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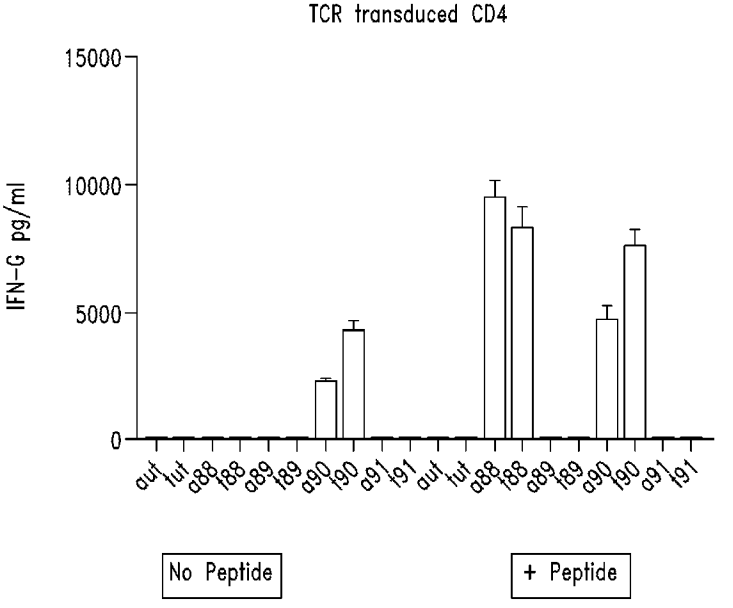

FIG. 5

(57) Abrégé/Abstract:
The present disclosure provides binding proteins, such as TCRs, that specifically bind various tumor associated antigens (including human BRAFV600E epitope), cells expressing such antigen specific binding proteins, nucleic acids encoding the same, and compositions for use in treating diseases or disorders in which cells express BRAFV600E, such as in cancer.

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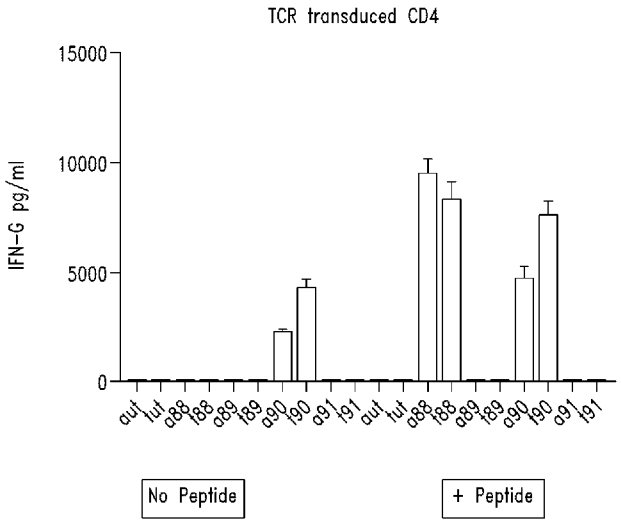

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(57) Abstract: The present disclosure provides binding proteins, such as TCRs, that specifically bind various tumor associated antigens (including human BRAFV600E epitope), cells expressing such antigen specific binding proteins, nucleic acids encoding the same, and compositions for use in treating diseases or disorders in which cells express BRAFV600E, such as in cancer.

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BRAF-SPECIFIC TCRS AND USES THEREOF

STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under CA015704 awarded by the National Institutes of Health. The government has certain rights in the invention.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 360056_454WO_SEQUENCE_LISTING.txt. The text file is 126 KB, was created on August 9, 2018, and is being submitted electronically via EFS-Web.

BACKGROUND

Adoptive transfer of tumor-specific T cells is an appealing strategy to eliminate existing tumors and requires the establishment of a robust population of antigen-specific T cells *in vivo* to eliminate existing tumor and prevent recurrences (Stromnes *et al.*, *Immunol. Rev. 257*:145, 2014). In recent years, there is increasing evidence that immune responses to antigens created by mutations in cancer can be recognized by T cells and that these T cells can mediate clinical responses to treatment with adoptive cell therapy and immune checkpoint inhibitors. Antigens that arise from such mutations are particularly appealing targets for immunotherapies due to being completely specific for the cancer relative to normal tissue, and also because they lack central tolerance mechanisms that could limit T cell function against other antigen types.

However, although administration of autologous or engineered allogeneic tumor-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) can mediate direct anti-tumor activity in select patients (Chapuis *et al.*, *Cancer Res. 72*:LB-136, 2012; Chapuis *et al.*, *Sci. Transl. Med. 5*:174ra127, 2013; Chapuis *et al.*, *Proc. Nat'l. Acad. Sci. U.S.A. 109*:4592, 2012) [2-4], identifying and isolating tumor-reactive T cells with desired

characteristics is a laborious and complex endeavor (*see* Stone and Kranz, *Frontiers Immunol. 4*:244, 2013; Chapuis *et al.*, 2013; Schmitt *et al.*, *Hum. Gene Ther. 20:*1240, 2009; Ho *et al.*, *J. Immunol. Methods 310*:40, 2006). Further, the variability in the avidity of the CTLs isolated from each patient or donor limits the anti-tumor efficacy in clinical trials (Chapuis *et al.*, 2013). Moreover, most antigen-specific mutations that lead to immune responses are found only in the cancer of one individual, and not in multiple patients.

