

HIGH AVIDITY WT1 T CELL RECEPTORS AND USES THEREOF

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format
5 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_466WO_SEQUENCE_LISTING.txt. The text file is 243 KB, was created on
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BACKGROUND

10 Adoptive T cell immunotherapy with genetically engineered T cells has shown
promise in multiple trials in which an antigen receptor of sufficient affinity was used to
target a tumor-associated antigen, including antibody-based chimeric receptors¹⁻³ and
high affinity TCRs⁴⁻⁸. While the natural process of diversity generation in the thymus
employs RAG-mediated TCR gene rearrangements to generate highly diverse CDR3s
15 varying in length as well as amino acid composition, isolating an effective high affinity
TCR within the affinity limits imposed by central tolerance remains a substantive
roadblock to implementing adoptive T cell immunotherapy for the diversity of
malignancies in which candidate intracellular self/tumor antigens have been
identified^{9,10}. In addition, TCR adoptive immunotherapy has the ability to detect
20 intracellular antigens that are presented on the cell surface by MHC Class I.

The WT1 protein is an attractive target for clinical development due to its
immune characteristics (Cheever *et al.*, *Clin. Cancer Res.* 15:5323, 2009), and its
expression in many aggressive tumor-types that have associated poor prognoses. WT1
is involved in the regulation of gene expression that promotes proliferation and
25 oncogenicity (Oji *et al.*, *Jpn. J. Cancer Res.* 90:194, 1999), is over-expressed in most
high-risk leukemias (Menssen *et al.*, *Leukemia* 9:1060, 1995), up to 80% of NSCLCs
(Oji *et al.*, *Int. J. Cancer* 100:297, 2002), 100% of mesotheliomas (Tsuta *et al.*, *App.*
Immunohistochem. Mol. Morphol. 17:126, 2009), and $\geq 80\%$ of gynecological
malignancies (Coosemans and Van Gool, *Expert Rev. Clin. Immunol.* 10:705, 2014).

Several peptides of the WT1 protein are known to be tumor-associated antigen peptides that are HLA-A*0201-restricted antigens.

There is a clear need for alternative highly WT1 antigen-specific TCR immunotherapies directed against various cancers, such as leukemia and tumors.

- 5 Presently disclosed embodiments address these needs and provide other related advantages.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B show how WT1₃₇-specific TCRs were identified by a high-throughput sequencing-based strategy. (A) Schematic of initial sequencing-based strategy for identifying TCR clonotypes associated with high WT1₃₇₋₄₅ peptide/MHC tetramer-binding. (B) Enrichment in sort populations versus percentage of total population is shown, with selected TCR highlighted. All TCRs indicated by black circles were synthesized and evaluated for antigen-specificity (27 total).

Figure 2 shows results of functional evaluation of TCRs that bind high levels of CD8 independent (CD8i) tetramer. TCR constructs were expressed in Jurkat cells that lack endogenous TCR α/β chains. Tetramer staining versus CD3 expression for each TCR is shown (CD3 expression directly correlates with transgenic TCR surface expression).

Figures 3A – 3C show additional WT1₃₇-specific TCRs were identified by a modified high-throughput sequencing-based strategy using a CD8 independent (CD8i) tetramer. (A) Schematic of modified sequencing-based strategy for identifying TCR clonotypes associated with high CD8-independent WT1₃₇ peptide/MHC tetramer-binding. (B) Enrichment in original sort populations versus percentage of total population as compared with (C) a similar analysis when CD8i tetramer is used is shown. An additional 14 TCRs were selected based on decreased surface CD3 levels and CD8i tetramer binding. All TCRs indicated by shaded (diagonal line pattern) circles were synthesized and evaluated for antigen-specificity.

Figure 4 shows CD8i tetramer binding of selected WT1₃₇ TCRs. TCR constructs were expressed in Jurkat cells that lack endogenous TCR α/β chains.

Tetramer staining versus CD3 expression for each TCR is shown (CD3 expression directly correlates with transgenic TCR surface expression).

Figures 5A and 5B show calculation of peptide EC₅₀ for selected TCRs in IFN γ assay when transduced into primary CD8⁺ PBMCs. (A) Selected TCRs were transduced into CD8⁺ T cells isolated from donor PMBCs. After 1 week, cells were sorted for tetramer⁺ CD8⁺ T cells and expanded. Expanded antigen-specific cells were cultured for 4-6 hours with peptide-pulsed T2 target cells and IFN γ production was determined by flow cytometry. (B) Percentage of IFN γ -producing cells was fit to dose-response curves by non-linear regression to calculate peptide EC₅₀ for each TCR.

Figure 6 shows that primary CD8⁺ T cells expressing WT1₃₇-specific TCRs efficiently kill the WT1⁺ HLA-A2⁺ breast cancer cell line MDA-MB-468. Sort-purified for high tetramer binding, CD8⁺ primary T cells were transduced with TCR and mixed at an 8:1 ratio (in triplicate) with the breast cancer cell line MDA-MB-468, which had been stained with CytoLight® Rapid Red dye. Total red object area (which correlates with the total number of live target cells) was calculated at the time points indicated for each TCR-transduced T cell population over a 72 hour period. In order to assess ongoing responsiveness of TCR-transduced T cells to persistent antigen, additional MDA-MB-468 cells were added at 48 hours.

Figure 7 shows that both CD4⁺ and CD8⁺ T cells expressing TCR 10.1 can eliminate the WT1⁺ A2⁺ pancreatic adenocarcinoma cell line PANC-1 after repeat challenge *in vitro*. Both CD4⁺ and CD8⁺ T cells were transduced to express the WT1₃₇ TCR 10.1. CD4⁺ T cells were further transduced to express CD8 α and CD8 β genes. After 8 days, transduced cells were sorted to purify CD8⁺ tetramer⁺ and CD4⁺/CD8⁺ tetramer⁺ T cells. Antigen-specific cells that were either CD4⁺/CD8⁺, CD8⁺, or a mixture of these two populations (CD4 and CD8) were mixed 8:1 (in triplicate) with the pancreatic adenocarcinoma cell line PANC-1, which had been previously transduced to express NucLight® Red dye. Total red object area (which correlates with the total number of live target cells) was calculated at the time points indicated for each TCR-transduced T cell population. In order to assess ongoing responsiveness of TCR-transduced T cells to persistent antigen, additional PANC-1 cells were added at 48 hours.

Figures 8A-8D show a comparison of tumor cell line killing by T cells transduced with WT1 p126 peptide-specific C4 TCR from Schmitt *et al.* (*Nat. Biotechnol.* 35:1188, 2017) as compared to killing by T cells transduced with WT1 p37 peptide-specific TCR of the present disclosure (WT1₃₇₋₄₅ TCR15.1). Note that the C4 TCR has a lower affinity for its peptide:MHC complex as compared to the WT1 p37 peptide-specific TCRs of this disclosure.

DETAILED DESCRIPTION

The present disclosure provides T cell receptors (TCRs) having high functional avidity for antigenic peptide from WT1 comprised of amino acids 37-45 (also referred to as WT1₃₇₋₄₅ peptide or p37 peptide antigen; *e.g.*, VLDFAPPGA, SEQ ID NO:59) that is associated with a major histocompatibility complex (MHC) (*e.g.*, human leukocyte antigen, HLA). Such p37 peptide antigen specific TCRs are useful for, for example, adoptive immunotherapy to treat cancer, such as cancers that overexpress WT1.

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.

In the instant disclosure, a high throughput sequencing-based approach was applied to immune cells from about 15 healthy donors to identify TCRs having high functional avidity for a p37:MHC complex. This strategy also allows for selection of TCRs even if when expressed at low levels of TCRs on the T cell surface. Enrichment of sort populations versus percentage of the total population was used to select high affinity and high functional avidity (*i.e.*, those with the greatest anti-tumor efficacy) TCRs specific for p37 and compositions thereof of the present disclosure. Such high functional avidity TCRs specific for p37 were identified in T cells that: (a) bound p37 peptide/MHC tetramers independent of CD8, (b) underwent less *in vitro* peptide-driven expansion, and (c) in some cases expressed such TCRs at relatively low levels on the T cell surface as compared to other TCRs in T cells not having such characteristics. A total of 27 TCRs were synthesized and evaluated for p37 antigen-specificity (*see* Figure 1B).

In certain embodiments, a T cell receptor (TCR) specific for a WT1 peptide comprises a TCR α -chain and a TCR β -chain, wherein the TCR α -chain comprises a V_{α} domain comprising the amino acid sequence set forth in any one of SEQ ID NOS: 253-263 and 34-44 and an α -chain constant domain having the amino acid sequence of SEQ ID NO:47, and the TCR β -chain comprises a V_{β} domain comprising the amino acid sequence set forth in any one of SEQ ID NOS: 253-263 and 23-33, and a β -chain constant domain having the amino acid sequence of SEQ ID NO:45 or 46, and such TCRs specifically bind to a VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex on a T cell surface and promote IFN γ production with a pEC₅₀ of 8.5 or higher. In certain embodiments, selected TCRs specifically bind to a VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex with a K_D of less than or equal to about 10⁻⁸ M, or wherein the high affinity TCR dissociates from a VLDFAPPGA (SEQ ID NO:59):HLA complex at a reduced k_{off} rate as compared to a TCR disclosed by Schmitt *et al.*, *Nat. Biotechnol.* 35:1188, 2017.

The compositions and methods described herein will in certain embodiments have therapeutic utility for the treatment of diseases and conditions associated with WT1 expression or overexpression (*e.g.*, detectable WT1 expression at a level that is greater in magnitude, in a statistically significant manner, than the level of WT1

expression that is detectable in a normal or disease-free cell). Such diseases include various forms of hyperproliferative disorders or proliferative 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
5 WT1 antigen-specific T cell responses, such as by the use of recombinant T cells expressing an enhanced affinity TCR specific for a WT1 peptide (*e.g.*, VLDFAPPGA, SEQ ID NO:59, also known as WT1₃₇₋₄₅ peptide or p37 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.

10 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
15 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 $\pm 10\%$ 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
20 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
25 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.

30 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 the 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" in some aspects 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 $\gamma\delta$ T cell, a regulatory T cell, a stem cell memory T cell, a natural killer cell (*e.g.*, a NK cell or a NK-T cell), a B 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) in some aspects can refer 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 β 2 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" or "T lymphocyte" is an immune system cell that matures in the thymus and produces T cell receptors (TCRs). T cells can exhibit phenotypes or markers associated with naïve T cells (e.g., 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) (e.g., antigen-experienced and long-lived), and effector cells (antigen-experienced, cytotoxic). T_M can be further divided into subsets exhibiting phenotypes or markers associated with of central memory T cells (T_{CM}, e.g., 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}, e.g., 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) can refer to antigen-experienced CD8⁺ cytotoxic T lymphocytes that has decreased expression of CD62L, CCR7, CD28, and are positive for granzyme and perforin as compared to T_{CM}. Helper T cells (T_H) can include CD4⁺ T cells that influence the activity of other immune cells by releasing cytokines. CD4⁺ T cells can activate and suppress an adaptive immune response, and which of those two functions is induced will depend on presence of other cells and signals. T cells can be collected using known techniques, and the various subpopulations or combinations thereof can be enriched or depleted by known techniques, such as by affinity binding to antibodies, flow cytometry, or immunomagnetic selection. 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) in some aspects 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. In some aspects, a TCR refers to a binding protein comprising two

TCR variable domains (a $V\alpha$ and a $V\beta$) of the present disclosure. In some aspects, a TCR comprises a single-chain TCR (*i.e.*, a single-chain fusion protein comprising TCR variable domains of the present disclosure, or a CAR comprising TCR variable domains of the present disclosure (discussed herein). In some aspects, a TCR can be found on
5 the surface of a cell or in soluble form and generally is comprised of a heterodimer having α and β chains (also known as $TCR\alpha$ and $TCR\beta$, respectively), or γ and δ chains (also known as $TCR\gamma$ and $TCR\delta$, 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 $V\alpha$, β -chain variable domain or $V\beta$; 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 $C\alpha$, β -chain constant domain or $C\beta$, typically amino acids 81 to 259 based on Kabat) adjacent to the cell membrane.
10 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). In certain embodiments, a TCR is
15 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.

The term "variable region" or "variable domain" refers to the domain of an immunoglobulin superfamily binding protein (*e.g.*, a TCR α -chain or β -chain (or γ chain and δ chain for $\gamma\delta$ TCRs)) that is involved in binding of the immunoglobulin superfamily binding protein (*e.g.*, TCR) to antigen. The variable domains of the α -chain and β -chain ($V\alpha$ and $V\beta$, respectively) of a native TCR generally have similar structures, with each domain comprising four generally conserved framework regions (FRs) and three CDRs. The $V\alpha$ domain is encoded by two separate DNA segments, the variable gene segment and the joining gene segment (V-J); the $V\beta$ domain is encoded
25 by three separate DNA segments, the variable gene segment, the diversity gene
30

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.

5 The terms "complementarity determining region," and "CDR," are synonymous with "hypervariable region" or "HVR," and are known in the art to refer to sequences of amino acids within immunoglobulin (*e.g.*, TCR) variable regions, which confer antigen specificity and/or binding affinity and are separated from one another in primary amino acid sequence by framework regions. In general, there are three CDRs in each TCR α -chain variable region (α CDR1, α CDR2, α CDR3) and three CDRs in each TCR β -chain variable region (β CDR1, β CDR2, β CDR3). In TCRs, CDR3 is thought to be the main
10 CDR responsible for recognizing processed antigen. In general, CDR1 and CDR2 interact mainly or exclusively with the MHC.

 CDR1 and CDR2 are encoded within the variable gene segment of a TCR
15 variable region-coding sequence, whereas CDR3 is encoded by the region spanning the variable and joining segments for V α , or the region spanning variable, diversity, and joining segments for V β . Thus, if the identity of the variable gene segment of a V α or V β is known, the sequences of their corresponding CDR1 and CDR2 can be deduced; *e.g.*, according to a numbering scheme as described herein. Compared with CDR1 and
20 CDR2, CDR3 is typically significantly more diverse due to the addition and loss of nucleotides during the recombination process.

 TCR variable domain sequences can be aligned to a numbering scheme (*e.g.*, Kabat, Chothia, EU, IMGT, Enhanced Chothia, and Aho), allowing equivalent residue positions to be annotated and for different molecules to be compared using, for
25 example, 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 CDR of the present disclosure is identified according to the IMGT numbering scheme (Lefranc *et al.*, *Dev. Comp. Immunol.* 27:55, 2003; imgt.org/IMGTindex/V-QUEST.php). In certain embodiments,
30 a CDR3 amino acid sequence of the present disclosure comprises one or more junction

amino acid; *e.g.*, such as may arise during (RAG)-mediated rearrangement, discussed herein.

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.

5 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) known human CD8 beta chain isoforms (*see* UniProtKB identifier P10966) and a single known human CD8 alpha chain isoform (*see* UniProtKB
10 identifier P01732).

"CD4" is 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); UniProtKB identifier P01730). CD4 is found on the surface of immune cells such as T helper cells, monocytes, macrophages, and
15 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 β 2, while the TCR complex binds MHCII α 1/ β 1). Without wishing to be bound by theory, it is believed that close proximity to the TCR complex allows CD4-
20 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 or recruit various types immune system cells, including T helper cells, and immune responses.

As used herein, "D/N/P region" in some aspects refers to nucleotides, or amino
25 acids encoded by the nucleotides, predicted to be located within diversity (D) gene segment, which can include non-templated (N) nucleotides and palindromic (P) nucleotides that are inserted (or deleted) during the V(D)J recombination process that leads to diversity of T cell receptors. Recombination activating gene (RAG)-mediated rearrangement of variable (V), diversity (D) and joining (J) gene segments is an
30 inaccurate process that results in the variable addition or subtraction of nucleotides (referred to as palindromic or P nucleotides), which is followed by terminal

deoxynucleotidyl transferase (TdT) activity that adds further adds random non-templated (N) nucleotides. Finally, exonucleases remove unpaired nucleotides and gaps are filled by DNA synthesis and repair enzymes. Such a trim and repair mechanism leads to the junctional diversity that underpins the efficient and specific
5 recognition of different antigens by different TCRs. D gene segments can be identified using the annotation system from the international ImMunoGeneTics information system (IMGT; at imgt.org).

In some aspects, "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
10 comprises a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, and a homodimer of CD3 ζ chains. The CD3 γ , CD3 δ , and CD3 ϵ chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3 γ , CD3 δ , and CD3 ϵ chains are negatively charged, which is a characteristic that allows these chains to associate with the positively
15 charged regions of T cell receptor chains. The intracellular tails of the CD3 γ , CD3 δ , and CD3 ϵ chains each contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM, whereas each CD3 ζ chain has three. Without wishing to be bound by theory, it is believed the ITAMs are important for the signaling capacity of a TCR complex. CD3 as used in the present disclosure may be from various
20 animal species, including human, mouse, rat, or other mammals.

As used herein, "TCR complex" in some aspects refers to a complex formed by the association of CD3 with TCR. For example, a TCR complex can be composed of a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, a homodimer of CD3 ζ chains, a TCR α chain, and a TCR β chain. Alternatively, a TCR complex can be composed of a CD3 γ
25 chain, a CD3 δ chain, two CD3 ϵ chains, a homodimer of CD3 ζ chains, a TCR γ chain, and a TCR δ chain.

In some aspects, a "component of a TCR complex," as used herein, refers to a TCR chain (*i.e.*, TCR α , TCR β , TCR γ or TCR δ), a CD3 chain (*i.e.*, CD3 γ , CD3 δ , CD3 ϵ or CD3 ζ), or a complex formed by two or more TCR chains or CD3 chains (*e.g.*, a
30 complex of TCR α and TCR β , a complex of TCR γ and TCR δ , a complex of CD3 ϵ and

CD3 δ , a complex of CD3 γ and CD3 ϵ , or a sub-TCR complex of TCR α , TCR β , CD3 γ , CD3 δ , and two CD3 ϵ chains).

"Antigen" or "Ag" as used herein refers to an immunogenic molecule that provokes an immune response. This immune response may involve antibody
5 production, activation of specific immunologically competent cells (*e.g.*, T cells), or both. An antigen (immunogenic molecule) may be, for example, a peptide, glycopeptide, polypeptide, glycopolypeptide, polynucleotide, polysaccharide, lipid or the like. It is readily apparent that an antigen can be synthesized, produced recombinantly, or derived from a biological sample. Exemplary biological samples that
10 can contain one or more antigens include tissue samples, tumor samples, cells, biological fluids, or combinations thereof. Antigens can be produced by cells that have been modified or genetically engineered to express an antigen, or that endogenously (*e.g.*, without modification or genetic engineering by human intervention) express a mutation or polymorphism that is immunogenic.

15 A "neoantigen," as used herein, refers to a host cellular product containing a structural change, alteration, or mutation that creates a new antigen or antigenic epitope that has not previously been observed in the subject's genome (*i.e.*, in a sample of healthy tissue from the subject) or been "seen" or recognized by the host's immune system, which: (a) is processed by the cell's antigen-processing and transport
20 mechanisms and presented on the cell surface in association with an MHC (*e.g.*, HLA) molecule; and (b) elicits an immune response (*e.g.*, a cellular (T cell) response). Neoantigens may originate, for example, from coding polynucleotides having alterations (substitution, addition, deletion) that result in an altered or mutated product, or from the insertion of an exogenous nucleic acid molecule or protein into a cell, or
25 from exposure to environmental factors (*e.g.*, chemical, radiological) resulting in a genetic change. Neoantigens may arise separately from a tumor antigen, or may arise from or be associated with a tumor antigen. "Tumor neoantigen" (or "tumor-specific neoantigen") refers to a protein comprising a neoantigenic determinant associated with, arising from, or arising within a tumor cell or plurality of cells within a tumor. Tumor
30 neoantigenic determinants are found on, for example, antigenic tumor proteins or peptides that contain one or more somatic mutations or chromosomal rearrangements

encoded by the DNA of tumor cells (*e.g.*, pancreas cancer, lung cancer, colorectal cancers), as well as proteins or peptides from viral open reading frames associated with virus-associated tumors.

The term "epitope" or "antigenic epitope" includes any molecule, structure,
5 amino acid sequence or protein determinant that is recognized and specifically bound by a cognate binding molecule, such as an immunoglobulin, T cell receptor (TCR), chimeric antigen receptor, or other binding molecule, domain or protein. Epitopic determinants generally contain chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three dimensional structural
10 characteristics, as well as specific charge characteristics.

As used herein, "specifically binds" or "specific for" in some aspects refers to an association or union of a T cell receptor (TCR) or a binding domain thereof (*e.g.*, scTCR or a fusion protein thereof) to a target molecule with an apparent affinity or K_A (*i.e.*, an equilibrium association constant of a particular binding interaction with units of
15 $1/M$) equal to or greater than $10^9 M^{-1}$ (which equals the ratio of the on-rate [k_{on}] to the off-rate [k_{off}] for this association reaction), or a functional avidity or EC_{50} equal to or greater than $10^{-9} M$, while not significantly associating or uniting with any other molecules or components in a sample. TCRs may be classified as "high affinity" binding proteins or binding domains (or fusion proteins thereof) or as "low affinity"
20 binding proteins or binding domains (or fusion proteins thereof). "High affinity" TCRs or binding domains refer to those TCRs or binding domains thereof having a K_A of 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 $10^5 M^{-1}$.
25 Alternatively, affinity may be defined as an equilibrium dissociation constant (K_D) of a particular binding interaction with units of M (*e.g.*, $10^{-9} M$ to $10^{-13} M$ or less).

