and-Mouth disease virus 2A
(F2A) peptide (NA)

<400> 130
ggaagcggag tgaaacagac tttgaatttt gaccttctca agttggcggg agacgtggag 60
tccaaccctg gacct 75

<210> 131
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence Equine rhinitis A virus (ERAV)
2A (E2A) peptide (NA)

<400> 131

ggaagcggac agtgtactaa ttatgctctc ttgaaattgg ctggagatgt tgagagcaac 60
cctggacct 69

<210> 132
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence Thoseaasigna virus 2A (T2A)
peptide (NA)

<400> 132
ggaagcggag agggcagagg aagtctgcta acatgcggtg acgtcgagga gaatcctgga 60
cct 63

<210> 133
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Glycine-serine linker

<400> 133
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 134
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Glycine-serine linker

<400> 134
Gly Ser Thr Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15
Ser Ser

<210> 135
<211> 25
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic sequence sgRNA Forward Oligo:
TRAC_sgRNA_pLenti_F1

<400> 135
caccggagaa tcaaaatcgg tgaat 25

<210> 136
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence sgRNA Reverse Oligo:
TRAC_sgRNA_pLenti_R1

<400> 136
aaacattcac cgattttgat tctcc 25

<210> 137
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence sgRNA Forward Oligo:
PD1_sgRNA_F1

<400> 137
caccgcagtt gtgtgacacg gaag 24

<210> 138
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence sgRNA Reverse Oligo:
PD1_sgRNA_R1

<400> 138
aaaccttccg tgtcacacaa ctgc 24

<210> 139
<211> 25
<212> DNA
<213> Artificial Sequence

<220>

<223> Synthetic sequence sgRNA Forward Oligo:
CTLA4_sgRNA_F1

<400> 139
caccggcaaa ggtgagtgag acttt 25

<210> 140
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence sgRNA Reverse Oligo:
CTLA4_sgRNA_R1

<400> 140
aaacaaagtc tcactcacct ttgcc 25

<210> 141
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence sgRNA Forward Oligo:
LAG3_sgRNA_F1

<400> 141
caccggtttc tgcagccgct ttggg 25

<210> 142
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence sgRNA Reverse Oligo:
LAG3_sgRNA_R2

<400> 142
aaaccccaaa gcggctgcag aaacc 25

<210> 143
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence CD8 co-receptor alpha chain

<400> 143
Ser Gln Phe Arg Val Ser Pro Leu Asp Arg Thr Trp Asn Leu Gly Glu
 1   5   10   15
Thr Val Glu Leu Lys Cys Gln Val Leu Leu Ser Asn Pro Thr Ser Gly
            20   25   30
Cys Ser Trp Leu Phe Gln Pro Arg Gly Ala Ala Ala Ser Pro Thr Phe
        35   40   45
Leu Leu Tyr Leu Ser Gln Asn Lys Pro Lys Ala Ala Glu Gly Leu Asp
    50   55   60
Thr Gln Arg Phe Ser Gly Lys Arg Leu Gly Asp Thr Phe Val Leu Thr
65   70   75   80
Leu Ser Asp Phe Arg Arg Glu Asn Glu Gly Tyr Tyr Phe Cys Ser Ala
                85   90   95
Leu Ser Asn Ser Ile Met Tyr Phe Ser His Phe Val Pro Val Phe Leu
            100   105   110
Pro Ala Lys Pro Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala
        115   120   125
Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg
    130   135   140
Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys
145   150   155   160
Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu
                165   170   175
Leu Ser Leu Val Ile Thr Leu Tyr Cys Asn His Arg Asn Arg Arg Arg
            180   185   190
Val Cys Lys Cys Pro Arg Pro Val Val Lys Ser Gly Asp Lys Pro Ser
        195   200   205
Leu Ser Ala Arg Tyr Val
    210

<210> 144
<211> 189
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence "CD8 co-receptor beta chain
      isoform 1