There is a clear need for alternative antigen-specific TCR immunotherapies directed against various cancers, such as hairy cell leukemia, malignant melanoma, thyroid, lung, and colon cancers. In particular, TCR immunotherapies targeting antigens that are both cancer-specific and widely prevalent in cancers are needed. Presently disclosed embodiments address these needs and provide other related advantages.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1I show the identification and characterization of patient-derived $CD4^+$ T cells specific for $BRAF^{V600E}$. (1A) Positron emission tomography showing recurrent tumor in left iliac region (left) and left thigh (right). (1B-1D) Specificity and HLA restriction of $BRAF^{V600E}$-specific T cells: (1B) IFN-γ production by the patient-derived T cell line incubated with autologous B cells pulsed with wildtype and mutant BRAF peptide; (1C) recognition of autologous B cells pulsed with mutant BRAF peptide or transfected with mRNA encoding mutant or wildtype BRAF sequences; (1D) recognition of autologous B cells pulsed with mutant BRAF peptide in the presence or absence of HLA blocking antibodies. (1E) Recognition by $BRAF^{V600E}$ -specific $CD4^+$ T cells of the B-LCL line 1331, which is matched at HLA-DQ with the patient, and the HLA-mismatched B-LCL line VAVY, prior to and after transduction with HLA-DRB1*0404 (DR4) or HLA-DQB1*0302/DQA1*03 (DQ3). (1F) IFN-γ release by patient-derived $BRAF^{V600E}$-specific T cells incubated with allogeneic B-LCL cell lines expressing HLA DQB1*03 alleles and pulsed with the indicated amount of 21-mer $BRAF^{V600E}$ peptide. Three technical replicates were performed. (1G) IFN-γ release by

patient-derived $BRAF^{V600E}$-specific T cells incubated with autologous B cells pulsed with $BRAF^{V600E}$ peptide or the indicated tumor cell lines with and without pretreatment with human IFN-γ 500 U/ml for 3 days. (1H, 1I) Expression (mean fluorescence intensity) of HLA-DQ (1H) and HLA-DR (1I) on tumor cell lines with and without IFN-γ pre-treatment, quantitated by flow cytometry relative to the isotype control. Experiments were performed in technical duplicate or triplicate as indicated, and are representative of two independent experiments.

Figures 2A-2O show the specificity of $CD8^+$ T cells in TIL (tumor infiltrating lymphocytes) and TCR sequencing of T cell clonotypes in blood after adoptive transfer. (2A) IFN-γ production by TIL incubated with autologous CD40L-activated B cells pulsed with 13 pools of peptides encompassing 40 20-mer peptides containing the 20 nonsynonymous mutations present in the patient's melanoma by elispot assay. The final concentration of each peptide in the assay was 10 μg/ml. Three technical replicates were performed. (2B-2F) IFN-γ production by TILs incubated with B cells transduced with tandem minigenes encompassing 29 non-synonymous mutations or the coding sequences from self-antigens Tyrosinase, Mage A3, Mart1, SSX2, and GP100 in the presence of brefeldin A (2B-2E) or pulsed with tumor-associated self-antigens (2F). The final concentration of each peptide in the assay was 10 μg/ml. Three technical replicates were performed. (2G) Frequency of TCR Vβ sequences in peripheral blood mononuclear cells after mock stimulation, $BRAF^{V600E}$ peptide stimulation, or after sorting IFN-γ secreting cells following $BRAF^{V600E}$ peptide re-stimulation. (2H) TCR Vβ clonotypes of BRAF-specific T cells quantitated by TCRβ sequencing of pre-treatment blood, tumor single cell suspension, and the TIL product infused into the patient. (2I) TCRVβ sequences in TIL product ranked by prevalence (right-hand portion of graph). (2J) Frequency of the top 34 TIL TCR Vβ clonotypes from (2I) in pre-treatment blood and post-treatment blood obtained at 10 and 24 months. (2K) Frequency of TCR Vβ clonotypes of $CD4^+$ $BRAF^{V600E}$ and $CD8^+$ T cells specific for the specified antigens in pre-treatment and post-treatment blood. (2L-2O) TCR β sequencing on the TIL and T cells from TIL incubated with autologous B cells and tiled peptides. Tiled peptides spanned (2L) Tyrosinase, (2M) Mart1, (2N) Mage A3, and

(2O) TRP2 and sorted by IFN-γ capture. Antigen-specific TCR β sequences enriched in the sorted cells are marked with a box.

Figures 3A-3C show phenotypic analysis for BRAF-specific T cells following treatment with tumor-infiltrating lymphocytes (TILs). (3A) Dump channel gating scheme for excluding monocytes ($CD14^+$), B cells ($CD19^+$), and dead cells (ViaProbe) from TIL. Viable CD4+ T cells were plotted against tetramer and CD45RA. (3B) CD45RA- memory cells (88.1% of $CD4^+$) that were tetramer-positive and –negative were plotted against the indicated cell surface markers. Numbers indicate the percentage of cells in the gated regions or the percentage of tetramer-positive cells for each marker. (3C) Intracellular cytokine staining of activated (CD154+) BRAF-specific T cells.

Figures 4A-4B show that a synthetic TCR derived from the dominant Vα and Vβ sequences of melanoma-responsive patient TILs recognizes cells expressing $BRAF^{V600E}$. (4A) Frequency of TCRBVa sequences in peripheral blood mononuclear cells after mock stimulation, $BRAF^{V600E}$ stimulation, or after sorting IFN-γ secretion cells following $BRAF^{V600E}$ peptide re-stimulation. (4B) IFN-γ production by $CD4^+$ T cells from two normal donors transduced with a synthetic TCR construct and incubated with an HLA-DQB1*0302 B cell line 1331 pulsed with $BRAF^{V600E}$ peptide or transfected with mRNA encoding mutant or wildtype BRAF sequences. N=2 or 3 technical replicates as indicated.

Figure 5 shows IFN-γ production by $CD4^+$ T cells transduced with one of four TCRs (pJV88-pJV91) made using TCRβ and TCRα genes identified in the patient TILs. Left, no antigen peptide. Right, antigen peptide.

Figures 6A and 6B show the effects of CRISPR-mediated knockout of endogenous TCR sequences on expression of a heterologous BRAF-specific TCR in primary human CD4+ T cells. (6A) Stimulated T cells were transfected with Cas9-RNPs targeting TCRA and TCRB and transduced with DNA encoding $BRAF^{V600E}$-specific TCR. BRAF-specific TCR expression was measured by Vbeta3.1 and TCR expression was measured using anti-CD3. Top panels: unmodified cells without (left) or with (right) CRISPR-mediated TCR knockout. Bottom panels: transduced cells

without (left) or with (right) CRISPR-mediated TCR knockout. (6B) Tetramer staining: T cells modified with $BRAF^{V600E}$-specific TCR with or without deletion of endogenous TCR were compared to untransduced cells (left-most peaks) and a patient-derived antigen-specific T cell clone (right-most peaks) for binding to tetramer.