The term "functional avidity" refers to a biological measure or activation threshold of an *in vitro* T cell response to a given concentration of a ligand, wherein the biological measures can include cytokine production (*e.g.*, IFN γ production, IL-2
30 production, etc.), cytotoxic activity, and proliferation. For example, T cells that biologically (immunologically) respond *in vitro* to a very low antigen dose by

producing cytokines, being cytotoxic, or proliferating are considered to have high functional avidity, while T cells having lower functional avidity require higher amounts of antigen before an immune response, similar to the high-avidity T cells, is elicited. It will be understood that functional avidity is different from affinity and avidity. Affinity refers to the strength of any given bond between a binding protein and its antigen/ligand. Some binding proteins are multivalent and bind to multiple antigens – in this case, the strength of the overall connection is the avidity.

As used herein, "functional avidity" refers to a quantitative determinant of the activation threshold of a TCR expressed by a T cell. *In vivo*, T cells are exposed to similar antigen doses regardless of the TCR avidity (high or low), but numerous correlations exist between the functional avidity and the effectiveness of an immune response. Some *ex vivo* studies have shown that distinct T cell functions (*e.g.*, proliferation, cytokines production, etc.) can be triggered at different thresholds (*see, e.g.*, Betts *et al.*, *J. Immunol.* 172:6407, 2004; Langenkamp *et al.*, *Eur. J. Immunol.* 32:2046, 2002). Factors that affect functional avidity include (a) the affinity of a TCR for the pMHC-complex, that is, the strength of the interaction between the TCR and pMHC (Cawthon *et al.*, *J. Immunol.* 167:2577, 2001), (b) expression levels of the TCR and the CD4 or CD8 co-receptors, and (c) the distribution and composition of signaling molecules (Viola and Lanzavecchia, *Science* 273:104, 1996), as well as expression levels of molecules that attenuate T cell function and TCR signaling.

The concentration of antigen needed to induce a half-maximum response between the baseline and maximum response after a specified exposure time is referred to as the "half maximal effective concentration" or "EC₅₀". The EC₅₀ value is generally presented as a molar (moles/liter) amount, but it is often converted into a logarithmic value as follows – log₁₀(EC₅₀) – which provides a sigmoidal graph (*see, e.g.*, Figure 5A). For example, if the EC₅₀ equals 1 μM (10⁻⁶ M), the log₁₀(EC₅₀) value is -6. Another value used is pEC₅₀, which is defined as the negative logarithm of the EC₅₀ (-log₁₀(EC₅₀)). In the above example, the EC₅₀ equaling 1 μM has a pEC₅₀ value of 6. In certain embodiments, the functional avidity of the TCRs of this disclosure will be a measure of its ability to promote IFNγ production by T cells, which can be measured using assays described herein. "High functional avidity" TCRs or binding domains

thereof refer to those TCRs or binding domains thereof having a EC_{50} of at least 10^{-9} M, at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or at least about 10^{-13} M. In some embodiments, the response comprises IFN- γ production; *e.g.*, the production of IFN- γ by an immune cell (such as a T cell, NK cell, or NK-T cell) expressing the TCR in response to antigen.

In some aspects, "WT1₃₇₋₄₅ antigen" or "WT1₃₇₋₄₅ peptide" or "WT1₃₇₋₄₅ peptide antigen" or "p37 peptide" or "p37 antigen" or "p37 peptide antigen" each refer to a naturally or synthetically produced portion of a WT1 protein ranging in length from about 9 amino acids to about 15 amino acids and comprising the amino acid sequence of VLDFAPPGA (SEQ ID NO:59), which can form a complex with a MHC (*e.g.*, HLA) molecule and such a complex can bind with a TCR specific for a WT1 peptide:MHC (*e.g.*, HLA) complex. Since WT1 is an internal host protein, WT1 antigen peptides will be presented in the context of class I MHC. In particular embodiments, WT1 peptide VLDFAPPGA (SEQ ID NO:59) is capable of associating with human class I HLA allele HLA-A*201.

In some aspects, the phrases "WT1₃₇₋₄₅ peptide-specific binding protein" or "WT1₃₇₋₄₅ peptide-specific TCR" or "WT1₃₇₋₄₅ antigen-specific TCR," or "WT1₃₇₋₄₅ peptide antigen-specific TCR" or "WT1 p37 peptide-specific binding protein" or "WT1 p37 peptide-specific TCR" or "WT1 p37 antigen-specific TCR," or "WT1 p37 peptide antigen-specific TCR," which are interchangeable herein, refer to a protein or polypeptide that specifically binds to a WT1 p37 peptide complexed with an MHC or HLA molecule, *e.g.*, on a cell surface, with about, or at least about, a particular affinity or functional avidity, preferably a high functional avidity, as defined herein. Such a binding protein or polypeptide comprises TCR variable domains as provided herein. In certain embodiments, a WT1-specific binding protein binds a WT1-derived peptide:HLA complex (or WT1-derived peptide:MHC complex) have a functional avidity $\log[EC_{50}]$ ranging from about -2.5 μ M to about -3.75 μ M (which is equivalent to -8.5M to about -9.8M). The EC_{50} range for these values range from about 3.16×10^{-9} M to about 1.58×10^{-10} M as measured, for example, by the assay described in the following paragraphs and in Example 1 herein.

Assays for assessing affinity, apparent affinity, relative affinity, or functional avidity are known. As described herein, apparent affinity or functional avidity of a TCR of this disclosure is measured by assessing binding to various concentrations of tetramers associated with p37 peptide, for example, by flow cytometry using labeled tetramers. In some examples, apparent K_D or EC_{50} 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. For example, apparent K_D is determined as the concentration of ligand that yields half-maximal binding, whereas an EC_{50} is determined as the concentration of ligand that yields half-maximal production of, for example, a cytokine (*e.g.*, IFN γ , IL-2).

"MHC-peptide tetramer staining" in some aspects 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.*, WT1), 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 high affinity or high functional avidity 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.

In some aspects, the term "WT1 p37-specific binding domain" or "WT1₃₇₋₄₅-specific binding domain" or "WT1 p37-specific binding fragment" or "WT1₃₇₋₄₅-specific binding fragment" refer to a domain or portion of a WT1-specific TCR responsible for specific binding to WT1 p37 antigen complexed with an MHC or HLA molecule. A WT1 p37 antigen-specific binding domain from a TCR alone (*i.e.*, without any other portion of a WT1-specific TCR) can be soluble and can bind to a WT1 p37 peptide:MHC complex with a K_D of less than 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. In other embodiments, a WT1 p37 peptide-specific TCR has high functional avidity and specifically binds to a VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex on a T cell surface and promotes IFN γ production at a pEC₅₀ of 8.5 or higher (*e.g.*, up to about 9, up to about 9.5, up to about 10, about 10.5, about 11, about 11.5, about 12, about 12.5, or about 13). Exemplary WT1 p37 peptide-specific binding domains include WT1 p37 peptide-specific scTCR (*e.g.*, single chain $\alpha\beta$ TCR proteins such as V α -L-V β , V β -L-V α , V α -C α -L-V α , or V α -L-V β -C β , wherein V α and V β are TCR α and β variable domains respectively, C α and C β are TCR α and β constant domains, respectively, and L is a linker), which are or can be derived from an anti-WT1 p37 peptide TCR of this disclosure.

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, intracellular 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.

A "transmembrane domain," as used herein, means any amino acid sequence having a three-dimensional structure that is thermodynamically stable in a cell membrane, and generally ranges in length from about 15 amino acids to about 30 amino acids. The structure of a hydrophobic transmembrane domain may comprise an alpha helix, a beta barrel, a beta sheet, a beta helix, or any combination thereof. Exemplary transmembrane domains are transmembrane domains from CD4, CD8, CD28, or CD27.

As used herein, an "immune effector domain" is an intracellular portion of a scTCR or CAR fusion protein that can directly or indirectly promote an immunological response in a cell when receiving the appropriate signal. In certain embodiments, an immune effector domain is part of a protein or protein complex that receives a signal when bound, or it binds directly to a target molecule, which triggers a signal from the immune effector domain. An immune effector domain may directly promote a immune cell response when it contains one or more signaling domains or motifs, such as an immunoreceptor tyrosine-based activation motif (ITAM). In other embodiments, an effector domain will indirectly promote a cellular response by associating with one or more other proteins that directly promote a cellular response. Exemplary immune effector domains include intracellular signaling domains from 4-1BB, CD3 ϵ , CD3 δ , CD3 ζ , CD27, CD28, CD79A, CD79B, CARD11, DAP10, FcR α , FcR β , FcR γ , Fyn, HVEM, ICOS, Lck, LAG3, LAT, LRP, NOTCH1, Wnt, NKG2D, OX40, ROR2, Ryk, SLAMF1, Slp76, pT α , TCR α , TCR β , TRIM, Zap70, PTCH2, or any combination of two or three of such domains.

A "linker" in some aspects refers to an amino acid sequence that connects two proteins, polypeptides, peptides, domains, regions, or motifs. An exemplary linker is a "variable domain linker," which specifically refers to a five to about 35 amino acid sequence that connects T cell receptor V α/β and C α/β chains (*e.g.*, V α -C α , V β -C β , V α -V β) or connects each V α -C α , V β -C β , V α -V β pair to a hinge or transmembrane domain, which provides a spacer function and flexibility sufficient for interaction of the two sub-binding domains so that the resulting single chain polypeptide retains a specific binding affinity or functional avidity to the same target molecule as a T cell receptor.

In certain embodiments, a variable domain linker comprises from about ten to about 30 amino acids or from about 15 to about 25 amino acids. In particular embodiments, a variable domain linker peptide comprises from one to ten repeats of Gly_xSer_y, wherein x and y are independently an integer from 0 to 10 provided that x and y are not both 0
 5 (e.g., Gly₄Ser (SEQ ID NO:171), Gly₃Ser (SEQ ID NO:172), Gly₂Ser, or (Gly₃Ser)_n(Gly₄Ser)₁ (SEQ ID NO:173), (Gly₃Ser)_n(Gly₂Ser)_n, (SEQ ID NO:174) (Gly₃Ser)_n(Gly₄Ser)_n (SEQ ID NO:175), or (Gly₄Ser)_n (SEQ ID NO:171), wherein n is an integer of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) and wherein linked variable domains form a functional binding domain (e.g., scTCR).

10 In some aspects, "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.,
 15 amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a fusion protein), or in the process of a genetic recombination or rearrangement event (e.g., RAG-mediated rearrangement).

In some aspects, an "altered domain" or "altered protein" refers to a motif, region, domain, peptide, polypeptide, or protein with a non-identical sequence identity
 20 to a wild type motif, region, domain, peptide, polypeptide, or protein (e.g., a wild type TCR α chain, TCR β chain, TCR α constant domain, TCR β 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%), preferably wherein or wherein the CDR3 from each of the TCR α and β variable domains are not
 25 altered.

In any of the presently disclosed embodiments, a TCR constant domain can be modified to enhance pairing of desired TCR chains. For example, enhanced pairing in a host T cell between a heterologous TCR α -chain and a heterologous TCR β -chain due to a modification results in the preferential assembly of a TCR comprising two
 30 heterologous chains over an undesired mispairing of a heterologous TCR chain with an endogenous TCR chain (see, e.g., Govers *et al.*, *Trends Mol. Med.* 16(2):77 (2010), the

TCR modifications of which are herein incorporated by reference). Exemplary modifications to enhance pairing of heterologous TCR chains include the introduction of complementary cysteine residues in each of the heterologous TCR α -chain and β -chain. In some embodiments, a polynucleotide encoding a heterologous TCR α -chain encodes a cysteine at amino acid position 48 (corresponding to the full-length, mature human TCR α -chain sequence) and a polynucleotide encoding a heterologous TCR β -chain encodes a cysteine at amino acid position 57 (corresponding to the full-length mature human TCR β -chain sequence).

"Chimeric antigen receptor" (CAR) refers to a fusion protein that is engineered to contain two or more naturally occurring amino acid sequences, domains, or motifs, linked together in a way that does not occur naturally or does not occur naturally in a host cell, which fusion protein can function as a receptor when present on a surface of a cell. CARs can include an extracellular portion comprising an antigen-binding domain (*e.g.*, obtained or derived from an immunoglobulin or immunoglobulin-like molecule, such as a TCR binding domain derived or obtained from a TCR specific for a cancer antigen, a scFv derived or obtained from an antibody, or an antigen-binding domain derived or obtained from a killer immunoreceptor from an NK cell) linked to a transmembrane domain and one or more intracellular signaling domains (optionally containing co-stimulatory domain(s)) (*see, e.g., Sadelain et al., Cancer Discov., 3(4):388 (2013); see also Harris and Kranz, Trends Pharmacol. Sci., 37(3):220 (2016), Stone et al., Cancer Immunol. Immunother., 63(11):1163 (2014), and Walseng et al., Scientific Reports 7:10713 (2017), which CAR constructs and methods of making the same are incorporated by reference herein*). CARs of the present disclosure that specifically bind to a WT1 antigen (*e.g.*, in the context of a peptide:HLA complex) comprise a TCR V α domain and a V β domain.

As used herein, "nucleic acid" or "nucleic acid molecule" or "polynucleotide" in some aspects refer 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
5 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.

10 In some aspects, 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
15 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
20 sequences (introns) between individual coding segments (exons).

As used herein, the term "recombinant" in some aspects refers 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
25 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,
30 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" or "mutated" in some aspects 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" in some aspects is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well known in the art (*see, e.g.*, WO 97/09433 at page 10; Lehninger, *Biochemistry*, 2nd Edition; Worth Publishers, Inc. NY, NY, pp.71-77, 1975; Lewin, *Genes IV*, Oxford University Press, NY and Cell Press, Cambridge, MA, p. 8, 1990).

The term "construct" in some aspects 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).

Exemplary 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
5 Fundamental Virology, Third Edition, B. N. Fields *et al.*, Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

In some aspects, "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
10 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
15 transcription is a double-stranded linear viral DNA, which is the substrate for viral integration into the DNA of infected cells.

The term "operably-linked" in some aspects refers to the association of two or more nucleic acid molecules on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably-linked with a coding
20 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" in some aspects refers to a DNA construct
25 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.
30 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, in some aspects 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, in some aspects 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 in some aspects refers to a nucleic acid molecule or portion of a nucleic acid molecule that is not native to a host cell, but may be homologous to a nucleic acid molecule or portion of a nucleic acid molecule from the host cell. The source of the heterologous or exogenous nucleic acid molecule, construct or sequence may be from a different genus or species. In certain embodiments, a heterologous or exogenous nucleic acid molecule 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 nucleic acid molecule introduced into the host cell, even if the host cell encodes a homologous protein or activity. Moreover, a cell comprising a "modification" or a "heterologous" polynucleotide or binding protein includes progeny of that cell, regardless of whether

the progeny were themselves transduced, transfected, or otherwise manipulated or changed.

As described herein, more than one heterologous or exogenous nucleic acid molecule can be introduced into a host cell as separate nucleic acid molecules, as a plurality of individually controlled genes, as a polycistronic nucleic acid molecule, 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 nucleic acid molecules encoding desired TCR specific for a WT1 antigen peptide (*e.g.*, TCR α and TCR β). 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 nucleic acid molecule (*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 nucleic acid molecules introduced into a host cell.

As used herein, the term "endogenous" or "native" in some aspects 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.

In some aspects, 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.

In some aspects, "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" in some aspects can be 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⁻ CD81⁺ phenotype or those found in the thymus (referred to as progenitor thymocytes).

As used herein, the term "host" in some aspects 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-WT1 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 some embodiments, host cells are genetically modified to express a protein or fusion protein that modulates immune signaling in a host cell to, for example, promote survival and/or expansion advantage to the modified cell (*e.g.*, *see* immunomodulatory fusion proteins of WO 2016/141357, which are herein incorporated by reference in their entirety). In other embodiments, host cells are genetically modified to introduce a TCR as provided herein, or to knock-down or minimize immunosuppressive signals in a cell (*e.g.*, a checkpoint inhibitor), which modifications may be made using, for example, a CRISPR/Cas system (*see, e.g.*, US 2014/0068797, U.S. Pat. No. 8,697,359; WO 2015/071474). In certain embodiments, a host cell is a human hematopoietic progenitor cell transduced with a

heterologous or exogenous nucleic acid molecule encoding a TCR α chain specific for a WT1 antigen peptide.

As used herein, "hyperproliferative disorder" in some aspects refers to excessive growth or proliferation as compared to a normal or undiseased cell. Exemplary
 5 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). Certain
 10 diseases that involve abnormal or excessive growth that occurs more slowly than in the context of a hyperproliferative disease can be referred to as "proliferative diseases", and include certain tumors, cancers, neoplastic tissue, carcinoma, sarcoma, malignant cells, pre malignant cells, as well as non-neoplastic or non-malignant disorders.

Furthermore, "cancer" may refer to any accelerated proliferation of cells,
 15 including solid tumors, ascites tumors, blood or lymph or other malignancies; connective tissue malignancies; metastatic disease; minimal residual disease following transplantation of organs or stem cells; multi-drug resistant cancers, primary or secondary malignancies, angiogenesis related to malignancy, or other forms of cancer.

TCRs Specific for WT1 p37 Antigen Peptides

20 In certain aspects, the instant disclosure provides a WT1 p37 peptide-specific T cell receptor (TCR) comprising (a) a T cell receptor (TCR) α -chain variable (V_α) domain, and a TCR β -chain variable (V_β) having the CDR3 amino acid sequence shown in any one of SEQ ID NOS:1-11, 181, 187, 193, 199, 205, 211, 217, 223, 229, 235, and 241; (b) a TCR V_α domain having the CDR3 amino acid sequence shown in any one of
 25 SEQ ID NOS:12-22, 178, 184, 190, 196, 202, 208, 214, 220, 226, 232, and 238, and a TCR V_β domain; or (c) a TCR V_α domain having the CDR3 amino acid sequence shown in any one of SEQ ID NOS:12-22, 178, 184, 190, 196, 202, 208, 214, 220, 226, 232, and 238, and a TCR V_β domain having the CDR3 amino acid sequence shown in any one of SEQ ID NOS:1-11, 181, 187, 193, 199, 205, 211, 217, 223, 229, 235, and
 30 241. For example, any of the TCRs, or binding domains thereof, of this disclosure can specifically bind to a WT1 p37 peptide:HLA complex on a cell (*e.g.*, T cell) surface

and/or can promote IFN γ production pEC₅₀ of 8.5 or higher (*e.g.*, up to about 8.6, up to about 8.65, up to about 8.7, up to about 8.72, up to about 8.75, up to about 8.8, up to about 9, up to about 9.1, up to about 9.2, up to up to about 9.3, up to about 9.4, about 9.5, up to about 9.6, up to about 9.68 up to about 9.7, up to about 9.75, up to about 10, up to about 10.5, up to about 11, up to about 11.5, up to about 12, up to about 12.5, or up to about 13). In certain embodiments, a TCR of the present disclosure can specifically bind to a VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex with an IFN γ production pEC₅₀ of 9.0 or higher, or with an IFN γ production pEC₅₀ of 9.0 or higher. In certain embodiments, a TCR, or a binding domain thereof (*e.g.*, scTCR or a fusion protein thereof), of this disclosure can specifically bind to a WT1 p37 peptide:HLA complex and promote IFN γ production at a pEC₅₀ ranging from 8.5 to about 9.9, or from 8.6 to about 9.8, or from 8.7 to about 9.7, or from 8.75 to about 9.65, or the like. The EC₅₀ can range from about 1.1×10^{-9} M to about 3.0×10^{-10} M, or any value in between. In further examples, any of the TCRs of this disclosure can specifically bind to a WT1 peptide:HLA complex on a cell surface independent of CD8 or in the absence of CD8. In further embodiments, a TCR specifically binds to a VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex with a K_D of less than or equal to about 10^{-9} M. In certain embodiments, the HLA comprises HLA-A*201. The peptide antigen VLDFAPPGA (SEQ ID NO:59) is a WT1 peptide antigen and corresponds to amino acids 37-45 of the WT1 protein.

In any of the embodiments described herein, the present disclosure provides a T cell receptor (TCR) comprising an α -chain and a β -chain, wherein the TCR binds to a WT1:HLA-A*201 complex on a T cell surface and promotes (a) an IFN γ production pEC₅₀ of 8.5 or higher (*e.g.*, up to about 9, up to about 9.5, up to about 10, about 10.5, about 11, about 11.5, about 12, about 12.5, or about 13); or (b) binds a cell surface independent or in the absence of CD8.