<400> 144
Leu Gln Gln Thr Pro Ala Tyr Ile Lys Val Gln Thr Asn Lys Met Val
 1   5   10   15
Met Leu Ser Cys Glu Ala Lys Ile Ser Leu Ser Asn Met Arg Ile Tyr
            20   25   30
Trp Leu Arg Gln Arg Gln Ala Pro Ser Ser Asp Ser His His Glu Phe

```
        35                  40                  45
Leu Ala Leu Trp Asp Ser Ala Lys Gly Thr Ile His Gly Glu Glu Val
    50                  55                  60
Glu Gln Glu Lys Ile Ala Val Phe Arg Asp Ala Ser Arg Phe Ile Leu
65                  70                  75                  80
Asn Leu Thr Ser Val Lys Pro Glu Asp Ser Gly Ile Tyr Phe Cys Met
                85                  90                  95
Ile Val Gly Ser Pro Glu Leu Thr Phe Gly Lys Gly Thr Gln Leu Ser
            100                 105                 110
Val Val Asp Phe Leu Pro Thr Thr Ala Gln Pro Thr Lys Lys Ser Thr
        115                 120                 125
Leu Lys Lys Arg Val Cys Arg Leu Pro Arg Pro Glu Thr Gln Lys Gly
    130                 135                 140
Pro Leu Cys Ser Pro Ile Thr Leu Gly Leu Leu Val Ala Gly Val Leu
145                 150                 155                 160
Val Leu Leu Val Ser Leu Gly Val Ala Ile His Leu Cys Cys Arg Arg
                165                 170                 175
Arg Arg Ala Arg Leu Arg Phe Met Lys Gln Phe Tyr Lys
            180                 185

<210> 145
<211> 200
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence CD8 co-receptor beta chain
      isoform 2

<400> 145
Leu Gln Gln Thr Pro Ala Tyr Ile Lys Val Gln Thr Asn Lys Met Val
 1               5                  10                  15
Met Leu Ser Cys Glu Ala Lys Ile Ser Leu Ser Asn Met Arg Ile Tyr
            20                  25                  30
Trp Leu Arg Gln Arg Gln Ala Pro Ser Ser Asp Ser His His Glu Phe
        35                  40                  45
Leu Ala Leu Trp Asp Ser Ala Lys Gly Thr Ile His Gly Glu Glu Val
    50                  55                  60
Glu Gln Glu Lys Ile Ala Val Phe Arg Asp Ala Ser Arg Phe Ile Leu
65                  70                  75                  80
Asn Leu Thr Ser Val Lys Pro Glu Asp Ser Gly Ile Tyr Phe Cys Met
                85                  90                  95
Ile Val Gly Ser Pro Glu Leu Thr Phe Gly Lys Gly Thr Gln Leu Ser
            100                 105                 110
Val Val Asp Phe Leu Pro Thr Thr Ala Gln Pro Thr Lys Lys Ser Thr
        115                 120                 125
Leu Lys Lys Arg Val Cys Arg Leu Pro Arg Pro Glu Thr Gln Lys Gly
    130                 135                 140
```

```
Pro Leu Cys Ser Pro Ile Thr Leu Gly Leu Leu Val Ala Gly Val Leu
145                 150                 155                 160
Val Leu Leu Val Ser Leu Gly Val Ala Ile His Leu Cys Cys Arg Arg
                165                 170                 175
Arg Arg Ala Arg Leu Arg Phe Met Lys Gln Leu Arg Leu His Pro Leu
            180                 185                 190
Glu Lys Cys Ser Arg Met Asp Tyr
        195                 200
```

<210> 146
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence CD8 co-receptor beta chain
isoform 3