DETAILED DESCRIPTION

In certain aspects, the present disclosure provides binding proteins, such as T cell receptors (TCRs), that are capable of specifically binding to a $BRAF^{V600E}$ peptide antigen, such as a $BRAF^{V600E}$ peptide antigen associated with a major histocompatibility complex (MHC) (*e.g.*, human leukocyte antigen, HLA). Binding proteins of this disclosure are useful in, for example, therapies to treat hyperproliferative diseases, such as cancer, characterized by $BRAF^{V600E}$ expression.

By way of background, antigens created by cancer-associated mutations are appealing targets for therapeutic intervention, but are generally unique to an individual patient. Thus, antigens caused by essential "driver" mutations of cancer are of interest since they are both specific to cancer cells and occur at high frequencies in patient populations. *BRAF* protein is involved in cell growth signaling, while mutant BRAF is implicated in a number of cancers (*see, e.g.,* Frasca *et al.*, *Endocrine-Related Cancer 15*:191(2008)). In particular, the substitution mutation V600E ($BRAF^{V600E}$), arising in exon 15 of the *BRAF* gene, activates BRAF to drive a growth signaling pathway that is an early event in carcinogenesis. This mutation is found in all instances of hairy cell leukemia, about half of malignant melanoma cases, and significant numbers of patients with advanced thyroid, lung and colon cancer.

The compositions and methods described herein will in certain embodiments have therapeutic utility for the treatment of diseases and conditions associated with $BRAF^{V600E}$ expression. Such diseases include various forms of hyperproliferative disorders, such as hairy cell leukemia, melanoma, thyroid cancers including poorly differentiated thyroid cancer, non-small cell lung cancer, colorectal cancer, papillary cancer, non-Hodgkin lymphoma, glioblastoma, and pilocytic astrocytoma, breast cancer, ovarian cancer, Langerhans cell histiocytosis, and sarcomas (*e.g.*, fibrosarcoma

(fibroblastic sarcoma), Dermatofibrosarcoma protuberans (DFSP), osteosarcoma, rhabdomyosarcoma, Ewing's sarcoma, a gastrointestinal stromal tumor, Leiomyosarcoma; angiosarcoma (vascular sarcoma), Kaposi's sarcoma, liposarcoma, pleomorphic sarcoma, and synovial sarcoma). Non-limiting examples of these and related uses are described herein and include *in vitro*, *ex vivo* and *in vivo* stimulation of $BRAF^{V600E}$ antigen-specific T cell responses, such as by the use of recombinant T cells expressing TCR specific for a $BRAF^{V600E}$ peptide.

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

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

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

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

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

"Major histocompatibility complex" (MHC) refers to glycoproteins that deliver peptide antigens to a cell surface. MHC class I molecules are heterodimers having a membrane spanning α chain (with three α domains) and a non-covalently associated β2 microglobulin. MHC class II molecules are composed of two transmembrane glycoproteins, α and β, both of which span the membrane. Each chain has two

domains. MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where a peptide:MHC complex is recognized by $CD8^+$ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where a peptide:MHC complex is recognized by $CD4^+$ T cells. Human MHC is referred to as human leukocyte antigen (HLA). HLA-II types include DP, DM, DOA, DOB, DQ, and DR. Numerous alleles encoding the subunits of the various HLA types are known, including, for example, HLA-DQA1*03, HLA-DQB1*0301, HLA-DQB1*0302, HLA-DQB1*0303. In certain embodiments, a binding protein according to the present disclosure is capable of recognizing a $BRAF^{V600E}$ peptide complexed with HLA-DQ. In certain embodiments, the HLA complex comprises HLA-DQB1*0301, *0302, or *0303. In certain embodiments, the HLA complex comprises HLA-DQB1*0302. In further embodiments, the HLA complex comprises HLA-DQA1*03.

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 be naïve (not exposed to antigen; increased expression of CD62L, CCR7, CD28, CD3, CD127, and CD45RA, and decreased expression of CD45RO as compared to T_{CM}), memory T cells (T_M) (antigen-experienced and long-lived), and effector cells (antigen-experienced, cytotoxic). T_M can be further divided into subsets of: central memory T cells (T_{CM}, increased expression of CD62L, CCR7, CD28, CD127, CD45RO, and CD95, and decreased expression of CD54RA as compared to naïve T cells); and effector memory T cells (T_{EM}, decreased expression of CD62L, CCR7, CD28, CD45RA, and increased expression of CD127 as compared to naïve T cells or T_{CM}).

Effector T cells (T_E) refers to antigen-experienced $CD8^+$ cytotoxic T lymphocytes that have decreased expression of CD62L ,CCR7, CD28, and are positive for granzyme and perforin as compared to T_{CM}. Helper T cells (T_H) are $CD4^+$ 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) refers to an immunoglobulin superfamily member (having a variable binding domain, a constant domain, a transmembrane region, and a short cytoplasmic tail; *see*, *e.g.*, Janeway *et al.*, Immunobiology: The Immune System in Health and Disease, 3rd Ed., Current Biology Publications, p. 4:33, 1997) capable of specifically binding to an antigen peptide bound to a MHC receptor. A TCR can be found on the surface of a cell or in soluble form and generally is comprised of a heterodimer having α and β chains (also known as TCRα and TCRβ, respectively), or γ and δ chains (also known as TCRγ and TCRδ, respectively). Like immunoglobulins, the extracellular portion of TCR chains (*e.g.*, α-chain, β-chain) contain two immunoglobulin domains: a variable domain (*e.g.*, α-chain variable domain or V_α, β-chain variable domain or V_β; typically amino acids 1 to 116 based on Kabat numbering (Kabat *et al.*, "Sequences of Proteins of Immunological Interest, US Dept. Health and Human Services, Public Health Service National Institutes of Health, 1991, 5th ed.)) at the N-terminus; and one constant domain (*e.g.*, α-chain constant domain or C_α, typically amino acids 117 to 259 based on Kabat, β-chain constant domain or C_β, typically amino acids 117 to 295 based on Kabat) adjacent to the cell membrane. Also, like immunoglobulins, the variable domains contain complementary determining regions (CDRs) separated by framework regions (FRs) (*see*, *e.g.*, Jores *et al.*, *Proc. Nat'l Acad. Sci. U.S.A. 87*:9138, 1990; Chothia *et al.*, *EMBO J. 7*:3745, 1988; *see also* Lefranc *et al.*, *Dev. Comp. Immunol. 27*:55, 2003). TCR variable domain sequences can be aligned to a numbering scheme (*e.g.*, Kabat, EU, International Immunogenetics Information System (IMGT) and Aho), which can allow equivalent residue positions to be annotated and for different molecules to be compared using Antigen receptor Numbering And Receptor Classification (ANARCI) software tool (2016, Bioinformatics 15:298-300). A numbering scheme provides a standardized delineation of framework regions and CDRs in the TCR variable domains.