In certain embodiments, a V β domain comprises or is derived from a TRBV7-6*01 / TRBJ2-7*01, TRBV20-1*02 / TRBJ2-7*01, TRBV15*02 / TRBJ1-5*01, TRBV13*01 / TRBJ2-5*01, TRAJ50*01 / TRBJ2-7*01, TRBV11-3*01 / TRBJ1-1*01, TRBV19*01 / TRBJ1-6*02, TRBV27*01 / TRBJ2-7*01, TRBV13*01 / TRBJ2-7*01, TRBV11-1*01 / TRBJ1 4*01, or TRBV4-3*01 / TRBJ1-3*01. In further embodiments,

a V_{α} domain comprises or is derived from a TRAV21*02 / TRAJ58*01, TRAV38-1*01 / TRAJ40*01, TRAV29/DV5*01 / TRAJ6*01, TRAV29/DV5*01 / TRAJ20*01, TRAV41*01 / TRAJ50*01, TRAV12-2*01 / TRAJ11*01, TRAV1-2*01 / TRAJ20*01, TRAV20*02 / TRAJ8*01, TRAV26-1*02 / TRAJ26*01, TRAV24*01 / TRAJ48*01, or
 5 TRAV20*02 / TRAJ37*02. In particular embodiments, a TCR comprises (a) a V_{β} domain comprising or derived from TRBV7-6*01 / TRBJ2-7*01 and a V_{α} domain comprises or is derived from a TRAV21*02 / TRAJ58*01; (b) a V_{β} domain comprises or is derived from a TRBV27*01 / TRBJ2-7*01 and a V_{α} domain comprises or is derived from a TRAV20*02 / TRAJ8*01; or (c) a V_{β} domain comprises or is derived
 10 from a TRBV13*01 / TRBJ2-5*01 and a V_{α} domain comprises or is derived from a TRAV29/DV5*01 / TRAJ20*01.

In certain embodiments, a TCR of the present disclosure further comprises: (i) the CDR1 α amino acid sequence set forth in any one of SEQ ID NOs.:194, 176, 182, 188, 200, 206, 212, 218, 224, 230, and 236, or a variant thereof comprising one or two
 15 amino acid substitutions, wherein, optionally, the one or two amino acid substitutions comprise a conservative amino acid substitution; and/or (ii) the CDR2 α amino acid sequence set forth in any one of SEQ ID NOs.:195, 177, 183, 189, 201, 207, 213, 219, 225, 231, and 237, or a variant thereof comprising one or two amino acid substitutions, wherein, optionally, the one or two amino acid substitutions comprise a conservative
 20 amino acid substitution.

In certain embodiments, a TCR of the present disclosure further comprises: (i) the CDR1 β amino acid sequence set forth in any one of SEQ ID NOs.: 197, 179, 185, 191, 197, 203, 209, 215, 221, 227, 233, and 239, or a variant thereof comprising one or two amino acid substitutions, wherein, optionally, the one or two amino acid
 25 substitutions comprise a conservative amino acid substitution; and/or (ii) the CDR2 β amino acid sequence set forth in any one of SEQ ID NOs.:198, 180, 186, 192, 204, 210, 216, 222, 228, 234, and 240, or a variant thereof comprising one or two amino acid substitutions, wherein, optionally, the one or two amino acid substitutions comprise a conservative amino acid substitution.

30 In certain embodiments, a TCR of the present disclosure comprises the CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences set forth in: (i)

SEQ ID NOs. 194, 195, 196 or 12, 197, 198, and 199 or 1, respectively; (ii) SEQ ID NOs.: 176, 177, 178 or 18, 179, 180, and 181 or 7, respectively; (iii) SEQ ID NOs.: 182, 183, 184 or 20, 185, 186, and 187 or 9, respectively; (iv) SEQ ID NOs.: 188, 189, 190 or 21, 191, 192, and 193 or 10, respectively; (v) SEQ ID NOs.: 200, 201, 202 or 13, 203, 204, and 205 or 2, respectively; (vi) SEQ ID NOs.: 206, 207, 208 or 14, 209, 210, and 211 or 3, respectively; (vii) SEQ ID NOs.: 212, 213, 214 or 15, 215, 216, and 217 or 4, respectively; (viii) SEQ ID NOs.: 218, 219, 220 or 17, 221, 222, and 223 or 6, respectively; (ix) SEQ ID NOs.: 224, 225, 226 or 19, 227, 228, and 229 or 8, respectively; (x) SEQ ID NOs.: 230, 231, 232 or 22, 233, 234, and 235 or 11, respectively; or (xi) SEQ ID NOs.: 236, 237, 238 or 16, 238, 240, and 241 or 5, respectively.

Any polypeptide of this disclosure can, as encoded by a polynucleotide sequence, comprise a "signal peptide" (also known as a leader sequence, leader peptide, or transit peptide). Signal peptides target newly synthesized polypeptides to their appropriate location inside or outside the cell. A signal peptide may be removed from the polypeptide during or once localization or secretion is completed. Polypeptides that have a signal peptide are referred to herein as a "pre-protein" and polypeptides having their signal peptide removed are referred to herein as "mature" proteins or polypeptides. In any of the herein disclosed embodiments, a binding protein or fusion protein comprises, or is, a mature protein, or is or comprises a pre-protein.

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:23 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:23 with amino acid residues 1-19 of SEQ ID NO.:23 removed (i.e., the TCR V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:242).

In certain embodiments, amino acid residues 1-15 of SEQ ID NO.:24 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:23 with amino acid residues 1-15 of SEQ ID NO.:24 removed (i.e., the TCR V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:243).

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:25 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:25 with amino acid residues 1-15 of SEQ ID NO.:25 removed (i.e., the TCR V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:244).

In certain embodiments, amino acid residues 1-29 of SEQ ID NO.:26 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:26 with amino acid residues 1-29 of SEQ ID NO.:26 removed (i.e., the TCR V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:245).

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:27 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:27 with amino acid residues 1-19 of SEQ ID NO.:27 removed (i.e., the TCR V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:246).

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:28 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:28 with amino acid residues 1-19 of SEQ ID NO.:28 removed (i.e., the TCR V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:247).

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:29 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:29 with amino acid residues 1-19 of SEQ ID NO.:29 removed (i.e., the TCR V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:248).

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:30 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:30 with amino acid residues 1-19 of SEQ ID NO.:30 removed (i.e., the TCR V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:249).

In certain embodiments, amino acid residues 1-29 of SEQ ID NO.:31 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:31 with amino acid residues 1-29 of SEQ ID NO.:31 removed (i.e., the TCR V β domain
5 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:250).

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:32 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:32 with amino acid residues 1-19 of SEQ ID NO.:32 removed (i.e., the TCR V β domain
10 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:251).

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:33 are or comprise a signal peptide. In some embodiments, a TCR V β domain is a mature TCR V β domain and comprises or consists of the amino acid sequence of SEQ ID NO.:33 with amino acid residues 1-19 of SEQ ID NO.:33 removed (i.e., the TCR V β domain
15 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:252).

In certain embodiments, amino acid residues 1-19 of SEQ ID NO.:34 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:34 with amino acid residues 1-19 of SEQ ID NO.:34 removed (i.e., the TCR V α domain
20 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:253).

In certain embodiments, amino acid residues 1-20 of SEQ ID NO.:35 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:35 with amino acid residues 1-20 of SEQ ID NO.:35 removed (i.e., the TCR V α domain
25 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:254).

In certain embodiments, amino acid residues 1-26 of SEQ ID NO.:36 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:36 with amino acid residues 1-26 of SEQ ID NO.:36 removed (i.e., the TCR V α domain
30 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:255).

In certain embodiments, amino acid residues 1-26 of SEQ ID NO.:37 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:37 with amino acid residues 1-26 of SEQ ID NO.:37 removed (i.e., the TCR V α domain
5 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:256).

In certain embodiments, amino acid residues 1-22 of SEQ ID NO.:38 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:38 with amino acid residues 1-22 of SEQ ID NO.:38 removed (i.e., the TCR V α domain
10 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:257).

In certain embodiments, amino acid residues 1-21 of SEQ ID NO.:39 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:39 with amino acid residues 1-21 of SEQ ID NO.:39 removed (i.e., the TCR V α domain
15 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:258).

In certain embodiments, amino acid residues 1-17 of SEQ ID NO.:40 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:40 with amino acid residues 1-17 of SEQ ID NO.:40 removed (i.e., the TCR V α domain
20 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:259).

In certain embodiments, amino acid residues 1-21 of SEQ ID NO.:41 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:41 with amino acid residues 1-21 of SEQ ID NO.:41 removed (i.e., the TCR V α domain
25 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:260).

In certain embodiments, amino acid residues 1-17 of SEQ ID NO.:42 are or comprise a signal peptide. In some embodiments, a TCR V α domain is a mature TCR V α domain and comprises or consists of the amino acid sequence of SEQ ID NO.:42 with amino acid residues 1-17 of SEQ ID NO.:42 removed (i.e., the TCR V α domain
30 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:261).

In certain embodiments, amino acid residues 1-22 of SEQ ID NO.:43 are or comprise a signal peptide. In some embodiments, a TCR V_{α} domain is a mature TCR V_{α} domain and comprises or consists of the amino acid sequence of SEQ ID NO.:43 with amino acid residues 1-22 of SEQ ID NO.:43 removed (i.e., the TCR V_{α} domain
5 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:262).

In certain embodiments, amino acid residues 1-21 of SEQ ID NO.:44 are or comprise a signal peptide. In some embodiments, a TCR V_{α} domain is a mature TCR V_{α} domain and comprises or consists of the amino acid sequence of SEQ ID NO.:44 with amino acid residues 1-21 of SEQ ID NO.:44 removed (i.e., the TCR V_{α} domain
10 comprises or consists of the amino acid sequence set forth in SEQ ID NO.:263).

In certain embodiments, a T cell receptor (TCR) specific for a WT1 peptide:HLA complex has a V_{α} domain that comprises or consists of the amino acid sequence as set forth in any one of SEQ ID NOS:253-263 and 34-33, has a V_{β} domain that comprises or consists of the amino acid sequence as set forth in any one of SEQ ID
15 NOS:242-252 and 23-33, or any combination thereof. In particular embodiments, a V_{α} domain comprises or consists of the amino acid sequence of SEQ ID NO:34 and a V_{β} domain comprises or consists of the amino acid sequence of SEQ ID NO:23. In further particular embodiments, (a) a V_{α} domain comprises or consists of the amino acid sequence of SEQ ID NO:41 and a V_{β} domain comprises or consists of the amino acid
20 sequence of SEQ ID NO:30; (b) a V_{α} domain comprises or consists of the amino acid sequence of SEQ ID NO:37 and a V_{β} domain comprises or consists of the amino acid sequence of SEQ ID NO:26; or (c) a V_{α} domain comprises or consists of the amino acid sequence of SEQ ID NO:42 and a V_{β} domain comprises or consists of the amino acid sequence of SEQ ID NO:31. In further particular embodiments, a V_{α} domain comprises
25 or consists of the amino acid sequence of SEQ ID NO:24 and a V_{β} domain comprises or consists of the amino acid sequence of SEQ ID NO:35.

In some embodiments, the V_{α} domain and the V_{β} domain comprise or consist of the amino acid sequences set forth in SEQ ID NOS.: (i) 253 and 242, respectively; (ii) 259 and 248, respectively; (iii) 261 and 250, respectively; (iv) 262 and 251,
30 respectively; (v) 257 and 246, respectively; (vi) 254 and 243, respectively; (vii) 255 and 244, respectively; (viii) 256 and 245, respectively; (ix) 258 and 247, respectively; (x)

260 and 249, respectively; (xi) 263 and 252, respectively; (xii) 34 and 23, respectively; (xiii) 40 and 29, respectively; (xiv) 42 and 31, respectively; (xv) 43 and 32, respectively; (xvi) 35 and 24, respectively; (xvii) 36 and 25, respectively; (xviii) 37 and 26, respectively; (xix) 39 and 28, respectively; (xx) 41 and 30, respectively; (xxi) 44 and 33, respectively; or (xxii) 38 and 27, respectively.

In certain embodiments, a high functional avidity recombinant TCR specific for WT1 p37 peptide as described herein includes variant polypeptide species that have one or more amino acid substitutions, insertions, or deletions in the amino acid sequence relative to the amino acid sequences of any one or more of SEQ ID NOS:48-58, as presented herein, provided that the CDR3s are not changed and the TCR retains or substantially retains its specific WT1 p37 binding function.

Conservative substitutions of amino acids are well known and may occur naturally or may be introduced when the 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*).

A variety of criteria known to persons skilled in the art indicate whether an amino acid that is substituted at a particular position in a peptide or polypeptide is conservative (or similar). For example, a similar amino acid or a conservative amino acid substitution is one in which an amino acid residue is replaced with an amino acid residue having a similar side chain. Similar amino acids may be included in the following categories: amino acids with basic side chains (*e.g., lysine, arginine, histidine*); amino acids with acidic side chains (*e.g., aspartic acid, glutamic acid*); amino acids with uncharged polar side chains (*e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, histidine*); amino acids with nonpolar side chains (*e.g.,*

alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); amino acids with beta-branched side chains (*e.g.*, threonine, valine, isoleucine), and amino acids with aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan). Proline, which is considered more difficult to classify, shares properties with amino acids that have aliphatic side chains (*e.g.*, leucine, valine, isoleucine, and alanine). In certain circumstances, substitution of glutamine for glutamic acid or asparagine for aspartic acid may be considered a similar substitution in that glutamine and asparagine are amide derivatives of glutamic acid and aspartic acid, respectively. As understood in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and conserved amino acid substitutes thereto of the polypeptide to the sequence of a second polypeptide (*e.g.*, using GENEWORKS, Align, the BLAST algorithm, or other algorithms described herein and practiced in the art).

Variants of a wild-type TCR, or a binding domain thereof, specific for WT1 p37 antigen:MHC complex may include a TCR that has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% amino acid sequence identity to any of the exemplary amino acid sequences disclosed herein (*e.g.*, SEQ ID NOS:23-58), provided that neither the CDR3 of the V_{β} domain nor the CDR3 of the V_{α} domain contain an alteration, and the alterations to the other portions do not reduce the functional avidity (or relative affinity) any more than 10%, 15%, or 20% as compared to the wild-type TCR. In some optional embodiments, a variant TCR further comprises no change in amino acid sequence of the V_{α} domain CDR1, the V_{α} domain CDR2, the V_{β} domain CDR1, the V_{β} domain CDR2, or any combination thereof, as set forth in any one of SEQ ID NOS:34-44 (parental V_{α} domain) or as set forth in any one of SEQ ID NOS:23-33 (parental V_{β} domain). In each of these embodiments, the TCR retains its ability to specifically induce IFN γ production at a pEC₅₀ of 8.5, 8.6, 8.7, 8.8, 8.9 or higher, or the TCR retains its ability to specifically bind to a peptide antigen:HLA complex (*e.g.*, VLDFAPPGA (SEQ ID NO:59):HLA complex) with a K_D of less than or equal to about 10⁻⁹ M, and specifically binds 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.3-fold, 3.5-fold, up to 5-fold better than the wild-type TCR consisting of any one of SEQ ID NOS:48-58.

In further embodiments, the present disclosure provides a p37-specific TCR, or a binding domain thereof, comprising (a) a TCR α -chain variable (V_α) domain having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:34-35 and 38-44, and a TCR β -chain variable (V_β) domain having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:23-25, 27, 28, 30, 32, and 33; (b) a TCR V_α domain has at least 92% sequence identity to the amino acid sequence of SEQ ID NO:36 or 37, and a TCR V_β domain having at least 90% *e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence as set forth in any one of SEQ ID NOS:23-25, 27, 28, 30, 32, and 33; or (c) a TCR V_α domain comprising or consisting of an amino acid sequence of SEQ ID NOS:34-44, and a TCR V_β domain having at least 90% sequence identity to the amino acid sequence as set forth in any one of SEQ ID NOS:23-25, 27, 28, 30, 32, and 33.

In still further embodiments, the present disclosure provides a p37-specific TCR, or a binding domain thereof, comprising (a) a TCR V_α domain having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:34-35 and 38-44, and a V_β domain having at least 92% (*e.g.*, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of SEQ ID NO:29; (b) a TCR V_α domain has at least 92% sequence identity to the amino acid sequence of SEQ ID NO:36 or 37, and a TCR V_β domain having at least 92% sequence identity to the amino acid sequence of SEQ ID NO:29; or (c) a TCR V_α domain comprising or consisting of an amino acid sequence of SEQ ID NOS:34-44, and a TCR V_β domain having at least 92% sequence identity to the amino acid sequence of SEQ ID NO:29.

In yet further embodiments, the present disclosure provides a p37-specific TCR, or a binding domain thereof, comprising (a) a TCR V_α domain having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:34-35 and 38-44, and a V_β domain having at least 93% sequence identity to the amino acid sequence of SEQ ID NO:31; (b) a TCR V_α domain has at least 92% sequence identity to the amino acid sequence of SEQ ID NO:36 or 37, and a TCR V_β domain having at least 93% sequence identity to the amino acid sequence of SEQ ID NO:31; or (c) a TCR V_α

domain comprising or consisting of an amino acid sequence of SEQ ID NOS:34-44, and a TCR V β domain having at least 93% sequence identity to the amino acid sequence of SEQ ID NO:31.

In more embodiments, the present disclosure provides a p37-specific TCR, or a
5 binding domain thereof, comprising (a) a TCR V α domain having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:34-35 and 38-44, and a V β domain having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:26; (b) a TCR V α domain has at least 92% sequence identity to the amino acid sequence of SEQ ID NO:36 or 37, and a TCR V β domain having at least
10 95% sequence identity to the amino acid sequence of SEQ ID NO:26; or (c) a TCR V α domain comprising or consisting of an amino acid sequence of SEQ ID NOS:34-44, and a TCR V β domain having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:26.

In still more embodiments, the present disclosure provides a p37-specific TCR,
15 or a binding domain thereof, comprising (a) a TCR V α domain having at least 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:34-35 and 38-44, and a V β domain comprising or consisting of the amino acid sequence set forth in any one of SEQ ID NOS:23-33; (b) a TCR V α domain has at least 92% sequence identity to the amino acid sequence of SEQ ID NO:36 or 37, and a TCR V β
20 domain comprising or consisting of the amino acid sequence set forth in any one of SEQ ID NOS:23-33; or (c) a TCR V α domain comprising or consisting of an amino acid sequence of SEQ ID NOS:34-44, and a TCR V β domain comprising or consisting of the amino acid sequence set forth in any one of SEQ ID NOS:23-33.

In any of the aforementioned embodiments, the TCR has the ability to bind to a
25 cell (*e.g.*, T cell) surface WT1 p37 peptide VLDFAPPGA (SEQ ID NO:59):HLA complex and specifically induce IFN γ production at a pEC₅₀ of 8.5, 8.6, 8.7, 8.8, 8.9, or higher, and/or the TCR is capable of specifically binding to a WT1 peptide VLDFAPPGA (SEQ ID NO:59):HLA cell surface complex independent, or in the absence, of CD8. In any of the aforementioned embodiments, the V β domain comprises
30 no change in the amino acid sequence of CDR1 and/or CDR2 as compared to the CDR1 and/or CDR2, respectively, present in any one of SEQ ID NOS:23-33.

In certain embodiments, any of the aforementioned WT1 p37 peptide-specific T cell receptors (TCRs) can be an antigen-binding fragment of a TCR. In further embodiments, an antigen-binding fragment of the TCR comprises a single chain TCR (scTCR), which can be contained in a chimeric antigen receptor (CAR). In some
5 embodiments, a WT1 p37 peptide-specific TCR is a multi-chain binding protein, for example, comprising a TCR α -chain comprising a V_α domain and an α -chain constant domain, wherein the TCR α -chain constant domain has at least about 90% sequence identity (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to the amino acid sequence of SEQ ID NO:47; and a TCR β -chain comprising a V_β domain
10 and a β -chain constant domain, wherein the TCR β -chain constant domain has at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of SEQ ID NO:45 or 46. In further embodiments, the present disclosure provides a WT1 p37 peptide-specific TCR comprising or consisting of an α -chain constant domain having the amino acid sequence
15 of SEQ ID NO:47, and/or comprising or consisting of a β -chain constant domain having the amino acid sequence of SEQ ID NO:45 or 46.

In further embodiments, the present disclosure provides a WT1 p37 peptide-specific TCR comprising a TCR α -chain comprising a V_α domain and an α -chain constant domain, wherein: (a) the V_α domain has at least 90% sequence identity
20 (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to the amino acid sequence set forth in any one of SEQ ID NOS:34-35 and 38-44, and the α -chain constant domain has at least about 98% sequence identity to the amino acid sequence of SEQ ID NO:47; or (b) the V_α domain has at least 92% sequence identity to the amino acid sequence of SEQ ID NO:36 or 37, and the α -chain constant domain has at least
25 98% sequence identity to the amino acid sequence of SEQ ID NO:47.

In some embodiments, the TCR comprises a TCR α -chain comprising a V_α domain and an α -chain constant domain, wherein: (a) the V_α domain comprises the amino acid sequence set forth in any one of SEQ ID NOS: 242-252 and 34-44, and the α -chain constant domain comprises the amino acid sequence of SEQ ID NO:47; or (b)
30 the V_α domain consists of the amino acid sequence set forth in any one of SEQ ID NOS: 242-252 and 34-44, and the α -chain constant domain has at least 90% identity

(*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to, comprises, or consists of the amino acid sequence of SEQ ID NO:47.

In some embodiments, the α -chain constant domain is present and the V α domain and the α -chain constant domain together form a TCR α -chain. In some
5 embodiments, the β -chain constant domain is present and the V β domain and the β -chain constant domain together form a TCR β -chain.