<400> 146

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Leu Gln Gln Thr Pro Ala Tyr Ile Lys Val Gln Thr Asn Lys Met Val
 1               5                  10                  15
Met Leu Ser Cys Glu Ala Lys Ile Ser Leu Ser Asn Met Arg Ile Tyr
            20                  25                  30
Trp Leu Arg Gln Arg Gln Ala Pro Ser Ser Asp Ser His His Glu Phe
        35                  40                  45
Leu Ala Leu Trp Asp Ser Ala Lys Gly Thr Ile His Gly Glu Glu Val
    50                  55                  60
Glu Gln Glu Lys Ile Ala Val Phe Arg Asp Ala Ser Arg Phe Ile Leu
65                  70                  75                  80
Asn Leu Thr Ser Val Lys Pro Glu Asp Ser Gly Ile Tyr Phe Cys Met
                85                  90                  95
Ile Val Gly Ser Pro Glu Leu Thr Phe Gly Lys Gly Thr Gln Leu Ser
            100                 105                 110
Val Val Asp Phe Leu Pro Thr Thr Ala Gln Pro Thr Lys Lys Ser Thr
        115                 120                 125
Leu Lys Lys Arg Val Cys Arg Leu Pro Arg Pro Glu Thr Gln Lys Gly
    130                 135                 140
Pro Leu Cys Ser Pro Ile Thr Leu Gly Leu Leu Val Ala Gly Val Leu
145                 150                 155                 160
Val Leu Leu Val Ser Leu Gly Val Ala Ile His Leu Cys Cys Arg Arg
                165                 170                 175
Arg Arg Ala Arg Leu Arg Phe Met Lys Gln Lys Phe Asn Ile Val Cys
            180                 185                 190
Leu Lys Ile Ser Gly Phe Thr Thr Cys Cys Cys Phe Gln Ile Leu Gln
        195                 200                 205
Ile Ser Arg Glu Tyr Gly Phe Gly Val Leu Leu Gln Lys Asp Ile Gly
    210                 215                 220
Gln
```

225

<210> 147
<211> 225
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence CD8 co-receptor beta chain
isoform 4

<400> 147
Leu Gln Gln Thr Pro Ala Tyr Ile Lys Val Gln Thr Asn Lys Met Val
 1   5   10   15
Met Leu Ser Cys Glu Ala Lys Ile Ser Leu Ser Asn Met Arg Ile Tyr
            20   25   30
Trp Leu Arg Gln Arg Gln Ala Pro Ser Ser Asp Ser His His Glu Phe
        35   40   45
Leu Ala Leu Trp Asp Ser Ala Lys Gly Thr Ile His Gly Glu Glu Val
    50   55   60
Glu Gln Glu Lys Ile Ala Val Phe Arg Asp Ala Ser Arg Phe Ile Leu
65   70   75   80
Asn Leu Thr Ser Val Lys Pro Glu Asp Ser Gly Ile Tyr Phe Cys Met
                85   90   95
Ile Val Gly Ser Pro Glu Leu Thr Phe Gly Lys Gly Thr Gln Leu Ser
            100   105   110
Val Val Asp Phe Leu Pro Thr Thr Ala Gln Pro Thr Lys Lys Ser Thr
        115   120   125
Leu Lys Lys Arg Val Cys Arg Leu Pro Arg Pro Glu Thr Gln Lys Gly
    130   135   140
Pro Leu Cys Ser Pro Ile Thr Leu Gly Leu Leu Val Ala Gly Val Leu
145   150   155   160
Val Leu Leu Val Ser Leu Gly Val Ala Ile His Leu Cys Cys Arg Arg
                165   170   175
Arg Arg Ala Arg Leu Arg Phe Met Lys Gln Lys Phe Asn Ile Val Cys
            180   185   190
Leu Lys Ile Ser Gly Phe Thr Thr Cys Cys Cys Phe Gln Ile Leu Gln
        195   200   205
Ile Ser Arg Glu Tyr Gly Phe Gly Val Leu Leu Gln Lys Asp Ile Gly
    210   215   220
Gln
225

<210> 148
<211> 222
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence CD8 co-receptor beta chain
isoform 5

<400> 148

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Leu Gln Gln Thr Pro Ala Tyr Ile Lys Val Gln Thr Asn Lys Met Val
 1               5                  10                  15
Met Leu Ser Cys Glu Ala Lys Ile Ser Leu Ser Asn Met Arg Ile Tyr
            20                  25                  30
Trp Leu Arg Gln Arg Gln Ala Pro Ser Ser Asp Ser His His Glu Phe
        35                  40                  45
Leu Ala Leu Trp Asp Ser Ala Lys Gly Thr Ile His Gly Glu Glu Val
    50                  55                  60
Glu Gln Glu Lys Ile Ala Val Phe Arg Asp Ala Ser Arg Phe Ile Leu
65                  70                  75                  80
Asn Leu Thr Ser Val Lys Pro Glu Asp Ser Gly Ile Tyr Phe Cys Met
                85                  90                  95
Ile Val Gly Ser Pro Glu Leu Thr Phe Gly Lys Gly Thr Gln Leu Ser
            100                 105                 110
Val Val Asp Phe Leu Pro Thr Thr Ala Gln Pro Thr Lys Lys Ser Thr
        115                 120                 125
Leu Lys Lys Arg Val Cys Arg Leu Pro Arg Pro Glu Thr Gln Lys Gly
    130                 135                 140
Pro Leu Cys Ser Pro Ile Thr Leu Gly Leu Leu Val Ala Gly Val Leu
145                 150                 155                 160
Val Leu Leu Val Ser Leu Gly Val Ala Ile His Leu Cys Cys Arg Arg
                165                 170                 175
Arg Arg Ala Arg Leu Arg Phe Met Lys Gln Pro Gln Gly Glu Gly Ile
            180                 185                 190
Ser Gly Thr Phe Val Pro Gln Cys Leu His Gly Tyr Tyr Ser Asn Thr
        195                 200                 205
Thr Thr Ser Gln Lys Leu Leu Asn Pro Trp Ile Leu Lys Thr
    210                 215                 220
```