In certain embodiments, a TCR is found on the surface of T cells (or T lymphocytes) and associates with the CD3 complex. The source of a TCR as used in the present disclosure may be from various animal species, such as a human, mouse, rat, rabbit or other mammal.

"CD3" is known in the art as a multi-protein complex of six chains (*see*, Abbas and Lichtman, 2003; Janeway *et al.*, p. 172 and 178, 1999). In mammals, the complex comprises a CD3γ chain, a CD3δ chain, two CD3ε chains, and a homodimer of CD3ζ chains. The CD3γ, CD3δ, and CD3ε chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3γ, CD3δ, and CD3ε chains are negatively charged, which is a characteristic that allows these chains to associate with the positively charged T cell receptor chains. The intracellular tails of the CD3γ, CD3δ, and CD3ε chains each contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM, whereas each CD3ζ chain has three ITAMs. Without wishing to be bound by theory, it is believed that the ITAMs are important for the signaling capacity of a TCR complex. CD3 as used in the present disclosure may be from various animal species, including human, mouse, rat, or other mammals.

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

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

"CD4" refers to an immunoglobulin co-receptor glycoprotein that assists the TCR in communicating with antigen-presenting cells (*see,* Campbell & Reece, Biology 909 (Benjamin Cummings, Sixth Ed., 2002); UniProtKB P01730). CD4 is found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells, and includes four immunoglobulin domains (D1 to D4) that are expressed at the cell surface. During antigen presentation, CD4 is recruited, along with the TCR complex, to bind to different regions of the MHCII molecule (CD4 binds MHCII β2, while the TCR complex binds MHCII α1/β1). Without wishing to be bound by theory, it is believed that close proximity to the TCR complex allows CD4-associated kinase molecules to phosphorylate the immunoreceptor tyrosine activation motifs (ITAMs) present on the cytoplasmic domains of CD3. This activity is thought to amplify the signal generated by the activated TCR in order to produce various types of T helper cells.

As used herein, the term "CD8 co-receptor" or "CD8" means the cell surface glycoprotein CD8, either as an alpha-alpha homodimer or an alpha-beta heterodimer. The CD8 co-receptor assists in the function of cytotoxic T cells ($CD8^+$) and functions through signaling via its cytoplasmic tyrosine phosphorylation pathway (Gao and Jakobsen, *Immunol. Today 21*:630-636, 2000; Cole and Gao, *Cell. Mol. Immunol. 1*:81-88, 2004). In humans, there are five (5) different CD8 beta chains (see UniProtKB identifier P10966) and a single CD8 alpha chain (see UniProtKB identifier P01732)

The term "variable region" or "variable domain" refers to the domain of a TCR α-chain or β-chain (or γ-chain and δ-chain for γδ TCRs) that is involved in binding of the TCR to antigen. The variable domains of the α-chain and β-chain (Vα and Vβ, respectively) of a native TCR generally have similar structures, with each domain comprising four generally conserved framework regions (FRs) and three CDRs. The Vα domain is encoded by two separate DNA segments, the variable gene segment and the joining gene segment (V-J); the Vβ domain is encoded by three separate DNA segments, the variable gene segment, the diversity gene segment, and the joining gene segment (V-D-J). A single Vα or Vβ domain may be sufficient to confer antigen-binding specificity. Furthermore, TCRs that bind a particular antigen may be isolated

using a Vα or Vβ domain from a TCR that binds the antigen to screen a library of complementary Vα or Vβ domains, respectively.

The terms "complementarity determining region," and "CDR," are synonymous with "hypervariable region" or "HVR," and are known in the art to refer to non-contiguous sequences of amino acids within TCR variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each α-chain variable region (αCDR1, αCDR2, αCDR3) and three CDRs in each β-chain variable region (βCDR1, βCDR2, βCDR3). CDR3 is thought to be the main CDR responsible for recognizing processed antigen. CDR1 and CDR2 mainly interact with the MHC. In certain embodiments, a binding protein of the present disclosure comprises an αCDR1, an αCDR2, and/or an αCDR3 amino acid sequence of a Vα domain as set forth in any one of SEQ ID NOs:1-4. In certain embodiments, a binding protein of the present disclosure comprises a βCDR1, a βCDR2, and/or a βCDR3 amino acid sequence of a Vβ domain as set forth in any one of SEQ ID NOs:5-7.

"Antigen" or "Ag" as used herein refers to an immunogenic molecule that provokes an immune response. This immune response may involve antibody 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 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. Exemplary antigens include $BRAF^{V600E}$.

The term "epitope" or "antigenic epitope" includes any molecule, structure, 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 characteristics, as well as specific charge characteristics.