In some embodiments, the TCR comprises a scTCR, or an scTCR is provided which is derived from a presently disclosed TCR. In some embodiments, the TCR comprises a CAR, or a CAR is provided which is derived from (*e.g.*, includes one or
10 more variable domains from) a presently disclosed TCR.

In more embodiments, there is provided a composition comprising a WT1-specific high functional avidity recombinant TCR, or binding domain thereof, according to any one of the aforementioned embodiments and a pharmaceutically acceptable carrier, diluent, or excipient.

15 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 a 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
20 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
25 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 high affinity or high
30 functional avidity TCR specific for WT1 p37 peptide complexed with MHC were 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

5 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.*,

10 *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 WT1 peptide antigens complexed with an HLA receptor.

15 The WT1-specific TCRs, or binding domains thereof, as described herein (*e.g.*, SEQ ID NOS:23-58, and non-CDR3 variants thereof), 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.

20 Examples include determination of T cell proliferation, T cell cytokine release, antigen-specific T cell stimulation, MHC restricted T cell stimulation, cytotoxic T lymphocyte (CTL) activity (*e.g.*, by detecting ⁵¹Cr 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

25 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

30 therein.

Polynucleotides Encoding TCRs Specific for WT1 p37 Antigen Peptides

Heterologous, isolated or recombinant nucleic acid molecules encoding a high affinity or high functional avidity recombinant T cell receptor (TCR), or binding domain thereof (*e.g.*, scTCR or fusion protein thereof) specific for WT1 p37 peptide as described herein may be produced and prepared according to various methods and techniques described herein (*see* Examples). Construction of an expression vector that is used for recombinantly producing a high affinity or high functional avidity engineered TCR or binding domain thereof specific for a WT1 p37 peptide of interest can be accomplished by using any suitable molecular biology engineering techniques known in the art, 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 (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 immunogen.

Certain embodiments relate to nucleic acids that encode the polypeptides contemplated herein, for instance, high affinity or high functional avidity engineered TCRs or binding domain thereof specific for WT1 p37 peptide::MHC complex. As one of skill in the art will recognize, a nucleic acid may refer to 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.

In certain embodiments, provided herein are isolated polynucleotides that encode an engineered (*e.g.*, codon optimized) high functional avidity TCR or binding domain thereof of this disclosure specific for a WT1 p37 peptide, wherein a V α domain can be encoded by a polynucleotide that is at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99%, 99.9%, or 100% identical to the nucleotide sequence set forth in any one of SEQ ID NOS:97, 98, and 101-107. In particular embodiments, a polynucleotide encodes a V_{α} domain that comprises or consists of the nucleotide sequence set forth in any one of SEQ ID NO:97-107. In further embodiments, provided herein are

5 polynucleotides that encode a high functional avidity engineered TCR or binding domain thereof of this disclosure specific for a WT1 p37 peptide, wherein a V_{β} domain is encoded by a polynucleotide that is at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% identical to the nucleotide sequence set forth in any

10 one of SEQ ID NOS:75-77, 79, 82, 84, and 85. In particular embodiments, a V_{β} domain is encoded by a polynucleotide that comprises or consists of the nucleotide sequence as set forth in any one of SEQ ID NOS:75-85.

In some embodiments, a TCR, or a binding domain thereof, provided herein comprises a V_{α} domain encoded by a polynucleotide that has at least 75% (75%, 76%,

15 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100%) sequence identity to the polynucleotide sequence set forth in any one of SEQ ID NOS:97, 98, and 101-107, or a V_{α} domain encoded by a polynucleotide that has at least 94% sequence identity to the polynucleotide sequence of SEQ ID NO:99 or 100, or a V_{α} domain encoded by a

20 polynucleotide that comprises or consists of a sequence set forth in any one of SEQ ID NOS:97-107; and a V_{β} domain encoded by a polynucleotide that has at least 75% sequence identity to the polynucleotide sequence set forth in any one of SEQ ID NOS:75-77, 79, 82, 84 and 85, or a V_{β} domain encoded by a polynucleotide that has at least 95% sequence identity to the polynucleotide sequence set forth in any one of SEQ

25 ID NOS:78, 80, 81 and 83, or a V_{β} domain encoded by the polynucleotide that comprises or consists of the nucleotide sequence as set forth in any one of SEQ ID NOS:75-85.

In any of the aforementioned embodiments, a polynucleotide encoding a V_{α} domain, V_{β} domain, or both, may further encode an α -chain constant domain or a β -chain constant domain, respectively. In certain embodiments, a TCR of this

30 disclosure comprises a TCR α -chain constant domain, wherein the α -chain constant domain is encoded by a polynucleotide comprising at least 98% to 100% sequence

identity to SEQ ID NO:110. In particular embodiments, an α -chain constant domain is encoded by a polynucleotide that comprises or consists of the nucleotide sequence of SEQ ID NO:110. In further embodiments, provided herein a β -chain constant domain encoded by a polynucleotide at least 99.9% to 100% sequence identity to SEQ ID NO:108 or 109. In particular embodiments, a β -chain constant domain is encoded by a polynucleotide that comprises or consists of the nucleotide sequence of SEQ ID NO:108 or 109.

In any of the aforementioned embodiments, a polynucleotide encoding a TCR comprises a TCR α -chain, a TCR β -chain, or both. In certain embodiments, a TCR of this disclosure is encoded by a polynucleotide comprising a nucleotide sequence encoding a self-cleaving peptide disposed between the polynucleotide sequence encoding the TCR α -chain and the polynucleotide sequence encoding the TCR β -chain. Exemplary self-cleaving peptides comprise an amino acid sequence of any one of SEQ ID NOS:60-63; or consist of an amino acid sequence of any one of SEQ ID NOS:60-63. Such self-cleaving peptides can be encoded by a polynucleotide comprising a polynucleotide sequence of any one of SEQ ID NOS:166-170; or encoded by a polynucleotide consisting of a polynucleotide sequence of any one of SEQ ID NOS:166-170.

In certain embodiments, a TCR α -chain, self-cleaving peptide, and TCR β -chain are encoded by a polynucleotide comprising at least 95% (*e.g.*, 95%, 96%, 97%, 98%, 99%, or 100%) identity to any one of SEQ ID NOS:155-165. In further embodiments, a TCR α -chain, self-cleaving peptide, and TCR β -chain are encoded by a polynucleotide comprising a polynucleotide sequence of any one of SEQ ID NOS:155-165; or encoded by a polynucleotide consisting of a sequence of any one of the polynucleotides of SEQ ID NOS:155-165. In still further embodiments, the encoded TCR α -chain, self-cleaving peptide, and TCR β -chain comprise an amino acid sequence having at least 95% (*e.g.*, 95%, 96%, 97%, 98%, 99%, or 100%) identity to any one of the polypeptides of SEQ ID NOS: 48-58, or the encoded TCR α -chain, self-cleaving peptide, and TCR β -chain comprise or consist of an amino acid sequence of any one of SEQ ID NOS: 48-58.

In any of the presently disclosed embodiments, a polynucleotide encoding a binding protein can further comprise: (i) a polynucleotide encoding a polypeptide that

comprises an extracellular portion of a CD8 co-receptor α chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain; (ii) a polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; or (iii) a polynucleotide of (i) and a polynucleotide of (ii). Without being bound by theory, in certain embodiments, co-expression or concurrent expression of a binding protein and a CD8 co-receptor protein or portion thereof functional to bind to an HLA molecule may improve one or more desired activity of a host cell (*e.g.*, immune cell, such as a T cell, optionally a CD4⁺ T cell) as compared to expression of the binding protein alone. It will be understood that the binding protein-encoding polynucleotide and the CD8 co-receptor polypeptide-encoding polynucleotide may be present on a single nucleic acid molecule (*e.g.*, in a same expression vector), or may be present on separate nucleic acid molecules in a host cell.

In certain further embodiments, a polynucleotide comprises: (a) the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain; (b) the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain; and (c) a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide of (a) and the polynucleotide of (b). In further embodiments, a polynucleotide comprises a polynucleotide that encodes a self-cleaving peptide and is disposed between: (1) the polynucleotide encoding a binding protein (*e.g.*, TCR of the present disclosure) and the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain; and/or (2) the polynucleotide encoding a binding protein and the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain.

In still further embodiments, a polynucleotide can comprise, operably linked in-frame: (i) (pnCD8 α)-(pnSCP1)-(pnCD8 β)-(pnSCP2)-(pnTCR); (ii) (pnCD8 β)-(pnSCP1)-(pnCD8 α)-(pnSCP2)-(pnTCR); (iii) (pnTCR)-(pnSCP1)-(pnCD8 α)-(pnSCP2)-(pnCD8 β); (iv) (pnTCR)-(pnSCP1)-(pnCD8 β)-(pnSCP2)-(pnCD8 α); (v) (pnCD8 α)-(pnSCP1)-(pnTCR)-(pnSCP2)-(pnCD8 β); or (vi) (pnCD8 β)-(pnSCP1)-(pnTCR)-(pnSCP2)-(pnCD8 α), wherein pnCD8 α is the polynucleotide encoding a

polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein pnCD8 β is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein pnTCR is the polynucleotide encoding a TCR, and wherein pnSCP1 and pnSCP2 are each

5 independently a polynucleotide encoding a self-cleaving peptide, wherein the polynucleotides and/or the encoded self-cleaving peptides are optionally the same or different (*e.g.*, P2A, T2A, F2A, E2A).

In some embodiments, the encoded TCR comprises a TCR α chain and a TCR β chain, wherein the polynucleotide comprises a polynucleotide encoding a self-cleaving

10 peptide disposed between the polynucleotide encoding a TCR α chain and the polynucleotide encoding a TCR β chain. In some embodiments, the polynucleotide comprises, operably linked in-frame: (i) (pnCD8 α)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnTCR β)-(pnSCP₃)-(pnTCR α); (ii) (pnCD8 β)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnTCR β)-(pnSCP₃)-(pnTCR α); (iii) (pnCD8 α)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnTCR α)-(pnSCP₃)-(pnTCR β); (iv) (pnCD8 β)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnTCR α)-(pnSCP₃)-(pnTCR β); (v) (pnTCR β)-(pnSCP₁)-(pnTCR α)-(pnSCP₂)-(pnCD8 α)-(pnSCP₃)-(pnCD8 β); (vi) (pnTCR β)-(pnSCP₁)-(pnTCR α)-(pnSCP₂)-(pnCD8 β)-(pnSCP₃)-(pnCD8 α); (vii) (pnTCR α)-(pnSCP₁)-(pnTCR β)-(pnSCP₂)-(pnCD8 α)-(pnSCP₃)-(pnCD8 β); or (viii) (pnTCR α)-(pnSCP₁)-(pnTCR β)-(pnSCP₂)-(pnCD8 β)-(pnSCP₃)-(pnCD8 α), wherein pnCD8 α is the polynucleotide encoding a

15 polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein pnCD8 β is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein pnTCR α is the polynucleotide encoding a TCR α chain, wherein pnTCR β is the polynucleotide

20 encoding a TCR β chain, and wherein pnSCP₁, pnSCP₂, and pnSCP₃ are each independently a polynucleotide encoding a self-cleaving peptide, wherein the polynucleotides and/or the encoded self-cleaving peptides are optionally the same or different.

In further embodiments, a binding protein is expressed as part of a transgene

30 construct that encodes, and/or a host cell of the present disclosure can encode: one or more additional accessory protein, such as a safety switch protein; a tag, a selection

marker; a CD8 co-receptor β -chain; a CD8 co-receptor α -chain or both; or any combination thereof. Polynucleotides and transgene constructs useful for encoding and expressing binding proteins and accessory components (*e.g.*, one or more of a safety switch protein, a selection marker, CD8 co-receptor β -chain, or a CD8 co-receptor α -chain) are described in PCT application PCT/US2017/053112, the polynucleotides, transgene constructs, and accessory components, including the nucleotide and amino acid sequences, of which are hereby incorporated by reference. It will be understood that any or all of a binding protein of the present disclosure, a safety switch protein, a tag, a selection marker, a CD8 co-receptor β -chain, or a CD8 co-receptor α -chain may be encoded by a single nucleic acid molecule or may be encoded by polynucleotide sequences that are, or are present on, separate nucleic acid molecules.

Exemplary safety switch proteins include, for example, a truncated EGF receptor polypeptide (huEGFRt) that is devoid of extracellular N-terminal ligand binding domains and intracellular receptor tyrosine kinase activity, but that retains its native amino acid sequence, has type I transmembrane cell surface localization, and has a conformationally intact binding epitope for pharmaceutical-grade anti-EGFR monoclonal antibody, cetuximab (Erbix) tEGF receptor (tEGFr; Wang *et al.*, *Blood* 118:1255-1263, 2011); a caspase polypeptide (*e.g.*, iCasp9; Straathof *et al.*, *Blood* 105:4247-4254, 2005; Di Stasi *et al.*, *N. Engl. J. Med.* 365:1673-1683, 2011; Zhou and Brenner, *Exp. Hematol.* pii:S0301-472X(16)30513-6. doi:10.1016/j.exphem.2016.07.011), RQR8 (Philip *et al.*, *Blood* 124:1277-1287, 2014); a 10-amino-acid tag derived from the human c-myc protein (Myc) (Kieback *et al.*, *Proc. Natl. Acad. Sci. USA* 105:623-628, 2008); and a marker/safety switch polypeptide, such as RQR (CD20 + CD34; Philip *et al.*, 2014).

Other accessory components useful for modified host cells of the present disclosure comprise a tag or selection marker that allows the cells to be identified, sorted, isolated, enriched, or tracked. For example, marked host cells having desired characteristics (*e.g.*, an antigen-specific TCR and a safety switch protein) can be sorted away from unmarked cells in a sample and more efficiently activated and expanded for inclusion in a product of desired purity.

As used herein, the term "selection marker" comprises a nucleic acid construct (and the encoded gene product) that confers an identifiable change to a cell permitting detection and positive selection of immune cells transduced with a polynucleotide comprising a selection marker. RQR is a selection marker that comprises a major
5 extracellular loop of CD20 and two minimal CD34 binding sites. In some embodiments, an RQR-encoding polynucleotide comprises a polynucleotide that encodes the 16-amino-acid CD34 minimal epitope. In some embodiments, the CD34 minimal epitope is incorporated at the amino terminal position of a CD8 co-receptor stalk domain (Q8). In further embodiments, the CD34 minimal binding site sequence
10 can be combined with a target epitope for CD20 to form a compact marker/suicide gene for T cells (RQR8) (Philip *et al.*, 2014, incorporated by reference herein). This construct allows for the selection of host cells expressing the construct, with for example, CD34 specific antibody bound to magnetic beads (Miltenyi) and that utilizes clinically accepted pharmaceutical antibody, rituximab, that allows for the selective
15 deletion of a transgene expressing engineered T cell (Philip *et al.*, 2014). Further exemplary selection markers also include several truncated type I transmembrane proteins normally not expressed on T cells: the truncated low-affinity nerve growth factor, truncated CD19, and truncated CD34 (see for example, Di Stasi *et al.*, *N. Engl. J. Med.* 365:1673-1683, 2011; Mavilio *et al.*, *Blood* 83:1988-1997, 1994; Fehse *et al.*, *Mol. Ther.* 1:448-456, 2000; each incorporated herein in their entirety).
20 A useful feature of CD19 and CD34 is the availability of the off-the-shelf Miltenyi CliniMACs™ selection system that can target these markers for clinical-grade sorting. However, CD19 and CD34 are relatively large surface proteins that may tax the vector packaging capacity and transcriptional efficiency of an integrating vector. Surface
25 markers containing the extracellular, non-signaling domains or various proteins (e.g., CD19, CD34, LNGFR) also can be employed. Any selection marker may be employed and should be acceptable for Good Manufacturing Practices. In certain embodiments, selection markers are expressed with a polynucleotide that encodes a gene product of interest (e.g., a binding protein of the present disclosure, such as a TCR or CAR).
30 Further examples of selection markers include, for example, reporters such as GFP, EGFP, β -gal or chloramphenicol acetyltransferase (CAT). In certain embodiments, a

selection marker, such as, for example, CD34 is expressed by a cell and the CD34 can be used to select enrich for, or isolate (*e.g.*, by immunomagnetic selection) the transduced cells of interest for use in the methods described herein. As used herein, a CD34 marker is distinguished from an anti-CD34 antibody, or, for example, a scFv, 5 TCR, or other antigen recognition moiety that binds to CD34.

In certain embodiments, a selection marker comprises an RQR polypeptide, a truncated low-affinity nerve growth factor (tNGFR), a truncated CD19 (tCD19), a truncated CD34 (tCD34), or any combination thereof.

Regarding RQR polypeptides, without wishing to be bound by theory, distance 10 of an epitope or target sequence from the host cell surface may be important for RQR polypeptides to function as selection markers/safety switches (Philip *et al.*, 2010 (*supra*)). In some embodiments, the encoded RQR polypeptide is contained in a β -chain, an α -chain, or both, or a fragment or variant of either or both, of the encoded CD8 co-receptor. In specific embodiments, a modified host cell comprises a 15 heterologous polynucleotide encoding iCasp9 and a heterologous polynucleotide encoding a recombinant CD8 co-receptor protein that comprises a β -chain containing a RQR polypeptide and further comprises a CD8 α -chain.

In any of the aforementioned embodiments, a polynucleotide encoding, *e.g.*, a TCR, or a binding domain thereof, or a CD8 co-receptor or extracellular portion thereof, of 20 the instant disclosure is codon optimized for efficient expression in a target host cell. In some embodiments, the host cell comprises a human immune system cell, such as a T cell, a NK cell, or a NK-T cell (Scholten *et al.*, *Clin. Immunol.* 119:135, 2006). Codon optimization can be performed using known techniques and tools, *e.g.*, using the GenScript® OptimumGene™ tool, or GeneArt (Life Technologies). Codon-optimized 25 sequences include sequences that are partially codon-optimized (*i.e.*, one or more of the codons, but less than all of the codons, is optimized for expression in the host cell) and those that are fully codon-optimized. It will be appreciated that in embodiments wherein a polynucleotide encodes more than one polypeptide (*e.g.*, a TCR α chain, a TCR β chain, a CD8 co-receptor α chain, a CD8 co-receptor β chain, and one or more self-cleaving 30 peptides), each polypeptide can independently fully codon optimized, partially codon optimized, or not codon optimized.

In certain embodiments, the present disclosure provides a host cell comprising a heterologous polynucleotide encoding any one or more of the TCRs, or binding domains thereof, of this disclosure, wherein the modified or recombinant host cell expresses on its cell surface the TCR, or binding domain thereof, encoded by the heterologous
5 polynucleotide.

Various techniques may be used for recombinant (*i.e.*, engineered) DNA, peptide and oligonucleotide synthesis, immunoassays and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as
10 commonly accomplished in the art or as described herein. These and related techniques and procedures may be generally performed according to conventional methods well-known in the art and as described in various general and more specific references in microbiology, molecular biology, biochemistry, molecular genetics, cell biology, virology and immunology techniques that are cited and discussed throughout the
15 present specification. *See, e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 3d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (John Wiley and Sons, updated July 2008); *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience;
20 Glover, *DNA Cloning: A Practical Approach*, vol. I & II (IRL Press, Oxford Univ. Press USA, 1985); *Current Protocols in Immunology* (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober 2001 John Wiley & Sons, NY, NY); *Real-Time PCR: Current Technology and Applications*, Edited by Julie Logan, Kirstin Edwards and Nick Saunders, 2009, Caister Academic
25 Press, Norfolk, UK; Anand, *Techniques for the Analysis of Complex Genomes*, (Academic Press, New York, 1992); Guthrie and Fink, *Guide to Yeast Genetics and Molecular Biology* (Academic Press, New York, 1991); *Oligonucleotide Synthesis* (N. Gait, Ed., 1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, Eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, Eds., 1984); *Animal Cell*
30 *Culture* (R. Freshney, Ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984); *Next-Generation Genome Sequencing* (Janitz, 2008 Wiley-VCH); *PCR*

Protocols (Methods in Molecular Biology) (Park, Ed., 3rd Edition, 2010 Humana Press);
Immobilized Cells And Enzymes (IRL Press, 1986); the treatise, *Methods In*
Enzymology (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian*
Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Harlow
5 and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor,
N.Y., 1998); *Immunochemical Methods In Cell And Molecular Biology* (Mayer and
Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental*
Immunology, Volumes I-IV (D. M. Weir and CC Blackwell, eds., 1986); Roitt, *Essential*
Immunology, 6th Edition, (Blackwell Scientific Publications, Oxford, 1988); *Embryonic*
10 *Stem Cells: Methods and Protocols* (Methods in Molecular Biology) (Kurstad Turksen,
Ed., 2002); *Embryonic Stem Cell Protocols: Volume I: Isolation and Characterization*
(Methods in Molecular Biology) (Kurstad Turksen, Ed., 2006); *Embryonic Stem Cell*
Protocols: Volume II: Differentiation Models (Methods in Molecular Biology) (Kurstad
Turksen, Ed., 2006); *Human Embryonic Stem Cell Protocols* (Methods in Molecular
15 Biology) (Kursad Turksen Ed., 2006); *Mesenchymal Stem Cells: Methods and*
Protocols (Methods in Molecular Biology) (Darwin J. Prockop, Donald G. Phinney,
and Bruce A. Bunnell Eds., 2008); *Hematopoietic Stem Cell Protocols* (Methods in
Molecular Medicine) (Christopher A. Klug, and Craig T. Jordan Eds., 2001);
Hematopoietic Stem Cell Protocols (Methods in Molecular Biology) (Kevin D. Bunting
20 Ed., 2008) *Neural Stem Cells: Methods and Protocols* (Methods in Molecular Biology)
(Leslie P. Weiner Ed., 2008).

In any of the aforementioned embodiments, polynucleotides of this disclosure
 are contained in a host cell or, in certain embodiments, are contained in a vector and the
 vector containing the polynucleotide may be in a host cell. Accordingly, vectors are
25 provided that comprise a polynucleotide as provided herein. In some embodiments, the
 polynucleotide is operably linked to an expression control sequence. Suitable vectors
 for use with certain embodiments disclosed herein are known and can be selected for a
 particular purpose or cell. An exemplary vector may comprise a nucleic acid molecule
 capable of transporting another nucleic acid molecule to which it has been linked, or
30 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
5 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 high affinity or high functional avidity recombinant TCRs, or a binding
10 domain thereof, specific for WT1 p37, 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 a high affinity or high
15 functional avidity recombinant TCR, or a binding domain thereof, specific for WT1 p37 peptide::MHC of this disclosure may be operatively linked to certain expression control 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
20 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
25 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 TCRs, or binding domains thereof, 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 or an adenoviral vector.

30 In particular embodiments, the recombinant expression vector is delivered to an appropriate 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, the host cell is a hematopoietic progenitor cell or a human immune system cell. For example, the immune system cell can be a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a dendritic cell, or any combination thereof, wherein, optionally, the combination if present comprises a CD4+ T cell and a CD8+ T cell. 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. The recombinant expression vectors 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 a heterologous polynucleotide or vector as presently disclosed. In certain embodiments, the host cell expresses on its cell surface the TCR encoded by the polynucleotide, and wherein the polynucleotide is heterologous to the host cell. 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).

In certain embodiments, the V α domain of the TCR expressed by the host cell is encoded by a polynucleotide comprising at least 75% (*e.g.*, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to any one of the polynucleotides of SEQ ID

NOS:97, 98, and 101-107, or at least 94% sequence identity to SEQ ID NO:99 or 100. In certain embodiments, the V_{α} domain is encoded by a polynucleotide: (a) comprising the sequence of any one of the polynucleotides of SEQ ID NOS:97-107; or (b) consisting of the sequence of any one of the polynucleotides of SEQ ID NOS:97-107.

5 In certain embodiments, the V_{β} domain of the host cell is encoded by a polynucleotide comprising at least 75% sequence identity to any one of the polynucleotides of SEQ ID NOS:75-77, 79, 82, 84 and 85, or at least 95% sequence identity to any one of the polynucleotides to SEQ ID NOS:78, 80, 81, and 83. In certain embodiments, V_{β} domain is encoded by a polynucleotide: (a) comprising the
10 sequence of any one of the polynucleotides of SEQ ID NOS:75-85; or (b) consisting of the sequence of any one of the polynucleotides of SEQ ID NOS:75-85.

 In certain embodiments, the TCR α -chain comprises an α -chain constant domain encoded by a polynucleotide comprising at least 98% identity to SEQ ID NO:110. In certain embodiments, the TCR α -chain comprises an α -chain constant domain encoded
15 by a polynucleotide: (a) comprising the polynucleotide sequence of SEQ ID NO:110; or (b) consisting of the polynucleotide sequence of SEQ ID NO:110. In certain embodiments, the TCR β -chain comprises a β -chain constant domain is encoded by a polynucleotide comprising at least 99.9% sequence identity to SEQ ID NO:108 or 109. In some embodiments, the TCR β -chain comprises a β -chain constant domain encoded
20 by a polynucleotide: (a) comprising the polynucleotide sequence of SEQ ID NO:108 or 109; or (b) consisting of the polynucleotide sequence of SEQ ID NO:108 or 109.

 In some embodiments, wherein the polynucleotide comprises a nucleotide sequence encoding a self-cleaving peptide disposed between the polynucleotide sequence encoding the TCR α -chain and the polynucleotide sequence encoding the
25 TCR β -chain.

 In some embodiments, the encoded self-cleaving peptide: (a) comprises the amino acid sequence of any one of the polypeptides of SEQ ID NOS:60-63; or (b) consists of the sequence of any one of the polypeptides of SEQ ID NOS:60-63.

 In some embodiments, the polynucleotide encoding the self-cleaving peptide:
30 (a) comprises the sequence of any one of the polynucleotides of SEQ ID NOS:166-170;

or (b) consists of the sequence of any one of the polynucleotides of SEQ ID NOS:166-170.

In some embodiments, the TCR α -chain, self-cleaving peptide, and TCR β -chain are encoded by a polynucleotide comprising at least 95% identity to any one of SEQ ID NOS:155-165.

In some embodiments, the TCR α -chain, self-cleaving peptide, and TCR β -chain are encoded by a polynucleotide that: (a) comprises the sequence of any one of the polynucleotides of SEQ ID NOS:155-165; or (b) consists of the sequence of any one of the polynucleotides of SEQ ID NOS:155-165.

In some embodiments, the encoded TCR α -chain, self-cleaving peptide, and TCR β -chain comprise the amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, 99.1%, 99.5%, 99.9%, or 100% identity to any one of the polypeptides of SEQ ID NOS: 48-58. In some embodiments, the encoded TCR α -chain, self-cleaving peptide, and TCR β -chain: (a) comprise the amino acid sequence of any one of the polypeptides of SEQ ID NOS:48-58; or (b) consist of the amino acid sequence of any one of the polypeptides of SEQ ID NOS: 48-58.

In some embodiments, host cell is a hematopoietic progenitor cell or a human immune system cell. In some embodiments, the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a dendritic cell, or any combination thereof, wherein, optionally, the combination comprises a CD4⁺ T cell and a CD8⁺ T cell.

In some embodiments, wherein the host immune system cell is a T cell. In some embodiments, the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

In certain embodiments, the TCR has higher surface expression on a T cell as compared to an endogenous TCR (*e.g.*, when the endogenous TCR is not artificially inhibited or prevented from expression).

In certain embodiments, the host cell further comprises: (i) a heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain; (ii) a heterologous polynucleotide encoding a polypeptide

that comprises an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; or (iii) the polynucleotide of (i) and the polynucleotide of (ii), wherein, optionally, the host cell comprises a CD4⁺ T cell.

5 In some embodiments, the host cell comprises: (a) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain; (b) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain; and (c) a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide of
10 (a) and the polynucleotide of (b).

 In any of the presently disclosed embodiments, the host cell (*e.g.*, immune cell, such as a human T cell) is capable of killing: (i) a tumor cell of breast cancer cell line MDA-MB-468; (ii) a tumor cell of pancreatic adenocarcinoma cell line PANC-1; (iii) a tumor cell of breast cancer cell line MDA-MB-231; (iv) a tumor cell of myelogenous
15 leukemia cell line K562 expressing an HLA-A2, wherein, optionally, the HLA-A2 comprises HLA-A*201; (v) a tumor cell of colon carcinoma cell line RKO expressing an HLA-A2, wherein, optionally, the HLA-A2 comprises HLA-A*201; or (vi) any combination of tumor cells of (i)-(v), when the host cell and the tumor cell are both present in a sample. In some embodiments,

20 In particular embodiments, the host cell is capable of killing the tumor cell when the host cell and the tumor cell are present in the sample at a ratio of 32:1 host cell:tumor cell, 16:1, 8:1, 4:1, 2:1, or 1.5:1. Killing of a target cell can be determined, for example, the Incucyte® bioimaging platform (Essen Bioscience). In certain embodiments, this platform uses activated caspase and labelled (*e.g.*, RapidRed or
25 NucRed) tumor cell signals, wherein overlap is measured and increased overlap area equals tumor cell death by apoptosis. Killing can also be determined using a 4-hour assay in which target cells are loaded with labeled chromium (⁵¹Cr), and ⁵¹Cr in the supernatant is measured following 4-hour co-incubation with an immune cell expressing a binding protein of the present disclosure.

30 In any of the foregoing embodiments, a host cell (*e.g.*, an immune cell) may be 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, TIGIT, FasL, an HLA molecule, a TCR molecule, or the like. Without wishing to be bound by theory, certain endogenously expressed immune cell proteins may be

5 recognized as foreign by an allogeneic host receiving the modified immune cells, which may result in elimination of the modified immune cells (*e.g.*, an HLA allele), or may downregulate the immune activity of the modified immune cells (*e.g.*, PD-1, LAG-3, CTLA4, FasL, TIGIT, TIM3), or may interfere with the binding activity of a heterologously expressed binding protein of the present disclosure (*e.g.*, an endogenous

10 TCR of a modified T cell that binds a non-Ras antigen and thereby interferes with the modified immune cell binding a cell that expresses a Ras antigen).

Accordingly, decreasing or eliminating expression or activity of such endogenous genes or proteins can improve the activity, tolerance, or persistence of the modified cells in an autologous or allogeneic host setting, and may allow for universal

15 administration of the cells (*e.g.*, to any recipient regardless of HLA type). In certain embodiments, a modified cell is a donor cell (*e.g.*, allogeneic) or an autologous cell. In certain embodiments, a host cell of this disclosure comprises a chromosomal gene knockout of one or more of a gene that encodes PD-1, LAG-3, CTLA4, TIM3, TIGIT, FasL, an HLA component (*e.g.*, a gene that encodes an $\alpha 1$ macroglobulin, an $\alpha 2$

20 macroglobulin, an $\alpha 3$ macroglobulin, a $\beta 1$ microglobulin, or a $\beta 2$ 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, compositions, and adoptive cell therapies of which are herein incorporated

25 by reference in their entirety).

As used herein, the term "chromosomal gene knockout" refers to a genetic alteration or introduced inhibitory agent in a host cell that prevents (*e.g.*, reduces, delays, suppresses, or abrogates) production, by the host cell, of a functionally active endogenous polypeptide product. Alterations resulting in a chromosomal gene

30 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 host cell.

In certain embodiments, a chromosomal gene knock-out or gene knock-in is made by chromosomal editing of a host 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 a donor gene "knock-in", for target gene "knock-out", and optionally to inactivate a target gene through a donor gene knock in or target gene knock out event. 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. Examples of endonucleases include zinc finger nucleases, TALE-nucleases, CRISPR-Cas nucleases, meganucleases, and megaTALs.

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.

5 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 12th and 13th
10 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 (histine-aspartic acid) sequence at positions 12 and 13 of the TALE leads to the
15 TALE binding to cytosine (C), NG (asparagine-glycine) binds to a T nucleotide, NI (asparagine-isoleucine) to A, NN (asparagine-asparagine) binds to a G or A nucleotide, and NG (asparagine-glycine) binds to a T nucleotide. 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 their entirety). TALENs can be used to
20 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
25 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
30 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 programmed 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; each of which is 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. Exemplary gRNA sequences and methods of using the same to knock out endogenous genes that encode immune cell proteins include those described in Ren *et al.*, *Clin. Cancer Res.* 23(9):2255-2266 (2017), the gRNAs, CAS9 DNAs, vectors, and gene knockout techniques of which are hereby incorporated by reference in their entirety.

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, TIGIT, FasL, 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 further embodiments, a chromosomal gene knockout is generated using a homing endonuclease that has been modified with modular DNA binding domains of TALENs to make a fusion protein known as a megaTAL. MegaTALs can be utilized to not only knock-out one or more target genes, but to also introduce (knock in) heterologous or exogenous polynucleotides when used in combination with an exogenous donor template encoding a polypeptide of interest.

In certain embodiments, a chromosomal gene knockout comprises an inhibitory nucleic acid molecule that is introduced into a host cell (e.g., an immune 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 (e.g., of PD-1, TIM3, LAG3, CTLA4, TIGIT, FasL, an HLA component, or a TCR component, or any combination thereof) in the host cell.

A chromosomal gene knockout can be confirmed directly by DNA sequencing of the host immune 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
5 the knockout.

In certain embodiments, a chromosomal gene knockout comprises a knockout of an HLA component gene selected from an $\alpha 1$ macroglobulin gene, an $\alpha 2$ macroglobulin gene, an $\alpha 3$ macroglobulin gene, a $\beta 1$ microglobulin gene, or a $\beta 2$ microglobulin gene.

In certain embodiments, a chromosomal gene knockout comprises a knockout of
10 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.

Moreover, it will be appreciated that any of the presently disclosed gene editing techniques and tools may be used to introduce a TCR-encoding and/or CD8 co-receptor-encoding polynucleotide of the present disclosure into a host cell genome.

15 In another aspect, compositions and unit doses are provided herein that comprise a modified host cell of the present disclosure and a pharmaceutically acceptable carrier, diluent, or excipient.

In certain embodiments, a host cell composition or unit dose comprises (i) a composition comprising at least about 30%, at least about 40%, at least about 50%, at
20 least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% modified 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% modified CD8⁺ T cells, in about a 1:1 ratio, wherein
25 the 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).

30 In some embodiments, a host cell composition or unit dose comprises (i) a composition comprising at least about 50% modified CD4⁺ T cells, combined with (ii)

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

It will be appreciated that a host cell composition or unit dose of the present disclosure may comprise any host cell as described herein, or any combination of host cells. In certain embodiments, for example, a host cell composition or unit dose comprises modified CD8⁺ T cells, modified CD4⁺ T cells, or both, wherein these T cells are modified to encode a binding protein specific for a Ras peptide:HLA-A*02:01 complex, and further comprises modified CD8⁺ T cells, modified CD4⁺ T cells, or both, wherein these T cells are modified to encode a binding protein specific for a WT1

peptide:HLA-A*02:01 complex. In addition or alternatively, a host cell composition or unit dose of the present disclosure can comprise any host cell or combination of host cells as described herein, and can further comprise a modified cell (e.g., immune cell, such as a T cell) expressing a binding protein specific for a different antigen (e.g., a different WT1 antigen, or an antigen from a different protein or target, such as, for example, BCMA, CD3, CEACAM6, c-Met, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, FLT1, KDR, FLT4, CD44v6, CD151, CA125, CEA, CTLA-4, GITR, BTLA, TGFB2, TGFB1, IL6R, gp130, Lewis A, Lewis Y, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A (e.g., including MAGE-A1, MAGE-A3, and MAGE-A4), mesothelin, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, HLA, tumor- or pathogen- associated peptide bound to HLA, hTERT peptide bound to HLA, tyrosinase peptide bound to HLA, WT-1 peptide bound to HLA, LT β R, LIFR β , LRP5, MUC1, OSMR β , TCR α , TCR β , CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD79a, CD79b, CD80, CD81, CD86, CD123, CD171, CD276, B7H4, TLR7, TLR9, PTCH1, WT-1, HA1-H, Robo1, α -fetoprotein (AFP), Frizzled, OX40, PRAME, and SSX-2. or the like). For example, a unit dose can comprise modified CD8⁺ T cells expressing a binding protein that specifically binds to a WT1-HLA complex and modified CD4⁺ T cells (and/or modified CD8⁺ T cells) expressing a binding protein (e.g., a CAR) that specifically binds to a HER2 antigen. It will also be appreciated that any of the host cells disclosed herein may be administered in a combination therapy.

In any of the embodiments described herein, a host cell composition or unit dose comprises equal, or approximately equal numbers of engineered CD45RA⁺ CD3⁺ CD8⁺ and modified CD45RA⁺ CD3⁺ CD4⁺ TM cells.

25 Uses and Methods of Treatment

In certain aspects, the instant disclosure is directed to methods for treating a hyperproliferative or proliferative disorder or a condition characterized by Wilms tumor protein 1 (WT1) expression or overexpression by administering to human subject in need thereof a composition comprising a high affinity or high functional avidity recombinant TCR, or a binding domain thereof, specific for human WT1 according to any of the aforementioned TCRs or any binding domains described herein, or a host

cell, such as a T cell, engineered to express the same, or compositions comprising any of the TCRs, or a binding domain thereof, or host cells described herein. In some embodiments, the TCR is expressed by a host cell, such as a hematopoietic progenitor cell or a human immune system cell. In some embodiments, the immune system cell is
5 a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a dendritic cell, or any combination thereof

The presence of a hyperproliferative disorder or proliferative 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
10 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.*
15 *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).

20 In certain embodiments, there are provided methods for treating a hyperproliferative or proliferative disorder, such as a hematological malignancy or a solid cancer (*see, e.g.*, Nakatsuka *et al.*, *Modern Pathology* 19:804-714 (2006)). Exemplary hematological malignancies include acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic
25 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 or proliferative disorder, such as a solid cancer is selected from biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast
30 cancer, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal 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, malignant melanoma, osteosarcoma, ovarian cancer (*see, e.g., Hylander et al., Gynecologic Oncology 101:12-17 (2006)*), pancreatic cancer, pancreatic ductal
5 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.

In some embodiments, the TCR is capable of promoting an antigen-specific T cell response against a human WT1 in a class I HLA-restricted manner. In some
10 embodiments, the class I HLA-restricted response is transporter-associated with antigen processing (TAP) independent. In some embodiments, the antigen-specific T cell response comprises at least one of a CD4⁺ helper T lymphocyte (Th) response and a CD8⁺ cytotoxic T lymphocyte (CTL) response. In some embodiments, the CTL response is directed against a WT1-overexpressing cell.

15 Also provided herein are any of the TCRs, polynucleotides, compositions, vectors, and host cells (including in any combination) for use in a method of treating a proliferative or hyperproliferative disorder associated with Wilms tumor protein 1 (WT1) expression or overexpression.

Also provided herein are any of the TCRs, polynucleotides, compositions,
20 vectors, and host cells (including in any combination) for use in a method of manufacturing a medicament for the treatment of a proliferative or hyperproliferative disorder associated with Wilms tumor protein 1 (WT1) expression or overexpression.

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
25 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 high functional avidity recombinant TCR, or a binding domain thereof, specific for human WT1 (*e.g., SEQ ID NOS:23-58, and variants thereof* provided herein) or a host cell expressing the same, and optionally an adjunctive
30 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 another aspect, the instant disclosure is directed to methods for treating a hyperproliferative disorder or proliferative disorder or a condition characterized by Wilms tumor protein 1 (WT1) overexpression or expression by administering to human subject in need thereof a composition comprising an isolated polynucleotide encoding a high affinity or high functional avidity recombinant TCR, or a binding domain thereof, specific for human WT1 according to any the aforementioned encoded TCRs, or a

binding domain thereof, or a host cell, such as a T cell, comprising the same, or a composition comprising any of the TCRs, or a binding domain thereof, or host cells described herein. In certain embodiments, the polynucleotide encoding a TCR, or a binding domain thereof, specific for human WT1 p37 peptide::MHC is codon optimized for a host cell of interest. In further embodiments, any of the aforementioned polynucleotides are operably linked to an expression control sequence and is optionally contained in an expression vector, such as a viral vector. Exemplary viral vectors include lentiviral vectors and γ -retroviral vectors. In related embodiments, the vector is capable of delivering the polynucleotide to a host cell, such as a hematopoietic progenitor cell or an immune system cell (*e.g.*, human hematopoietic progenitor cell or a human immune system cell). Exemplary immune system cells include a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a dendritic cell, or any combination thereof (*e.g.*, human). In certain embodiments, the immune system cell is a T cell, such as a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof, all of which are optionally human.

In still another aspect, the instant disclosure is directed to methods for treating a hyperproliferative disorder or proliferative disorder or a condition characterized by Wilms tumor protein 1 (WT1) overexpression by administering to human subject in need thereof an effective amount of a host cell comprising a heterologous polynucleotide or an expression vector according to any of the aforementioned embodiments, or any described herein, wherein the engineered or recombinant host cell expresses on its cell surface the TCR encoded by the heterologous polynucleotide that is specific for human WT1 p37::MHC. In certain embodiments, the instant disclosure is directed to methods for treating a hyperproliferative disorder or a proliferative disorder or a condition characterized by Wilms tumor protein 1 (WT1) p37 peptide production or the presence of WT1 p37 peptide::MHC complex by administering to human subject in need thereof an effective amount of a host cell comprising a heterologous polynucleotide or an expression vector according to any of the aforementioned embodiments, or any described herein, wherein the engineered or recombinant host cell expresses on its cell surface the TCR encoded by the heterologous polynucleotide that is specific for human WT1 p37::MHC.

Also provided is an adoptive immunotherapy method for treating a condition characterized by WT1 overexpression in cells of a subject having a hyperproliferative or proliferative disorder, comprising administering to the subject an effective amount of a host cell or composition of the present disclosure.

5 In some embodiments, the host cell is modified *ex vivo*. In some embodiments, the host cell is an allogeneic cell, a syngeneic cell, or an autologous cell to the subject. In some embodiments, the host cell is a hematopoietic progenitor cell or a human immune system cell. In some embodiments, the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a
10 dendritic cell, or any combination thereof.

In some embodiments, the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

In some embodiments, the hyperproliferative or proliferative disorder is a hematological malignancy or a solid cancer.

15 In some embodiments, the hematological malignancy is selected from acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM).

In some embodiments, the solid cancer is selected from breast cancer, ovarian
20 cancer, lung cancer, biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal 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, mesothelioma, malignant
25 melanoma, 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.

In some embodiments, the host cell is administered parenterally.