A "binding domain" (also referred to as a "binding region" or "binding moiety"), as used herein, refers to a molecule or portion thereof (*e.g.*, peptide, oligopeptide, polypeptide, protein) that possesses the ability to specifically and non-covalently associate, unite, or combine with a target (*e.g.*, $BRAF^{V600E}$). A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule, a molecular complex (*i.e.*, complex comprising two or more biological molecules), or other target of interest. Exemplary binding domains include single chain immunoglobulin variable regions (*e.g.*, scTCR, scFv), receptor ectodomains, ligands (*e.g.*, cytokines, chemokines), or synthetic polypeptides selected for their specific ability to bind to a biological molecule, a molecular complex or other target of interest.

As used herein, "specifically binds" or "specific for" refers to an association or union of a binding protein (*e.g.*, TCR receptor) or a binding domain (or fusion protein thereof) to a target molecule with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10^5 M^{-1} (which equals the ratio of the on-rate [k_{on}] to the off-rate [k_{off}] for this association reaction), while not significantly associating or uniting with any other molecules or components in a sample. Binding proteins or binding domains (or fusion proteins thereof) may be classified as "high-affinity" binding proteins or binding domains (or fusion proteins thereof) or as "low-affinity" binding proteins or binding domains (or fusion proteins thereof). "High-affinity" binding proteins or binding domains refer to those binding proteins or binding domains having a K_a of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, or at least 10^{13} M^{-1}. "Low-affinity" binding proteins or binding domains refer to those binding proteins or binding domains having a K_a of up to 10^7 M^{-1}, up to 10^6 M^{-1}, up to 10^5 M^{-1}. Alternatively, affinity may be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (*e.g.*, 10^{-5} M to 10^{-13} M).

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

A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, as well as determining binding domain or fusion protein affinities, such as Western blot, ELISA, analytical ultracentrifugation, spectroscopy and surface plasmon resonance (Biacore®) analysis (*see*, *e.g.*, Scatchard *et al.*, *Ann. N.Y. Acad. Sci. 51*:660, 1949; Wilson, *Science 295*:2103, 2002; Wolff *et al.*, *Cancer Res. 53*:2560, 1993; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent). Assays for assessing affinity or apparent affinity or relative affinity are also known. In certain examples, apparent affinity for a TCR is measured by assessing binding to various concentrations of tetramers, for example, by flow cytometry using labeled tetramers. In some examples, apparent K_D of a TCR is measured using 2-fold dilutions of labeled tetramers at a range of concentrations, followed by determination of binding curves by non-linear regression, apparent K_D being determined as the concentration of ligand that yielded half-maximal binding.

The term "$BRAF^{V600E}$-specific binding protein" refers to a protein or polypeptide that specifically binds to a $BRAF^{V600E}$ peptide antigen or a $BRAF^{V600E}$ peptide antigen:HLA complex, *e.g.*, on a cell surface, and does not bind a HLA complex on a cell surface comprising a BRAF peptide not containing the $BRAF^{V600E}$ mutation.

In certain embodiments, a $BRAF^{V600E}$ -specific binding protein binds a $BRAF^{V600E}$-containing peptide:HLA complex (or $BRAF^{V600E}$-containing peptide:MHC complex) with a K_d of less than about 10^{-8} M, less than about 10^{-9} M, less than about 10^{-10} M, less than about 10^{-11} M, less than about 10^{-12} M, or less than about 10^{-13} M, or with an affinity that is about the same as, at least about the same as, or is greater than at or about the affinity exhibited by an exemplary $BRAF^{V600E}$-specific binding protein provided herein, such as any of the $BRAF^{V600E}$-specific TCRs provided herein, for example, as measured by the same assay. In certain embodiments, a $BRAF^{V600E}$-specific binding protein comprises a $BRAF^{V600E}$-specific immunoglobulin superfamily binding protein or binding portion thereof.

The term "$BRAF^{V600E}$ binding domain" or "$BRAF^{V600E}$ binding fragment" refers to a domain or portion of a $BRAF^{V600E}$-specific binding protein responsible for the specific $BRAF^{V600E}$ binding. A $BRAF^{V600E}$-specific binding domain alone (*i.e.*, without any other portion of a $BRAF^{V600E}$-specific binding protein) can be soluble and can bind to $BRAF^{V600E}$ (*e.g.*, in complex with an MHC receptor molecule or functional fragment thereof) with a K_d of less than about 10^{-8} M, less than about 10^{-9} M, less than about 10^{-10} M, less than about 10^{-11} M, less than about 10^{-12} M, or less than about 10^{-13} M. Exemplary $BRAF^{V600E}$-specific binding domains include $BRAF^{V600E}$-specific scTCR (*e.g.*, single chain αβTCR proteins such as Vα-L-Vβ, Vβ-L-Vα, Vα-Cα-L-Vα, or Vα-L-Vβ-Cβ, wherein Vα and Vβ are TCRα and β variable domains respectively, Cα and Cβ are TCRα and β constant domains, respectively, and L is a linker) and scFv fragments as described herein, which can be derived from an anti- $BRAF^{V600E}$ TCR or antibody.

Principles of antigen processing by antigen presenting cells (APC) (such as dendritic cells, macrophages, lymphocytes or other cell types), and of antigen presentation by APC to T cells, including major histocompatibility complex (MHC)-restricted presentation between immunocompatible (*e.g.,* sharing at least one allelic form of an MHC gene that is relevant for antigen presentation) APC and T cells, are well established (*see, e.g.,* Murphy, Janeway's Immunobiology (8^{th} 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 generally vary in length from about 10 amino acids to about 25 amino acids and associate with class II MHC molecules.

"$BRAF^{V600E}$ antigen" or " $BRAF^{V600E}$ peptide antigen" or "$BRAF^{V600E}$-containing peptide antigen" refers to a naturally or synthetically produced portion of a BRAF protein ranging in length from about 7 amino acids to about 20 amino acids and comprising the V600E substitution mutation (*e.g.*, a peptide from $BRAF^{597-603}$, or $BRAF^{590-610}$, that includes a glutamic acid substituted for a valine at the residue corresponding to position 600 of the full-length wild-type BRAF; *see*, *e.g.*, Uniprot entry no. P15056 and NCBI Reference identifier NP_004324.2), which can form a complex with a MHC (*e.g.*, HLA) molecule and such a complex can bind with a binding protein specific for a $BRAF^{V600E}$ peptide:MHC (*e.g.*, HLA) complex. Exemplary $BRAF^{V600E}$ peptide antigens include those having the amino acid sequence set forth in SEQ ID NO.: 38 or 39.