In some embodiments, the method comprises administering a plurality of doses of the host cell to the subject. In some embodiments, the plurality of doses are administered at intervals between administrations of about two to about four weeks.

Cells expressing the recombinant TCR (*e.g.*, high affinity or high functional avidity), or a binding domain thereof, specific for human WT1 p37 peptide as described herein may be administered to a subject in a pharmaceutically or physiologically acceptable or suitable excipient or carrier. Pharmaceutically acceptable excipients are biologically compatible vehicles, *e.g.*, physiological saline, which are described in greater detail herein, that are suitable for administration to a human or other non-human mammalian subject.

A therapeutically effective dose is an amount of host cells (expressing a high affinity or high functional avidity recombinant TCR, or a binding domain thereof, specific for human WT1 p37 peptide::MHC) 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 WT1 or producing a WT1 p37 peptide (*e.g.*, a cytotoxic T cell response) in a statistically significant manner) in a treated human or non-human mammal. As is well known in the medical arts, 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 10^4 cells/m², about 5×10^4 cells/m², about 10^5 cells/m², about 5×10^5 cells/m², about 10^6 cells/m², about 5×10^6 cells/m², about 10^7 cells/m², about 5×10^7 cells/m², about 10^8 cells/m², about 5×10^8 cells/m², about 10^9 cells/m², about 5×10^9 cells/m², about 10^{10} cells/m², about 5×10^{10} cells/m², or about 10^{11} cells/m². In some embodiments, a dose comprises about 10^7 cells/m², about 5×10^7 cells/m², about 10^8 cells/m², about 5×10^8 cells/m², about 10^9 cells/m², about 5×10^9 cells/m², about 10^{10} cells/m², about 5×10^{10} cells/m², or about 10^{11} cells/m².

Pharmaceutical 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 disease, 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 immunogenic compositions administered according to the methods described herein can be determined by performing pre-clinical (including *in vitro* and *in vivo* animal studies) and clinical studies and analyzing data obtained therefrom by appropriate statistical, biological, and clinical methods and techniques, all of which can readily be practiced by a person skilled in the art.

A condition associated with WT1 overexpression (or, in some embodiments, expression) includes any disorder or condition in which underactivity, over-activity or improper activity of a WT1 cellular or molecular event is present, and typically results from unusually high (with statistical significance) levels of WT1 expression in afflicted cells (*e.g.*, leukemic 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 WT1 overexpression 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 WT1 overexpression include hyperproliferative disorders, which in some aspects 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.

According to certain embodiments, virtually any type of cancer that is characterized by WT1 overexpression may be treated through the use of compositions and methods disclosed herein, including hematological cancers (*e.g.*, leukemia including acute myeloid leukemia (AML), T or B cell lymphomas, myeloma, and others). Furthermore, "cancer" may refer to any accelerated proliferation of cells, including solid tumors, ascites tumors, blood or lymph or other malignancies; connective tissue malignancies; metastatic disease; minimal residual disease following transplantation of organs or stem cells; multi-drug resistant cancers, primary or secondary malignancies, angiogenesis related to malignancy, or other forms of cancer. Also contemplated within the presently disclosed embodiments are specific embodiments wherein only one of the above types of disease is included, or where specific conditions may be excluded regardless of whether or not they are characterized by WT1 overexpression.

Certain methods of treatment or prevention contemplated herein include administering a host cell (which may be autologous, allogeneic or syngeneic) comprising a desired nucleic acid molecule as described herein that is stably integrated into the chromosome of the cell. For example, such a cellular composition may be generated *ex vivo* using autologous, allogeneic or syngeneic immune system cells (*e.g.*, T cells, antigen-presenting cells, natural killer cells) in order to administer a desired, WT1-targeted T-cell composition to a subject as an adoptive immunotherapy.

As used herein, administration of a composition or therapy in some aspects 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.*, WT1 specific modified (*i.e.*,

recombinant or engineered) host cells with one or more cytokines; immunosuppressive therapy such as calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof). For example, a therapy of this disclosure can be combined with specific inhibitors or modulators of

5 immunosuppression components, such as inhibitors or modulators of immune checkpoint molecules (*e.g.*, anti-PD-1, anti-PD-L1, or anti-CTLA-4 antibodies; *see, e.g.*, Pardoll, *Nature Rev. Cancer* 12:252, 2012; Chen and Mellman, *Immunity* 39:1, 2013).

In some embodiments, the host cell is administered to the subject at a dose of

10 about 10^7 cells/m² to about 10^{11} cells/m². In some embodiments, the method further comprises administering a cytokine. In some embodiments, the cytokine is IL-2, IL-15, IL-21 or any combination thereof. In some embodiments, the cytokine is IL-2 and is administered concurrently or sequentially with the host cell. In some embodiments, the cytokine is administered sequentially, provided that the subject was administered the

15 host cell at least three or four times before cytokine administration.

In some embodiments, the cytokine is IL-2 and is administered subcutaneously.

In some embodiments, the subject is further receiving immunosuppressive therapy.

In some embodiments, the immunosuppressive therapy is selected from

20 calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof.

In some embodiments, the subject has received a non-myeloablative or a myeloablative hematopoietic cell transplant.

In some embodiments, the subject is administered the host cell at least three

25 months after the non-myeloablative hematopoietic cell transplant.

In some embodiments, the subject is administered the host cell at least two months after the myeloablative hematopoietic cell transplant. Techniques and regimens for performing HCT are known in the art and can comprise transplantation of any suitable donor cell, such as a cell derived from umbilical cord blood, bone marrow, or

30 peripheral blood, a hematopoietic stem cell, a mobilized stem cell, or a cell from amniotic fluid. Accordingly, in certain embodiments, a modified immune cell of the

present disclosure can be administered with or shortly after hematopoietic stem cells in a modified HCT therapy. In some embodiments, the HCT comprises a donor hematopoietic cell comprising a chromosomal knockout of a gene that encodes an HLA component, a chromosomal knockout of a gene that encodes a TCR component,
5 or both.

In further embodiments, the subject had previously received lymphodepleting chemotherapy prior to receiving the composition or HCT. In certain embodiments, a lymphodepleting chemotherapy comprises a conditioning regimen comprising cyclophosphamide, fludarabine, anti-thymocyte globulin, or a combination thereof.
10 In certain embodiments, a plurality of doses of a recombinant host 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 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
15 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 an antibody specific for PD-1 (*e.g.*, pidilizumab, nivolumab, or pembrolizumab), an antibody specific for PD-L1 (*e.g.*, MDX-1105,
20 BMS-936559, MEDI4736, MPDL3280A, or MSB0010718C), an antibody specific for CTLA4 (*e.g.*, tremelimumab or ipilimumab), 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
25 may be administered at least two to at least three months after the non-myeloablative hematopoietic cell transplant.

An effective amount of a therapeutic or pharmaceutical composition in some aspects refers to an amount sufficient, at dosages and for periods of time needed, to
30 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 administering an effective amount to a subject that is susceptible or at risk of developing a disease or disease-state (*e.g.*,
5 recurrence) as a preventative course.

The level of a cytotoxic T lymphocyte (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 WT1-specific
10 TCRs 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).

15 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,
20 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
25 presence and level of an immune response to a WT1-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
30 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.

The pharmaceutical 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. In certain embodiments, a unit dose comprises a recombinant host cell as described herein at a dose of about 10^7 cells/m² to about 10^{11} cells/m². 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 recombinant cells or active compound calculated to produce the desired therapeutic effect in association with an appropriate pharmaceutical carrier.

In general, an appropriate dosage and treatment regimen provides the active molecules or cells in an amount sufficient to provide therapeutic or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated subjects as compared to non-treated subjects. Increases in preexisting immune responses to a tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which are routine in the art and may be performed using samples obtained from a subject before and after treatment.

Methods according to this disclosure may 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 composition of the present disclosure with (concurrently, simultaneously, or sequentially) an immune checkpoint inhibitor. In some embodiments, a combination therapy comprises administering a composition of the present disclosure (*e.g.*, TCR, polynucleotide, vector, or host cell, or combination thereof) with an agonist of a stimulatory immune checkpoint agent. In further embodiments, a combination therapy comprises administering a composition of the present disclosure 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.

Techniques and regimens for performing HCT are known in the art and can comprise transplantation of any suitable donor cell, such as a cell derived from umbilical cord blood, bone marrow, or peripheral blood, a hematopoietic stem cell, a mobilized stem cell, or a cell from amniotic fluid. Accordingly, in certain embodiments, a modified immune cell of the present disclosure can be administered with or shortly after hematopoietic stem cells in a modified HCT therapy. In some embodiments, the HCT comprises a donor hematopoietic cell comprising a chromosomal knockout of a gene that encodes an HLA component, a chromosomal knockout of a gene that encodes a TCR component, or both.

In further embodiments, the subject had previously received lymphodepleting chemotherapy prior to receiving the composition or HCT. In certain embodiments, a lymphodepleting chemotherapy comprises a conditioning regimen comprising cyclophosphamide, fludarabine, anti-thymocyte globulin, or a combination thereof.

5 Methods according to this disclosure may 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 composition of the present disclosure with (concurrently, simultaneously, or sequentially) an immune checkpoint inhibitor. In some embodiments, a combination
10 therapy comprises administering a composition of the present disclosure with an agonist of a stimulatory immune checkpoint agent. In further embodiments, a combination therapy comprises administering a composition of the present disclosure with a secondary therapy, such as chemotherapeutic agent, a radiation therapy, a surgery, an antibody, or any combination thereof.

15 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
20 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,
25 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
30 the embodiments disclosed herein, a method may comprise a composition of the present

disclosure with one or more inhibitor of any one of the following immune suppression components, singly or in any combination.

In certain embodiments, a composition of the present disclosure is used in combination with a PD-1 inhibitor, for example a PD-1-specific antibody or binding
5 fragment thereof, such as pidilizumab, nivolumab, pembrolizumab, MEDI0680 (formerly AMP-514), AMP-224, BMS-936558 or any combination thereof. In further embodiments, a composition 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
10 any combination thereof. Also contemplated are cemiplimab; IBI-308; nivolumab + relatlimab; BCD-100; camrelizumab; JS-001; spartalizumab; tislelizumab; AGEN-2034; BGBA-333 + tislelizumab; CBT-501; dostarlimab; durvalumab + MEDI-0680; JNJ-3283; pazopanib hydrochloride + pembrolizumab; pidilizumab; REGN-1979 + cemiplimab; ABBV-181; ADUS-100 + spartalizumab; AK-104; AK-105; AMP-224;
15 BAT-1306; BI-754091; CC-90006; cemiplimab + REGN-3767; CS-1003; GLS-010; LZM-009; MEDI-5752; MGD-013; PF-06801591; Sym-021; tislelizumab + pamiparib; XmAb-20717; AK-112; ALPN-202; AM-0001; an antibody to antagonize PD-1 for Alzheimer's disease; BH-2922; BH-2941; BH-2950; BH-2954; a biologic to antagonize CTLA-4 and PD-1 for solid tumor; a bispecific monoclonal antibody to target PD-1 and
20 LAG-3 for oncology; BLSM-101; CB-201; CB-213; CBT-103; CBT-107; a cellular immunotherapy + PD-1 inhibitor; CX-188; HAB-21; HEISCOIII-003; IKT-202; JTX-4014; MCLA-134; MD-402; mDX-400; MGD-019; a monoclonal antibody to antagonize PDCD1 for oncology; a monoclonal antibody to antagonize PD-1 for oncology; an oncolytic virus to inhibit PD-1 for oncology; OT-2; PD-1 antagonist + ropeginterferon
25 alfa-2b; PEGMP-7; PRS-332; RXI-762; STIA-1110; TSR-075; a vaccine to target HER2 and PD-1 for oncology; a vaccine to target PD-1 for oncology and autoimmune disorders; XmAb-23104; an antisense oligonucleotide to inhibit PD-1 for oncology; AT-16201; a bispecific monoclonal antibody to inhibit PD-1 for oncology; IMM-1802; monoclonal antibodies to antagonize PD-1 and CTLA-4 for solid tumor and hematological tumor;
30 nivolumab biosimilar; a recombinant protein to agonize CD278 and CD28 and antagonize PD-1 for oncology; a recombinant protein to agonize PD-1 for autoimmune disorders and

inflammatory disorders; SNA-01; SSI-361; YBL-006; AK-103; JY-034; AUR-012; BGB-108; drug to inhibit PD-1, Gal-9, and TIM-3 for solid tumor; ENUM-244C8; ENUM-388D4; MEDI-0680; monoclonal antibodies to antagonize PD-1 for metastatic melanoma and metastatic lung cancer; a monoclonal antibody to inhibit PD-1 for oncology; monoclonal antibodies to target CTLA-4 and PD-1 for oncology; a monoclonal antibody to antagonize PD-1 for NSCLC; monoclonal antibodies to inhibit PD-1 and TIM-3 for oncology; a monoclonal antibody to inhibit PD-1 for oncology; a recombinant protein to inhibit PD-1 and VEGF-A for hematological malignancies and solid tumor; a small molecule to antagonize PD-1 for oncology; Sym-016; inebilizumab + MEDI-0680; a vaccine to target PDL-1 and IDO for metastatic melanoma; an anti-PD-1 monoclonal antibody plus a cellular immunotherapy for glioblastoma; an antibody to antagonize PD-1 for oncology; monoclonal antibodies to inhibit PD-1/PD-L1 for hematological malignancies and bacterial infections; a monoclonal antibody to inhibit PD-1 for HIV; or a small molecule to inhibit PD-1 for solid tumor.

15 In certain embodiments, a composition of the present disclosure 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 composition of the present disclosure is used in combination with an inhibitor of CTLA4. In particular embodiments, a composition of the present disclosure 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.

25 In certain embodiments, a composition of the present disclosure 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.

30 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of CD244.

In certain embodiments, a composition of the present disclosure 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.

5 In certain embodiments, a composition of the present disclosure cell is used in combination with an inhibitor of TIM3.

 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of Gal9.

 In certain embodiments, a composition of the present disclosure is used in
10 combination with an inhibitor of adenosine signaling, such as a decoy adenosine receptor.

 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of A2aR.

 In certain embodiments, a composition of the present disclosure is used in
15 combination with an inhibitor of KIR, such as lirilumab (BMS-986015).

 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of an inhibitory cytokine (typically, a cytokine other than TGF β) or Treg development or activity.

 In certain embodiments, a composition of the present disclosure is used in
20 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.

25 In certain embodiments, a composition of the present disclosure 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-boronoheptanoic acid (ABH), S-(2-boronoethyl)-L-cysteine (BEC), or any combination thereof.

30 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of VISTA, such as CA-170 (Curis, Lexington, Mass.).

In certain embodiments, a composition of the present disclosure 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.

5 In certain embodiments, a composition of the present disclosure 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 composition of the present disclosure is used in
10 combination with a LAIR1 inhibitor.

 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of CEACAM-1, CEACAM-3, CEACAM-5, or any combination thereof.

 In certain embodiments, a composition of the present disclosure is used in
15 combination with an agent that increases the activity (*i.e.*, is an agonist) of a stimulatory immune checkpoint molecule. For example a composition of the present disclosure 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,
20 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,
25 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 composition of the present disclosure 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 composition of the
30 present disclosure 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 composition of the present disclosure and further administering a radiation treatment
5 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
10 a composition of the present disclosure 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
15 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-
20 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, Cytosan,
25 dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylmelamineoxaliplatin, iphosphamide, melphalan, merchlorehtamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, temozolamide, teniposide, triethylenethiophosphoramidate and etoposide (VP 16)); antibiotics such as dactinomycin
30 (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— dacarbazine (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, methylprednisolone, prednisone, and prednisolone); 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 may be 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 composition of the present disclosure.

Also provided herein are methods for modulating an adoptive immunotherapy, wherein the methods comprise administering, to a subject who has previously received a modified host cell of the present disclosure that comprises a heterologous polynucleotide encoding a safety switch protein, a cognate compound of the safety switch protein in an amount effective to ablate in the subject the previously administered modified host cell.

In certain embodiments, the safety switch protein comprises tEGFR and the cognate compound is cetuximab, or the safety switch protein comprises iCasp9 and the cognate compound is AP1903 (*e.g.*, dimerized AP1903), or the safety switch protein comprises a RQR polypeptide and the cognate compound is rituximab, or the safety switch protein comprises a myc binding domain and the cognate compound is an antibody specific for the myc binding domain.

In still further aspects, methods are provided for manufacturing a composition, or a unit dose of the present disclosure. In certain embodiments, the methods comprise combining (i) an aliquot of a host cell transduced with a vector of the present disclosure with (ii) a pharmaceutically acceptable carrier. In certain embodiments, vectors of the present disclosure are used to transfect/transduce a host cell (*e.g.*, a T cell) for use in adoptive transfer therapy (*e.g.*, targeting a cancer antigen).

In some embodiments, the methods further comprise, prior to the aliquotting, culturing the transduced host cell and selecting the transduced cell as having incorporated (*i.e.*, expressing) the vector. In further embodiments, the methods comprise, following the culturing and selection and prior to the aliquotting, expanding the transduced host cell. In any of the embodiments of the instant methods, the manufactured composition or unit dose may be frozen for later use. Any appropriate host cell can be used for manufacturing a composition or unit dose according to the instant methods, including, for example, a hematopoietic stem cell, a T cell, a primary T cell, a T cell line, a NK cell, or a NK-T cell. In specific embodiments, the methods comprise a host cell which is a CD8⁺ T cell, a CD4⁺ T cell, or both.

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

5 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of CTLA4. In particular embodiments, a composition of the present disclosure 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.

10 In certain embodiments, a composition of the present disclosure 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.

15 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of CD244.

In certain embodiments, a composition of the present disclosure 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.
20 WO 2010/084158.

In certain embodiments, a composition of the present disclosure cell is used in combination with an inhibitor of TIM3.

In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of Gal9.

25 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of adenosine signaling, such as a decoy adenosine receptor.

In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of A2aR.

30 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of KIR, such as lirilumab (BMS-986015).

In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of an inhibitory cytokine (typically, a cytokine other than TGF β) or Treg development or activity.

5 In certain embodiments, a composition of the present disclosure 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.

10 In certain embodiments, a composition of the present disclosure 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-borohexanoic acid (ABH), S-(2-boronoethyl)-L-cysteine (BEC), or any combination thereof.

15 In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of VISTA, such as CA-170 (Curis, Lexington, Mass.).

In certain embodiments, a composition of the present disclosure 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
20 (Compugen), or both.

In certain embodiments, a composition of the present disclosure 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.

25 In certain embodiments, a composition of the present disclosure is used in combination with a LAIR1 inhibitor.

In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of CEACAM-1, CEACAM-3, CEACAM-5, or any combination thereof.

30 In certain embodiments, a composition of the present disclosure 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 composition of the present disclosure 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 composition of the present disclosure 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 composition of the present disclosure 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 composition of the present disclosure 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 a composition of the present disclosure 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, Cytosan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylmelamineoxaliplatin, iphosphamide, melphalan, merchloroethamine, 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— dacarbazine (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;

5 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, methylprednisolone, prednisone, and prednisolone);

10 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 may be 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 composition of the present disclosure.

20 Also provided herein are methods for modulating an adoptive immunotherapy, wherein the methods comprise administering, to a subject who has previously received a modified host cell of the present disclosure that comprises a heterologous polynucleotide encoding a safety switch protein, a cognate compound of the safety switch protein in an amount effective to ablate in the subject the previously

25 administered modified host cell.

In certain embodiments, the safety switch protein comprises tEGFR and the cognate compound is cetuximab, or the safety switch protein comprises iCasp9 and the cognate compound is AP1903 (*e.g.*, dimerized AP1903), or the safety switch protein comprises a RQR polypeptide and the cognate compound is rituximab, or the safety

30 switch protein comprises a myc binding domain and the cognate compound is an antibody specific for the myc binding domain.

In still further aspects, methods are provided for manufacturing a composition, or a unit dose of the present disclosure. In certain embodiments, the methods comprise combining (i) an aliquot of a host cell transduced with a vector of the present disclosure with (ii) a pharmaceutically acceptable carrier. In certain embodiments, vectors of the present disclosure are used to transfect/transduce a host cell (*e.g.*, a T cell) for use in adoptive transfer therapy (*e.g.*, targeting a cancer antigen).

In some embodiments, the methods further comprise, prior to the aliquotting, culturing the transduced host cell and selecting the transduced cell as having incorporated (*i.e.*, expressing) the vector. In further embodiments, the methods comprise, following the culturing and selection and prior to the aliquotting, expanding the transduced host cell. In any of the embodiments of the instant methods, the manufactured composition or unit dose may be frozen for later use. Any appropriate host cell can be used for manufacturing a composition or unit dose according to the instant methods, including, for example, a hematopoietic stem cell, a T cell, a primary T cell, a T cell line, a NK cell, or a NK-T cell. In specific embodiments, the methods comprise a host cell which is a CD8⁺ T cell, a CD4⁺ T cell, or both.

EXAMPLES

EXAMPLE 1

METHODS

20 *Cell lines*

T2 is a TAP-deficient T cell leukemia/B-LCL hybrid cell line expressing only HLA A*02:01¹¹, and 293T/17 is a highly-transfectable cell line purchased from ATCC. Jurkat76 cells are a TCR α /TCR β deficient derivative of the parental Jurkat cell line, and do not naturally express CD8¹². Jurkat76 cells were previously transduced to express CD8 $\alpha\beta$ Jurkat-CD8). Cell lines were maintained in RPMI 1640 medium with HEPES (Invitrogen, GIBCO) supplemented with 10% heat-inactivated FBS (Hyclone, GE Healthcare Life Sciences), 100 U/mL penicillin and 100 μ g/mL streptomycin.