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

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

An "altered domain" or "altered protein" refers to a motif, region, domain, peptide, polypeptide, or protein with a non-identical sequence identity to a wild type motif, region, domain, peptide, polypeptide, or protein (*e.g.*, a wild type TCRα chain, TCRβ chain, TCRα constant domain, TCRβ constant domain) of at least 85% (*e.g.*, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%).

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

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

following the coding region ("leader and trailer") as well as intervening sequences (introns) between individual coding segments (exons).

As used herein, the term "recombinant" refers to a cell, microorganism, nucleic acid molecule, or vector that has been genetically engineered by human intervention – that is, modified by introduction of a heterologous nucleic acid molecule, or refers to a cell or microorganism that has been altered such that expression of an endogenous nucleic acid molecule or gene is controlled, deregulated, deleted, attenuated, or constitutive. Human generated genetic alterations may include, for example, modifications that introduce nucleic acid molecules (which may include an expression control element, such as a promoter) that encode one or more proteins or enzymes, or other nucleic acid molecule additions, deletions, substitutions, or other functional disruption of or addition to a cell's genetic material. Exemplary modifications include those in coding regions or functional fragments thereof of heterologous or homologous polypeptides from a reference or parent molecule.

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

A "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are 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" 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).

Viral vectors include retrovirus, adenovirus, parvovirus (*e.g.*, adeno-associated viruses), coronavirus, negative strand RNA viruses such as ortho-myxovirus (*e.g.*, influenza virus), rhabdovirus (*e.g.*, rabies and vesicular stomatitis virus), paramyxovirus (*e.g.*, measles and Sendai), positive strand RNA viruses such as picornavirus and alphavirus, and double-stranded DNA viruses including adenovirus, herpesvirus (*e.g.*, Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (*e.g.*, vaccinia, fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include avian leukosis-sarcoma, mammalian C-type, B-type viruses, D type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., Retroviridae: The viruses and their replication, In Fundamental Virology, Third Edition, B. N. Fields *et al.*, Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

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

The term "operably linked" 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 sequence when it is

capable of affecting the expression of that coding sequence (*i.e.*, the coding sequence is under the transcriptional control of the promoter). "Unlinked" means that the associated genetic elements are not closely associated with one another and the function of one does not affect the other.

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

The term "expression", as used herein, refers to the process by which a polypeptide is produced based on the encoding sequence of a nucleic acid molecule, such as a gene. The process may include transcription, post-transcriptional control, post-transcriptional modification, translation, post-translational control, post-translational modification, or any combination thereof.

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

As used herein, "heterologous" nucleic acid molecule, construct or sequence 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 nucleic acid molecule,

construct or sequence may be from a different genus or species. In certain embodiments, a heterologous nucleic acid molecule is added (*i.e.*, is 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 a heterologous polynucleotide introduced into the host cell, even if the host cell encodes a homologous protein or activity.

As described herein, more than one heterologous 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 nucleic acid molecules encoding desired binding proteins specific for a $BRAF^{V600E}$ antigen peptide (*e.g.*, TCRα and TCRβ). When two or more heterologous nucleic acid molecules are introduced into a host cell, it is understood that the two or more heterologous 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" refers to a gene, protein, or activity that is normally present in a host cell. Moreover, a gene, protein or activity that is mutated, overexpressed, shuffled, duplicated or otherwise altered as compared to a parent gene, protein or activity is still considered to be endogenous or native to that particular host cell. For example, an endogenous control sequence from a first gene (*e.g.*, promoter, translational attenuation sequences) may be used to alter or regulate expression of a second native gene or nucleic acid molecule, wherein the expression or

regulation of the second native gene or nucleic acid molecule differs from normal expression or regulation in a parent cell.

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

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

As used herein, a "hematopoietic progenitor cell" is a cell that can be derived from hematopoietic stem cells or fetal tissue and is capable of further differentiation into mature cells types (*e.g.*, immune system cells). Exemplary hematopoietic progenitor cells include those with a $CD24^{Lo}$ Lin^{-} $CD117^{+}$ phenotype or those found in the thymus (referred to as progenitor thymocytes).

As used herein, the term "host" refers to a cell (*e.g.*, T cell) or microorganism targeted for genetic modification with a heterologous nucleic acid molecule to produce a polypeptide of interest (*e.g.*, an anti-$BRAF^{V600E}$ 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, *e.g.*, biosynthesis of the heterologous protein (*e.g.*, inclusion of a detectable marker; deleted, altered or truncated endogenous TCR; or increased co-stimulatory factor expression). In certain embodiments, a host cell is a human hematopoietic progenitor cell transduced with a

heterologous nucleic acid molecule encoding a TCRα chain specific for a $BRAF^{V600E}$ antigen peptide.

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

Binding Proteins Specific for $BRAF^{V600E}$ Peptide:HLA Complexes

BRAF (also known as B-RAF1, BRAF1, NS7, RAFB1, B-Raf, B-Raf proto-oncogene, and serine/threonine kinase) refers to a 766-amino acid protein encoded by the *BRAF* gene. The transcript sequence for human wild-type *BRAF* is set forth in NCBI Reference identifier NM_004333.4 (SEQ ID NO:78), and the protein sequence is set forth in NCBI Reference identifier NP_004324.2 (SEQ ID NO:36). BRAF is a member of the Raf kinase family of growth signal transduction protein kinases, and plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion. In terms of structure, BRAF is composed of three conserved domains characteristic of Raf kinases: a Ras-GTP-binding self-regulatory domain; a serine-rich hinge region; and a catalytic kinase domain that phosphorylates a consensus sequence on protein substrates (CR1, CR2, and CR3, respectively). Active B-Raf forms dimers.