Human T cell culture:

Leukapheresis samples were collected from healthy donors at the Seattle Cancer Care Alliance after written informed consent in accordance with the Declaration of Helsinki and with approval of the institutional review board under protocol 868.01.

5 PBMCs were isolated from HLA-typed donors and 10 HLA-A*02:01-restricted T cell lines were generated per donor specific for peptide WT₁₃₇₋₄₅, VLDFAPPGA, (10 donors total) as previously described^{13, 14}. Briefly, CD8⁺ T cells were purified using the EasySep™ Human CD8⁺ T cell isolation kit (StemCell Technologies) and DC were generated from autologous PBMC by adhesion to plastic and culture with 1000 U/ml

10 IL-4 and 800 U/ml GMCSF for 2 days with the addition of a maturation cytokine cocktail for the last day before harvest. DC were loaded with 1 µg/ml peptide for 90 minutes and then washed to remove excess peptide and irradiated at 4000 Rad. Approximately 5×10^6 CD8⁺ T cells were co-cultured at a 2.5:1 ratio with peptide-pulsed DC plus 30 ng/ml IL-21. T cells were maintained in RPMI 1640 medium with

15 HEPES (Invitrogen, GIBCO) supplemented with 5% heat-inactivated pooled human serum (Bloodworks Northwest), 100 U/mL penicillin, 100 µg/mL streptomycin and 55 µM 2-β-mercaptoethanol. Cultures were fed every 2-3 days by exchanging half of the medium and adding 12.5 U/ml IL-2, 2250 U/ml IL-7 and IL-15. T cells were re-stimulated every 10 days by culturing at a 1:2 ratio with irradiated, peptide-pulsed,

20 autologous PBMCs.

Flow cytometry-based cell sorting

T cell lines from all donors were combined on ice at the end of the antigen-specific expansion. The pooled sample was divided and stained with peptide/HLA-A2 tetramer under 3 conditions: (1) a wild type tetramer concentration empirically

25 determined to give maximal separation of positive and negative populations as described in the 'Tetramer binding and affinity measurements' section; (2) a 100-fold dilution of the optimal tetramer dose; and 3) a separate modified tetramer made by mutating the HLA-A2 molecule at positions D227K and T228A of the α3-domain), which interact with CD8¹⁵. This tetramer has been shown to selectively bind high

30 affinity CD8-independent TCRs^{16, 17}. For each tetramer-stained sample, cells with the highest levels of tetramer binding (top ~2% of labelled cells) were flow cytometrically

sorted. The sorted populations were analyzed by Adaptive Biotechnologies immunoseq assay to quantitate the relative abundance of each clonotype. An additional sample containing the entire tetramer positive population was also sorted from the optimal tetramer stained sample and TCR $\alpha\beta$ pairing information determined by Adaptive
5 Biotechnologies pairSeq Assay¹⁸.

Data analysis

Enrichment calculations

The enrichment score for each clonotype was calculated as: (frequency in the sorted tetramer⁺ population)/(frequency in the unsorted pooled sample). Clonotypes
10 that were not detected in the pooled sample were assigned a frequency in the pooled sample corresponding to 1 cell for enrichment calculations.

TCR Sequencing and alpha/beta pairing:

TCR repertoire analysis was performed by Adaptive Biotechnologies ImmunoSeq assay. Single cell V(D)J analysis (TCR alpha/beta pairing) was performed
15 using Chromium Single Cell Immune Profiling by 10x genomics.

TCR Transduction

Codon-optimized TCR constructs in a TCR β -p2a-TCR α orientation were synthesized on the BioXpTM 3200 (SGI-DNA) and cloned into the pRRLSIN.cPPT.MSCV.WPRE lentiviral expression plasmid (gift from Dr. Richard
20 Morgan, NCI) by Gibson Assembly. The expression vector was then packaged in 293T cells using a 3rd generation lentiviral packaging system. Lentiviral supernatant was harvested after 48 hr and filtered to remove cell debris. Approximately 5×10^5 Jurkat76 cells were combined with 2 ml of lentiviral supernatant plus 5 μ g/ml polybrene. Cells were centrifuged at 1000 g for 90 min at 30° C to facilitate transduction. For TCR-
25 transduction of primary CD8⁺ T cells, HLA-A2⁺ PBMC were enriched for CD8⁺ T cells using the EasySepTM Human CD8⁺ T cell isolation kit (StemCell Technologies) and activated for 4 hours with DynabeadsTM Human T-Expander CD3/CD28 (Gibco). Approximately 2×10^6 CD8⁺ T cells were combined with 2 ml of lentiviral supernatant plus 5 μ g/ml protamine sulphate and 50 U/ml IL-2. Transgenic TCR⁺ cells were

FACSsorted using peptide/HLA-A*02:01 tetramers to obtain pure antigen-specific cell populations for downstream assays.

TCR Binding Data

Assessment of correct TCR pairing

- 5 Jurkat76 cells, were transduced with each TCR construct and analyzed for tetramer binding relative to CD3 surface expression, which reflects total transgenic TCR surface expression in these cells lacking an endogenous TCR.

Tetramer binding and affinity measurements

- 10 The optimal tetramer dose was determined by performing a tetramer titration on a positive T cell population and selecting the concentration, which best separated the positive and negative populations without increasing the background staining of the negative population.

TCR Functional Data

IFN- γ production

- 15 Primary CD8⁺ T cells were lentivirally transduced with each TCR expression construct and sorted to yield a uniformly tetramer positive cell population, then mixed at a 1:1 ratio with T2 target cells pulsed with decreasing doses of peptide (1-10⁻⁵ μ M). Autologous PBMC were alternatively used as APC where indicated. After 4 hours of incubation in the presence of golgi-inhibitors (BD GolgiPlug and GolgiStop), cells were
20 surface-stained with anti-CD8 and then fixed (BD Cytofix/Cytoperm) before intracellular labelling with anti-IFN- γ in BD Perm/Wash buffer. The cells were analyzed by flow cytometry to determine the percentage of IFN- γ ⁺ cells for each sample. These data were fit to a dose-response curve by non-linear regression using Graphpad Prism (four parameter-variable slope, with the bottom and top of the curve
25 constrained to 0 and 100, respectively).

- Figures 1(A) and 1(B) show how WT1₃₇₋₄₅ peptide-specific TCRs were identified by high-throughput sequencing-based strategy. TCR clonotypes that were enriched in the high tetramer-binding sort compared to the total tetramer-positive population were identified as likely to have a high affinity or high functional avidity for
30 the peptide/HLA-A2 ligand. (A) Schematic of initial sequencing-based strategy for

identifying TCR clonotypes associated with high WT1₃₇₋₄₅ peptide/MHC tetramer-binding. (B) Enrichment in sort populations versus percentage of total population is shown, with selected TCR highlighted. All TCRs indicated by black circles were synthesized and evaluated for antigen-specificity (27 total).

5 Figure 2 shows results of tetramer-binding studies evaluating the specificity and relative tetramer binding affinity of the selected TCRs. TCR constructs were expressed in Jurkat cells that lack endogenous TCR α/β chains. Tetramer staining versus CD3 expression for each TCR is shown (CD3 expression directly correlates with transgenic TCR surface expression).

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EXAMPLE 2

IDENTIFICATION OF HIGH FUNCTIONAL AVIDITY TCRs

Since some high affinity TCRs have been shown to bind tetramer independent of CD8, a second experiment was performed to identify additional TCRs that are specifically enriched in the high tetramer binding sort population when a CD8 independent (CD8i) tetramer was used. Figures 3A-3C show how additional WT1₃₇₋₄₅ peptide-specific TCRs were identified by a modified high-throughput sequencing-based strategy using a CD8 independent (CD8i) tetramer. A schematic of a modified sequencing-based strategy for identifying TCR clonotypes associated with high CD8 independent WT1₃₇ peptide/MHC tetramer-binding is shown in Figure 3A. Enrichment in original sort populations versus percentage of total population as compared with a similar analysis when CD8i tetramer used is shown in Figures 3B and 3C. An additional 14 TCRs were selected based on surface CD3 levels and CD8i tetramer binding. All named TCR clonotypes in Figures 3B and 3C were synthesized and evaluated for antigen-specificity. All TCRs indicated by shaded (diagonal line pattern) circles in Figure 3C represent additional TCRs identified using CD8i tetramer.

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EXAMPLE 3**TETRAMER STAINING VERSUS CD3 EXPRESSION**

CD8i tetramer binding of additional CD8i tetramer-selected WT1₃₇₋₄₅ peptide-specific TCRs is shown in Figure 4. TCR constructs were expressed in Jurkat cells that
5 lack endogenous TCR α/β chains (as well as lacking CD8 expression). Tetramer staining versus CD3 expression for each TCR is shown in Figure 4 (CD3 expression directly correlates with transgenic TCR surface expression). TCRs that bound most strongly to tetramer, resulting in high levels of tetramer staining relative to anti-CD3 staining, were selected for further analysis.

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EXAMPLE 4**IFN γ ASSAY TO MEASURE FUNCTIONAL AVIDITY (EC₅₀)**

The ability of a TCR to signal T cell activation at limiting concentrations of antigen was measured by the peptide EC₅₀, which is the amount of peptide that target cells need to be pulsed with to elicit a T cell response (*e.g.*, IFN γ production) from 50%
15 of the present TCR-transduced T cells. This value directly correlates with the ability of T cells expressing a given TCR to kill antigen-expressing target cells. To determine the peptide EC₅₀ for the selected TCRs, each TCR was transduced into CD8⁺ T cells isolated from donor PMBCs (Figure 5A). After 1 week, cells were sorted for tetramer⁺ CD8⁺ T cells and expanded. Expanded antigen-specific cells were cultured for 4-6
20 hours with peptide-pulsed T2 target cells and IFN γ production was determined by flow cytometry (Figure 5A). The percentage of IFN γ -producing cells was fit to dose-response curves by non-linear regression to calculate peptide EC₅₀ for each TCR (Figure 5B).

EXAMPLE 5***IN VITRO* KILLING OF HLA-A2⁺ WT1⁺ MDA-MB-468 CELLS BY PRIMARY CD8⁺ T CELLS EXPRESSING WT1₃₇₋₄₅ PEPTIDE-SPECIFIC TCRs**

In order to directly assess TCR-transduced CD8⁺ T cell-mediated lysis of tumor cells that naturally express and present WT1 p37 antigen on HLA-A2, donor-derived CD8⁺ T cells were transduced with one of each of the selected TCRs and sort-purified for high tetramer binding. TCR-transduced T cells were then mixed at an 8:1 ratio (in triplicate) with the breast cancer cell line MDA-MB-468, which had been stained with CytoLight® Rapid Red dye. Total red object area (which correlates with the total number of live target cells) was calculated at the time points indicated for each TCR-transduced T cell population over a 72 hour period. The most potent tumor-reactive T cells would remain responsive to tumor antigens for long periods after *in vivo* transfer into patients. Therefore, in order to assess ongoing responsiveness of TCR-transduced T cells to persistent antigen, additional MDA-MB-468 cells were added at 48 hours. See Figure 6.

EXAMPLE 6***IN VITRO* KILLING OF HLA-A2⁺ WT1⁺ PANC-1 CELLS BY PRIMARY CD4⁺ AND CD8⁺ T CELLS EXPRESSING WT1₃₇₋₄₅ PEPTIDE-SPECIFIC TCRs**

Both CD4⁺ and CD8⁺ T cells can play a role in tumor clearance *in vivo*. Therefore, an MHC class I-restricted TCR that can also signal an antigen-specific response in CD4⁺ T cells is preferable to a TCR that can only activate CD8⁺ T cells. The ability of MHC class I-restricted TCRs to function in CD4⁺ T cells appears to be, in part, dependent on the affinity of the TCR for peptide MHC. In many cases, transduction of the CD4⁺ T cells with genes encoding CD8α and CD8β helps to efficiently elicit an antigen-specific response. Therefore, to assess the ability CD4 (transduced with CD8α / CD8β) versus CD8 T cells that express TCR10.1 to target HLA-A2⁺ WT1⁺ tumor cells, both CD4⁺ and CD8⁺ T cells were transduced to express the WT1₃₇₋₄₅ TCR10.1. CD4⁺ T cells were further transduced to express CD8α and CD8β genes. After 8 days, transduced cells were sorted to purify CD8⁺ tetramer⁺ and

CD4⁺/CD8⁺ tetramer⁺ T cells. Antigen-specific cells that were either CD4⁺, CD8⁺, or a mixture of these two populations (CD4 and CD8) were mixed 8:1 (in triplicate) with the pancreatic adenocarcinoma cell line PANC-1, which had been previously transduced to express NucLight® Red dye. Total red object area (which correlates with the total number of live target cells) was calculated at the time points indicated for each TCR-transduced T cell population. In order to assess ongoing responsiveness of TCR-transduced T cells to persistent antigen, additional PANC-1 cells were added at 48 hours. Figure 7 shows that both CD4⁺ and CD8⁺ T cells expressing WT1₃₇₋₄₅ TCR10.1 can eliminate the WT1⁺ A2⁺ pancreatic adenocarcinoma cell line PANC-1 after repeat challenge *in vitro*.

The WT1 p126 epitope is not always processed/presented efficiently by cells expressing WT1 and HLA-A2 (Jaigirdar et al., J. Immunother. 39:105, 2017). In particular, several solid tumor-derived cell lines that express WT1 and HLA-A2 are not efficiently targeted by WT1-p126-specific TCRs, with or without pre-culture with IFN γ to up-regulate immunoproteasome expression. In some aspects, the present disclosure relates, in part, to the finding that the WT1-p37 epitope is more broadly processed and presented by a wide variety of tumor types as compared to the WT1-p126 epitope. **Figures 8A-8D** shows the lysis of various WT1⁺ A2⁺ tumor cell lines by a WT1-p126 peptide-specific TCR as compared to a WT1 p37 peptide-specific TCR. These data highlight the fact that WT1 p-37 peptide-specific TCRs appear to be generally more reliably able to target a broad set of WT1+A2⁺ tumors.

The various embodiments described herein can be combined to provide further embodiments. All of the patents, patent application publications, patent applications, and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including but not limited to U.S. Patent Application No. 62/816,746, filed March 11, 2019, are incorporated herein by reference in their entirety. In general, terms used in the following claims should not be construed as limited to specific embodiments disclosed herein, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled.

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CLAIMS

What is claimed is:

1. A T cell receptor (TCR), comprising:
 - (a) a TCR α -chain variable (V_α) domain, and a TCR β -chain variable (V_β) domain having the CDR3 amino acid sequence set forth in any one of SEQ ID NOS: 199, 1-11, 181, 187, 193, 205, 211, 217, 223, 229, 235, and 241;
 - (b) a TCR V_α domain having the CDR3 amino acid sequence set forth in any one of SEQ ID NOS: 196, 12-22, 178, 184, 190, 202, 208, 214, 220, 226, 232, and 238, and a TCR V_β domain; or
 - (c) a TCR V_α domain having the CDR3 amino acid sequence set forth in any one of SEQ ID NOS: 199, 1-11, 181, 187, 193, 205, 211, 217, 223, 229, 235, and 241, and a TCR V_β domain comprising the CDR3 amino acid sequence set forth in any one of SEQ ID NOS: 196, 12-22, 178, 184, 190, 202, 208, 214, 220, 226, 232, and 238;wherein the TCR specifically binds to a VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex with an IFN γ production pEC₅₀ of 8.5 or higher.
2. The TCR of claim 1, wherein the TCR specifically binds to the VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex with an IFN γ production pEC₅₀ of 9.0 or higher.
3. The TCR of claim 1 or 2, wherein the TCR specifically binds to the VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex with an IFN γ production pEC₅₀ of 9.5 or higher.
4. The TCR of any one of claims 1-3, wherein the TCR further specifically binds to the VLDFAPPGA (SEQ ID NO:59):human leukocyte antigen (HLA) complex on a cell surface independent of CD8 or in the absence of CD8.
5. The TCR of any one of claims 1-4, wherein the HLA comprises HLA-A*201.

6. The TCR according to any one of claims 1-5, wherein the V α domain comprises an amino acid sequence that has at least:

- (a) about 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:34-35 and 38-44; or
- (b) 92% sequence identity to the amino acid sequence of SEQ ID NO:36 or 37.

7. The TCR according to any one of claims 1-6, wherein the V α domain comprises no change in the amino acid sequence of CDR1 and/or CDR2 as compared to the CDR1 and/or CDR2, respectively, present in any one of SEQ ID NOS:34-44.

8. The TCR according to any one of claims 1-7, further comprising:

- (i) the CDR1 α amino acid sequence set forth in any one of SEQ ID NOS:194, 176, 182, 188, 200, 206, 212, 218, 224, 230, and 236, or a variant thereof comprising one or two amino acid substitutions, wherein, optionally, the one or two amino acid substitutions comprise a conservative amino acid substitution; and/or
- (ii) the CDR2 α amino acid sequence set forth in any one of SEQ ID NOS:195, 177, 183, 189, 201, 207, 213, 219, 225, 231, and 237, or a variant thereof comprising one or two amino acid substitutions, wherein, optionally, the one or two amino acid substitutions comprise a conservative amino acid substitution.

9. The TCR according to any one of claims 1-8, wherein the V β domain comprises an amino acid sequence that has at least:

- (a) 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:23-25, 27, 28, 30, 32, and 33;
- (b) 92% sequence identity to the amino acid sequence of SEQ ID NO:29;
- (c) 93% sequence identity to the amino acid sequence of SEQ ID NO:31; or
- (d) 95% sequence identity to the amino acid sequence of SEQ ID NO:26.

10. The TCR of claim any one of claims 1-9, wherein the V β domain comprises no change in the amino acid sequence of CDR1 and/or CDR2 as compared to the CDR1 and/or CDR2, respectively, present in any one of SEQ ID NOS:23-33.

11. The TCR according to any one of claims 1-10, further comprising:

- (i) the CDR1 β amino acid sequence set forth in any one of SEQ ID NOS.: 197, 179, 185, 191, 197, 203, 209, 215, 221, 227, 233, and 239, or a variant thereof comprising one or two amino acid substitutions, wherein, optionally, the one or two amino acid substitutions comprise a conservative amino acid substitution; and/or
- (ii) the CDR2 β amino acid sequence set forth in any one of SEQ ID NOS.:198, 180, 186, 192, 204, 210, 216, 222, 228, 234, and 240, or a variant thereof comprising one or two amino acid substitutions, wherein, optionally, the one or two amino acid substitutions comprise a conservative amino acid substitution.

12. The TCR according to any one of claims 1-11, comprising the CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences set forth in:

- (i) SEQ ID NOS. 194, 195, 196 or 12, 197, 198, and 199 or 1, respectively;
- (ii) SEQ ID NOS.: 176, 177, 178 or 18, 179, 180, and 181 or 7, respectively;
- (iii) SEQ ID NOS.: 182, 183, 184 or 20, 185, 186, and 187 or 9, respectively;
- (iv) SEQ ID NOS.: 188, 189, 190 or 21, 191, 192, and 193 or 10, respectively;
- (v) SEQ ID NOS.: 200, 201, 202 or 13, 203, 204, and 205 or 2, respectively;
- (vi) SEQ ID NOS.: 206, 207, 208 or 14, 209, 210, and 211 or 3, respectively;
- (vii) SEQ ID NOS.: 212, 213, 214 or 15, 215, 216, and 217 or 4, respectively;
- (viii) SEQ ID NOS.: 218, 219, 220 or 17, 221, 222, and 223 or 6, respectively;
- (ix) SEQ ID NOS.: 224, 225, 226 or 19, 227, 228, and 229 or 8, respectively;
- (x) SEQ ID NOS.: 230, 231, 232 or 22, 233, 234, and 235 or 11, respectively; or
- (xi) SEQ ID NOS.: 236, 237, 238 or 16, 238, 240, and 241 or 5, respectively.

13. The TCR according to any one of claims 1-12, wherein the V_{α} domain comprises the amino acid sequence set forth in any one of SEQ ID NOS.:253-263 and 34-44.

14. The TCR according to any one of claims 1-13, wherein the V_{α} domain consists of the amino acid sequence set forth in any one of SEQ ID NOS.:253-263 and 34-44.

15. The TCR according to any one of claims 1-14, wherein the V_{β} domain comprises the amino acid sequence set forth in any one of SEQ ID NOS.:242-252 and 23-33.

16. The TCR according to any one of claims 1-15, wherein the V_{β} domain consists of the amino acid sequence set forth in any one of SEQ ID NOS.:242-252 and 23-33.

17. The TCR according to any one of claims 1-16, wherein the TCR comprises a TCR α -chain constant domain having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO:47.

18. The TCR according to any one of claims 1-17, wherein the TCR comprises a TCR β -chain constant domain having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO:45 or 46.

19. The TCR according to any one of claims 1-18, wherein the TCR comprises a TCR α -chain comprising a V_{α} domain and an α -chain constant domain, wherein:

(a) the V_{α} domain has at least about 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:34-35 and 38-44, and the α -chain constant domain has at least about 98% sequence identity to the amino acid sequence of SEQ ID NO:47; or

(b) the V_{α} domain has 92% sequence identity to the amino acid sequence of SEQ ID NO:36 or 37, and the α -chain constant domain has at least about 98% sequence identity to the amino acid sequence of SEQ ID NO:47.

20. The TCR according to any one of claims 1-19, wherein the TCR comprises a TCR α -chain comprising a V_{α} domain and an α -chain constant domain, wherein:

(a) the V_{α} domain comprises the amino acid sequence set forth in any one of SEQ ID NOS: 242-252 and 34-44, and the α -chain constant domain comprises the amino acid sequence of SEQ ID NO:47; or

(b) the V_{α} domain consists of the amino acid sequence set forth in any one of SEQ ID NOS: 242-252 and 34-44, and the α -chain constant domain consists the amino acid sequence of SEQ ID NO:47.

21. The TCR according to any one of claims 1-20, wherein the TCR comprises a TCR β -chain comprising a V_{β} domain and a β -chain constant domain, wherein:

(a) the V_{β} domain has at least about 90% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS:23-25, 27, 28, 30, 32, and 33, and the β -chain constant domain comprises the amino acid sequence of SEQ ID NO:45 or has at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:46;

(b) the V_{β} domain has 92% sequence identity to the amino acid sequence of SEQ ID NO:29, and the β -chain constant domain comprises the amino acid sequence of SEQ ID NO:45 or has at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:46;

(c) the V_{β} domain has 93% sequence identity to the amino acid sequence of SEQ ID NO:31, and the β -chain constant domain comprises the amino acid sequence of SEQ ID NO:45 or has at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:46; or

(c) the V_{β} domain has 95% sequence identity to the amino acid sequence of SEQ ID NO:26, and the β -chain constant domain comprises the amino acid sequence of

SEQ ID NO:45 or has at least about 95% sequence identity to the amino acid sequence of SEQ ID NO:46.

22. The TCR according to any one of claims 1-21, wherein the TCR comprises a TCR β -chain comprising a V_{β} domain and an β -chain constant domain, wherein:

(a) the V_{β} domain comprises the amino acid sequence set forth in any one of SEQ ID NOS:253-263 and 23-33, and the β -chain constant domain comprises the amino acid sequence of SEQ ID NO:45 or 46;

(b) the V_{β} domain consists of the amino acid sequence set forth in any one of SEQ ID NOS: 253-263 and 23-33, and the β -chain constant domain consists of the amino acid sequence of SEQ ID NO:45 or 46;

(c) the V_{β} domain comprises the amino acid sequence set forth in any one of SEQ ID NOS:25, 28, 29, 32 and 33, and the β -chain constant domain comprises the amino acid sequence of SEQ ID NO:45;

(d) the V_{β} domain consists of the amino acid sequence set forth in any one of SEQ ID NOS:25, 28, 29, 32 and 33, and the β -chain constant domain consists of the amino acid sequence of SEQ ID NO:45;

(e) the V_{β} domain comprises the amino acid sequence set forth in any one of SEQ ID NOS:23, 24, 26, 27, 30 and 31, and the β -chain constant domain comprises the amino acid sequence of SEQ ID NO:46; or

(f) the V_{β} domain consists of the amino acid sequence set forth in any one of SEQ ID NOS:23, 24, 26, 27, 30 and 31, and the β -chain constant domain consists of the amino acid sequence of SEQ ID NO:46.

23. The TCR of any one of claims 1-22, wherein the V_{α} domain and the V_{β} domain comprise or consist of the amino acid sequences set forth in SEQ ID NOs.:

(i) 253 and 242, respectively;

(ii) 259 and 248, respectively;

(iii) 261 and 250, respectively;

(iv) 262 and 251, respectively;

- (v) 257 and 246, respectively;
- (vi) 254 and 243, respectively;
- (vii) 255 and 244, respectively;
- (viii) 256 and 245, respectively;
- (ix) 258 and 247, respectively;
- (x) 260 and 249, respectively;
- (xi) 263 and 252, respectively;
- (xii) 34 and 23, respectively;
- (xiii) 40 and 29, respectively;
- (xiv) 42 and 31, respectively;
- (xv) 43 and 32, respectively;
- (xvi) 35 and 24, respectively;
- (xvii) 36 and 25, respectively;
- (xviii) 37 and 26, respectively;
- (xix) 39 and 28, respectively;
- (xx) 41 and 30, respectively;
- (xxi) 44 and 33, respectively; or
- (xxii) 38 and 27, respectively.

24. The TCR of claim 23, further comprising an α -chain constant domain and/or a β -chain constant domain, wherein the α -chain constant domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:47, and wherein the β -chain constant domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence set forth SEQ ID NO:45 or 46.

25. The TCR of claim 24, wherein the α -chain constant domain is present and the $V\alpha$ domain and the α -chain constant domain together form a TCR α -chain.

26. The TCR of claim 24 or 25, wherein the β -chain constant domain is present and the $V\alpha$ domain and the β -chain constant domain together form a TCR β -chain.
27. The TCR of any one of claims 1-26, wherein the TCR comprises a scTCR.
28. The TCR of any one of claims 1-26, wherein the TCR comprises a CAR.
29. An isolated polynucleotide encoding the TCR according to any one of claims 1-28.
30. The polynucleotide according to claim 29, wherein the polynucleotide encoding the TCR is codon optimized for a host cell of interest.
31. The polynucleotide according to claim 29 or 30, wherein the polynucleotide encodes an amino acid sequence having at least 95% identity to, comprising, or consisting of the amino acid sequence set forth in any one of SEQ ID NOS: 48-58.
32. The polynucleotide according to any one of claims 29-31, comprising the polynucleotide sequence set forth in any one of SEQ ID NOS: 64-165.
33. The polynucleotide according to any one of claims 29-32, further comprising:
- (i) a polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain;
 - (ii) a polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; or
 - (iii) a polynucleotide of (i) and a polynucleotide of (ii).

34. The polynucleotide of claim 33, comprising:

- (a) the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain;
- (b) the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain; and
- (c) a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide of (a) and the polynucleotide of (b).

35. The polynucleotide of claim 33 or 34, further comprising a polynucleotide that encodes a self-cleaving peptide and is disposed between:

- (1) the polynucleotide encoding a binding protein and the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain; and/or
- (2) the polynucleotide encoding a binding protein and the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain.

36. The polynucleotide of any one of claims 33-35, comprising, operably linked in-frame:

- (i) (pnCD8 α)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnTCR);
- (ii) (pnCD8 β)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnTCR);
- (iii) (pnTCR)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnCD8 β);
- (iv) (pnTCR)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnCD8 α);
- (v) (pnCD8 α)-(pnSCP₁)-(pnTCR)-(pnSCP₂)-(pnCD8 β); or
- (vi) (pnCD8 β)-(pnSCP₁)-(pnTCR)-(pnSCP₂)-(pnCD8 α),

wherein pnCD8 α is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain,

wherein pnCD8 β is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain,

wherein pnTCR is the polynucleotide encoding a TCR,

and wherein pnSCP₁ and pnSCP₂ are each independently a polynucleotide encoding a self-cleaving peptide, wherein the polynucleotides and/or the encoded self-cleaving peptides are optionally the same or different.

37. The polynucleotide of any one of claims 33-36, wherein the encoded TCR comprises a TCR α chain and a TCR β chain, wherein the polynucleotide comprises a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide encoding a TCR α chain and the polynucleotide encoding a TCR β chain.

38. The polynucleotide of claim 37, comprising, operably linked in-frame:

- (i) (pnCD8 α)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnTCR β)-(pnSCP₃)-(pnTCR α);
- (ii) (pnCD8 β)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnTCR β)-(pnSCP₃)-(pnTCR α);
- (iii) (pnCD8 α)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnTCR α)-(pnSCP₃)-(pnTCR β);
- (iv) (pnCD8 β)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnTCR α)-(pnSCP₃)-(pnTCR β);
- (v) (pnTCR β)-(pnSCP₁)-(pnTCR α)-(pnSCP₂)-(pnCD8 α)-(pnSCP₃)-(pnCD8 β);
- (vi) (pnTCR β)-(pnSCP₁)-(pnTCR α)-(pnSCP₂)-(pnCD8 β)-(pnSCP₃)-(pnCD8 α);
- (vii) (pnTCR α)-(pnSCP₁)-(pnTCR β)-(pnSCP₂)-(pnCD8 α)-(pnSCP₃)-(pnCD8 β); or
- (viii) (pnTCR α)-(pnSCP₁)-(pnTCR β)-(pnSCP₂)-(pnCD8 β)-(pnSCP₃)-(pnCD8 α),

wherein pnCD8 α is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain,

wherein pnCD8 β is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain,

wherein pnTCR α is the polynucleotide encoding a TCR α chain,

wherein pnTCR β is the polynucleotide encoding a TCR β chain,
and wherein pnSCP₁, pnSCP₂, and pnSCP₃ are each independently a
polynucleotide encoding a self-cleaving peptide, wherein the polynucleotides and/or the
encoded self-cleaving peptides are optionally the same or different.

39. An expression vector, comprising the polynucleotide of any one of
claims 29-38 operably linked to an expression control sequence.

40. The expression vector according to claim 39, wherein the vector is
capable of delivering the polynucleotide to a host cell.

41. The expression vector according to claim 39, wherein the host cell is a
hematopoietic progenitor cell or a human immune system cell.

42. The expression vector according to claim 41, wherein the immune
system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$
T cell, a natural killer cell, a dendritic cell, or any combination thereof.

43. The expression vector according to claim 42, wherein the T cell is a
naïve T cell, a central memory T cell, an effector memory T cell, or any combination
thereof.

44. The expression vector according to any one of claims 39-43, wherein the
vector is a viral vector.

45. The expression vector according to claim 44, wherein the viral vector is
an adenoviral vector, a lentiviral vector, or a γ -retroviral vector.

46. A host cell, comprising the polynucleotide according to any one of
claims 29-38 or the expression vector according to any one of claims 29-45, wherein
the host cell expresses on its cell surface the TCR encoded by the polynucleotide, and
wherein the polynucleotide is heterologous to the host cell.

47. The host cell according to claim 46, wherein the V_{α} domain is encoded by a polynucleotide comprising at least 75% sequence identity to any one of the polynucleotides of SEQ ID NOS:97, 98, and 101-107, or at least 94% sequence identity to SEQ ID NO:99 or 100.

48. The host cell according to claim 46 or 47, wherein V_{α} domain is encoded by a polynucleotide:

- (a) comprising the sequence of any one of the polynucleotides of SEQ ID NOS:97-107; or
- (b) consisting of the sequence of any one of the polynucleotides of SEQ ID NOS:97-107.

49. The host cell according to any one of claims 46-48, wherein the V_{β} domain is encoded by a polynucleotide comprising at least 75% sequence identity to any one of the polynucleotides of SEQ ID NOS:75-77, 79, 82, 84 and 85, or at least 95% sequence identity to any one of the polynucleotides to SEQ ID NOS:78, 80, 81, and 83.

50. The host cell according to any one of claims 46-49, wherein the V_{β} domain is encoded by a polynucleotide:

- (a) comprising the sequence of any one of the polynucleotides of SEQ ID NOS:75-85; or
- (b) consisting of the sequence of any one of the polynucleotides of SEQ ID NOS:75-85.

51. The host cell according to any one of claims 46-50, wherein the TCR α -chain comprises an α -chain constant domain encoded by a polynucleotide comprising at least 98% identity to SEQ ID NO:110.

52. The host cell according to any one of claims 46-51, wherein the TCR α -chain comprises an α -chain constant domain encoded by a polynucleotide:

- (a) comprising the polynucleotide sequence of SEQ ID NO:110; or
- (b) consisting of the polynucleotide sequence of SEQ ID NO:110.

53. The host cell according to any one of claims 46-52, wherein the TCR β -chain comprises a β -chain constant domain is encoded by a polynucleotide comprising at least 99.9% sequence identity to SEQ ID NO:108 or 109.

54. The host cell according to any one of claims 46-53, wherein the TCR β -chain comprises a β -chain constant domain encoded by a polynucleotide:

- (a) comprising the polynucleotide sequence of SEQ ID NO:108 or 109; or
- (b) consisting of the polynucleotide sequence of SEQ ID NO:108 or 109.

55. The host cell according to any one of claims 46-54, wherein the polynucleotide comprises a nucleotide sequence encoding a self-cleaving peptide disposed between the polynucleotide sequence encoding the TCR α -chain and the polynucleotide sequence encoding the TCR β -chain.

56. The host cell according to claim 55, wherein the encoded self-cleaving peptide:

- (a) comprises the amino acid sequence of any one of the polypeptides of SEQ ID NOS:60-63; or
- (b) consists of the sequence of any one of the polypeptides of SEQ ID NOS:60-63.

57. The host cell according to claim 55 or 56, wherein the polynucleotide encoding the self-cleaving peptide:

- (a) comprises the sequence of any one of the polynucleotides of SEQ ID NOS:166-170; or
- (b) consists of the sequence of any one of the polynucleotides of SEQ ID NOS:166-170.

58. The host cell according to any one of claims 46-57, wherein the TCR α -chain, self-cleaving peptide, and TCR β -chain are encoded by a polynucleotide comprising at least 95% identity to any one of SEQ ID NOS:155-165.

59. The host cell according to any one of claims 46-58, wherein the TCR α -chain, self-cleaving peptide, and TCR β -chain are encoded by a polynucleotide that:

- (a) comprises the sequence of any one of the polynucleotides of SEQ ID NOS:155-165; or
- (b) consists of the sequence of any one of the polynucleotides of SEQ ID NOS:155-165.

60. The host cell of claims 58 or 59, wherein the encoded TCR α -chain, self-cleaving peptide, and TCR β -chain comprise the amino acid sequence having at least 95% identity to any one of the polypeptides of SEQ ID NOS: 48-58.

61. The host cell of any one of claims 58-60, wherein the encoded TCR α -chain, self-cleaving peptide, and TCR β -chain:

- (a) comprise the amino acid sequence of any one of the polypeptides of SEQ ID NOS:48-58; or
- (b) consist of the amino acid sequence of any one of the polypeptides of SEQ ID NOS: 48-58.

62. The host cell according to any one of claims 46-61, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

63. The host cell according to claim 62, wherein the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, dendritic cell, or any combination thereof, wherein, optionally, the combination, if present, comprises a CD4⁺ T cell and a CD8⁺ T cell.

64. The host cell according to claim 62, wherein the immune system cell is a T cell.

65. The host cell according to claim 64, wherein the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

66. The host cell according to any one of claims 46-65, wherein the TCR has higher surface expression on a T cell as compared to an endogenous TCR.

67. The host cell according to any one of claims 46-66, further comprising:

(i) a heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain;

(ii) a heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; or

(iii) the polynucleotide of (i) and the polynucleotide of (ii), wherein, optionally, the host cell comprises a CD4⁺ T cell.

68. The host cell of claim 67, comprising:

(a) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain;

(b) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain; and

(c) a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide of (a) and the polynucleotide of (b).

69. The host cell of any one of claims 46-68, wherein the host cell is capable of killing:

(i) a tumor cell of breast cancer cell line MDA-MB-468;

(ii) a tumor cell of pancreatic adenocarcinoma cell line PANC-1;

- (iii) a tumor cell of breast cancer cell line MDA-MB-231;
- (iv) a tumor cell of myelogenous leukemia cell line K562 expressing an HLA-A2, wherein, optionally, the HLA-A2 comprises HLA-A*201;
- (v) a tumor cell of colon carcinoma cell line RKO expressing an HLA-A2, wherein, optionally, the HLA-A2 comprises HLA-A*201; or
- (vi) any combination of tumor cells of (i)-(v),
when the host cell and the tumor cell are both present in a sample.

70. The host cell of claim 69, wherein the host cell is capable of killing the tumor cell when the host cell and the tumor cell are present in the sample at a ratio of 32:1 host cell:tumor cell, 16:1, 8:1, 4:1, 2:1, or 1.5:1.

71. A composition, comprising the host cell of any one of claims 46-70 and a pharmaceutically acceptable carrier, diluent, or excipient.

72. The composition of claim 71, comprising a host CD4⁺ T cell and a host CD8⁺ T cell.

73. A method for treating a hyperproliferative or proliferative disorder, comprising administering to human subject in need thereof a composition comprising the TCR specific for human Wilms tumor protein 1 (WT1) according to any one of claims 1- 28.

74. The method of claim 73, wherein the TCR is expressed on the surface of a host cell, wherein, optionally, the host cell is a hematopoietic progenitor cell or a human immune system cell, wherein, further optionally, the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a dendritic cell, or any combination thereof.

75. The method of claim 74, wherein the host cell comprises a host cell of any one of claims 46-70.

76. The method according to claim 73, wherein the hyperproliferative or proliferative disorder is a hematological malignancy or a solid cancer.

77. The method according to claim 76, wherein the hematological malignancy is selected from acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM).

78. The method according to claim 77, wherein the solid cancer is selected from breast cancer, ovarian cancer, lung cancer, biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal 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, mesothelioma, malignant melanoma, 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.

79. The method according to any one of claims 73-78, wherein the TCR is capable of promoting an antigen-specific T cell response against a human WT1 in a class I HLA-restricted manner.

80. The method according to claim 79, wherein the class I HLA-restricted response is transporter-associated with antigen processing (TAP)-independent.

81. The method according to claim 79 or 80, wherein the antigen-specific T cell response comprises at least one of a CD4⁺ helper T lymphocyte (Th) response and a CD8⁺ cytotoxic T lymphocyte (CTL) response.

82. The method according to claim 81, wherein the CTL response is directed against a WT1-overexpressing cell.

83. An adoptive immunotherapy method for treating a condition characterized by WT1 overexpression in cells of a subject having a hyperproliferative or proliferative disorder, comprising administering to the subject an effective amount of the host cell according to any one of claims 46-70, or the composition of claim 71 or 72.

84. The method according to claim 83, wherein the host cell is modified *ex vivo*.

85. The method according to claim 83 or 84, wherein the host cell is an allogeneic cell, a syngeneic cell, or an autologous cell to the subject.

86. The method according to any one of claims 83-85, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

87. The method according to claim 86, wherein the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a dendritic cell, or any combination thereof.

88. The method according to claim 87, wherein the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

89. The method according to any one of claims 83-88, wherein the hyperproliferative or proliferative disorder is a hematological malignancy or a solid cancer.

90. The method according to claim 89, wherein the hematological malignancy is selected from acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia

(CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM).

91. The method according to claim 89, wherein the solid cancer is selected from breast cancer, ovarian cancer, lung cancer, biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal 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, mesothelioma, malignant melanoma, 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.

92. The method according to any one of claims 83-91, wherein the host cell is administered parenterally.

93. The method according to any one of claims 83-92, wherein the method comprises administering a plurality of doses of the host cell to the subject.

94. The method according to claim 93, wherein the plurality of doses are administered at intervals between administrations of about two to about four weeks.

95. The method according to any one of claims 83-94, wherein the host cell is administered to the subject at a dose of about 10^7 cells/m² to about 10^{11} cells/m².

96. The method according to any one of claims 83-95, wherein the method further comprises administering a cytokine.

97. The method according to claim 96, wherein the cytokine is IL-2, IL-15, IL-21 or any combination thereof.

98. The method according to claim 97, wherein the cytokine is IL-2 and is administered concurrently or sequentially with the host cell.

99. The method according to claim 98, wherein the cytokine is administered sequentially, provided that the subject was administered the host cell at least three or four times before cytokine administration.

100. The method according to any one of claims 97-99, wherein the cytokine is IL-2 and is administered subcutaneously.

101. The method according to any one of claims 83-100, wherein the subject is further receiving immunosuppressive therapy.

102. The method according to claim 101, wherein the immunosuppressive therapy is selected from calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof.

103. The method according to any one of claims 83-102, wherein the subject has received a non-myeloablative or a myeloablative hematopoietic cell transplant.

104. The method according to claim 103, wherein the subject is administered the host cell at least three months after the non-myeloablative hematopoietic cell transplant.

105. The method according to claim 103, wherein the subject is administered the host cell at least two months after the myeloablative hematopoietic cell transplant.

106. The method of any one of claims 73-105, wherein the subject has received or is receiving an immune checkpoint inhibitor and/or an agonist of a stimulatory immune checkpoint agent.

107. A unit dose form comprising the host cell according to any one of claims 46-70 or the composition of claim 72.

108. The unit dose form according to claim 107, wherein the host cell is at a dose of about 10^7 cells/m² to about 10^{11} cells/m².

109. The TCR of any one of claims 1-28, the polynucleotide of any one of claims 29-38, the vector of any one of claims 39-45, the host cell of any one of claims 46-70, or the composition of claim 71 or 72, or any combination thereof, for use in a method of treating a proliferative or hyperproliferative disorder associated with Wilms tumor protein 1 (WT1) expression or overexpression.

110. The TCR of any one of claims 1-28, the polynucleotide of any one of claims 29-38, the vector of any one of claims 39-45, the host cell of any one of claims 46-70, or the composition of claim 71 or 72, or any combination thereof, for use in a method of manufacturing a medicament for the treatment of a proliferative or hyperproliferative disorder associated with Wilms tumor protein 1 (WT1) expression or overexpression.