A mutant form of BRAF kinase comprising a V600E mutation ($BRAF^{V600E}$) is an oncogenic driver present in numerous neoplastic conditions, including 40% of melanoma cases, 10% of colorectal cancer cases, and 1% of non-small cell lung cancer cases, and confers constitutive signaling that promotes tumor cell growth and survival. Small molecule BRAF inhibitors have some efficacy in melanoma, but resistance evolves by recruitment of alternative signaling pathways without loss of expression of $BRAF^{V600E}$ protein. *See* Shi *et al.*, *Cancer Disc.* *4*(1):80 (2014).

In certain aspects, the present disclosure provides a binding protein comprising: (a) a T cell receptor (TCR) α chain variable (Vα) domain having a CDR3 amino acid sequence set forth in any one of SEQ ID NOS:29-32, or a CDR3 amino acid sequence set forth in any one of SEQ ID NOS:29-32 with up to five amino acid substitutions, insertions, and/or deletions, and a TCR β chain variable (Vβ) domain; (b) a Vα domain, and a Vβ domain having a CDR3 amino acid sequence as set forth in any one of SEQ ID NOS:33-35, or a CDR3 amino acid sequence set forth in any one of SEQ ID NOS:33-35 with up to five amino acid substitutions, insertions, and/or deletions; or (c) a Vα domain of (a) and a Vβ domain of (b), wherein the binding protein is capable of specifically binding to a HLA complex on a cell surface comprising a BRAF peptide containing a $BRAF^{V600E}$ mutation, and does not bind a HLA complex on a cell surface comprising a BRAF peptide not containing the $BRAF^{V600E}$ mutation. In certain embodiments, the HLA complex comprises HLA-DQ.

In certain embodiments, (a) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 29 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:33, (b) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 30 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:34, (c) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 31 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:34, (d) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 32 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:35, (e) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 29 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:34, (f) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 29 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:35, (g) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 30 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:33, (h) the Vα domain comprises the CDR3 amino acid of SEQ ID NO: 30 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:35, (i) the Vα domain comprises the CDR3 amino acid of SEQ ID NO: 31 and the Vβ domain comprises the

CDR3 amino acid sequence of SEQ ID NO:33, (j) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 31 and the Vβ domain comprises the CDR3 amino acid sequence ofn SEQ ID NO:35, (k) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO:32 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:33, or (l) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO:32 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:34.

Peptide-MHC complexes, such as $BRAF^{V600E}$peptide:HLA complexes, are recognized by and bound by a TCR through the Vα and Vβ domains. During lymphocyte development, Vα exons are assembled from different variable and joining gene segments (V-J), and Vβ exons are assembled from different variable, diversity, and joining gene segments (V-D-J). The TCRα chromosomal locus has 70-80 variable gene segments and 61 joining gene segments. The TCRβ chromosomal locus has 52 variable gene segments, and two separate clusters of each containing a single diversity gene segment, together with six or seven joining gene segments. Functional Vα and Vβ gene exons are generated by the recombination of a variable gene segment with a joining gene segment for Vα, and a variable gene segment with a diversity gene segment and a joining gene segment for Vβ.

The Vα and Vβ domains each comprise three hypervariable loops, also referred to as complementary determining regions (CDRs) that contact the peptide-MHC complex. CDR1 and CDR2 are encoded within the variable gene segment, whereas CDR3 is encoded by the region spanning the variable and joining segments for Vα, or the region spanning variable, diversity, and joining segments for Vβ. Compared with CDR1 and CDR2, CDR3 is significantly more diverse because of the addition and loss of nucleotides during the recombination process.

TCR variable domain sequences can be aligned to a numbering scheme (Kabat, Chothia, Enhanced Chothia, and Aho), allowing equivalent residue positions to be annotated and for different molecules to be compared using 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.

Accordingly, CDR1 and CDR2 sequences may be deduced from the corresponding variable gene segments (*e.g.*, TCRBV28-01, TCRAV21-01, TCRAV26-01, TCRAV12-02 alleles). In certain embodiments, (a) the Vα CDR3 amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO:29 and the Vα domain further comprises a CDR1 amino acid sequence and CDR2 amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:11, (b) the Vα CDR3 amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO:30 and the Vα domain further comprises a CDR1 amino acid sequence and CDR2 amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:12, (c) the Vα CDR3 amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO:31 and the Vα domain further comprises a CDR1 amino acid sequence and CDR2 amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:13, or (d) the Vα CDR3 amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO:32 and the Vα domain further comprises a CDR1 amino acid sequence and CDR2 amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:13. In certain embodiments, the Vβ CDR3 amino acid sequence comprises the amino acid sequence set forth in any one of SEQ ID NOS:33-35 and the Vβ domain further comprises a CDR1 amino acid sequence and CDR2 amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:8.

Methods of identifying binding pairs of TCR Vα and Vβ domains include, for example, those described in PCT Patent Publication No. WO 2016/161273; Redmond et al., 2016, Genome Med. 8: 80; Munson et al., 2016, Proc. Natl. Acad. Sci. 113:8272-7; Kim et al., 2012, PLoS ONE 7:e37338 (each of the methods from which are incorporated by reference in its entirety). Accordingly, a Vα domain for the $BRAF^{V600E}$-specific Vβ domains described herein (e.g., a Vβ domain comprising CDR3 as set forth in any one of SEQ ID NOS:33-35), or vice versa, may be identified.

A $BRAF^{V600E}$-specific binding protein described herein may possess one or more amino acid substitutions, deletions, or insertions relative to a naturally occurring binding protein (*e.g.*, TCR). Conservative substitutions of amino acids are known and

may occur naturally or may be introduced when the binding protein or TCR is recombinantly produced. Amino acid substitutions, deletions, and insertions may be introduced into a protein using mutagenesis methods (*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 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).

Accordingly, in certain embodiments, the binding protein of the instant disclosure comprises a Vα domain that is at least about 90% (*e.g.*, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or at least about 99.9%) identical to the amino acid sequence set forth in any one of SEQ ID NOS:1-4, and comprises a Vβ domain that is at least about 90% (*e.g.*, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or at least about 99.9%) identical to the amino acid sequence set forth in any one of SEQ ID NOS:5-7, provided that (a) at least three or four of the CDRs have no mutations and (b) the CDRs that do have mutations have only up to three amino acid substitutions, insertions, deletions or combinations thereof. In certain embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOS:1-4. In certain embodiments, the Vβ domain comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOS:5-7.

In particular embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:1 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:5.

In other embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:1 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:6.

In other embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:1 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:7.

In particular embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:2 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:5.

In other embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:2 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:6.

In other embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:2 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:7.

In particular embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:3 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:5.

In other embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:3 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:6.

In other embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:3 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:7.

In particular embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:4 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:5.

In other embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:4 and the Vβ domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:6.

In other embodiments, the Vα domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:4 and the Vβ domain comprises or consists of of the amino acid sequence set forth in SEQ ID NO:7.

In further embodiments, a $BRAF^{V600E}$-specific binding protein is a TCR, an antigen-binding fragment of a TCR, or a chimeric antigen receptor. A "chimeric antigen receptor" (also called a CAR) is a fusion protein comprising an antigen binding domain (*e.g.*, obtained or derived from an immunoglobulin or immunoglobulin-like molecule, such as an scFv derived from an antibody or TCR specific for a cancer antigen, or an antigen-binding domain obtained or derived 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): 388-398, 2013; *see also* Harris and Kranz, *Trends Pharmacol. Sci.*, *37*(3): 220-230, 2016; Stone *et al.*, *Cancer Immunol. Immunother.*, *63*(11):1163-1176,2014). In certain embodiments, a binding protein comprises a CAR comprising a $BRAF^{V600E}$-specific TCR binding domain (*see*, *e.g.*, Walseng *et al.*, *Scientific Reports 7*:10713, 2017; the TCR CAR constructs and methods of which are hereby incorporated by reference in their entirety). Methods of making CARs are described, for example, in U.S. Patent No. 6,410,319; U.S. Patent No. 7,446,191; U.S. Patent Publication No. 2010/065818; U.S. Patent No. 8,822,647; PCT Publication No. WO 2014/031687; U.S. Patent No. 7,514,537; and Brentjens *et al.*, *Clin. Cancer Res. 13*:5426, 2007.

In certain embodiments, the antigen-binding fragment of the TCR comprises a single chain TCR (scTCR), which comprises both the TCR Vα and Vβ domains TCR, but only a single TCR constant domain (Cα or Cβ). In certain embodiments, the antigen-binding fragment of the TCR, or chimeric antigen receptor is chimeric (*e.g.*, comprises amino acid residues or motifs from more than one donor or species), humanized (*e.g.*, comprises residues from a non-human organism that are altered or substituted so as to reduce the risk of immunogenicity in a human), or human. Binding proteins according to the present disclosure, *e.g.*, TCRs, may further comprise a TCR constant domain, *e.g.*, joined to the C-terminus of a Vα domain, a Vβ domain, or both. A TCR β-chain constant domain may be encoded by a TRBC1 gene or TRBC2 gene, and a TCRα-chain may be encoded by a TRAC gene. In certain embodiments, the TCR comprises an α chain constant (Cα) domain having at least 90% (*e.g.*, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or at least about 99.9%) sequence identity to the amino acid sequence set forth in SEQ ID NO:25. In a particular embodiment, the Cα domain comprises the amino acid sequence set forth in SEQ ID NO: 25. In certain embodiments, the TCR comprises a β chain (Cβ) constant domain having at least 90% (*e.g.*, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%,

99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or at least about 99.9%) sequence identity to the amino acid sequence set forth in SEQ ID NO:26. In a particular embodiment, Cβ domain comprises the amino acid sequence set forth in SEQ ID NO: 26.

In certain embodiments, a binding protein comprises a α-chain (comprised of a Vα domain and a Cα domain) comprising the amino acid sequence set forth in any one of SEQ ID NOS: 55-58. In certain embodiments, a binding protein comprises a β-chain (comprised of a Vβ domain and a Cβ domain) comprising the amino acid sequence set forth in any one of SEQ ID NOS:59-61.

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

In certain embodiments, nucleic acid molecules encoding a binding protein (*e.g.*, a TCR) specific for a $BRAF^{V600E}$ peptide:HLA complex) are used to transfect/transduce a host cell (*e.g.*, a T cell) 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 embodiments according to the present disclosure. Similarly,

methods for transfecting/transducing T cells with desired nucleic acids have been described (*e.g.*, U.S. Patent Application Pub. No. US 2004/0087025) as have adoptive transfer procedures using T cells of desired antigen-specificity (*e.g.*, Schmitt *et al.*, *Hum. Gen. 20*:1240, 2009; Dossett *et al.*, *Mol. Ther. 17*:742, 2009; Till *et al.*, *Blood 112*:2261, 2008; Wang *et al.*, *Hum. Gene Ther. 18*:712, 2007; Kuball *et al.*, *Blood 109*:2331, 2007; US 2011/0243972; US 2011/0189141; Leen *et al.*, *Ann. Rev. Immunol. 25*:243, 2007), such that adaptation of these methodologies to the presently disclosed embodiments is contemplated, based on the teachings herein, including those directed to TCRs specific for $BRAF^{V600E}$ peptide antigens complexed with an HLA receptor.

The $BRAF^{V600E}$-specific binding proteins or domains as described herein may be functionally characterized according to any of a large number of art-accepted methodologies for assaying T cell activity, including determination of T cell binding, activation or induction and also including determination of T cell responses that are antigen-specific. Examples include determination of T cell proliferation, T cell cytokine release, antigen-specific T cell stimulation, MHC restricted T cell stimulation, CTL activity (*e.g.*, by detecting ^{51}Cr release from pre-loaded target cells), changes in T cell phenotypic marker expression, and other measures of T-cell functions. Procedures for performing these and similar assays are may be found, for example, in Lefkovits (*Immunology Methods Manual: The Comprehensive Sourcebook of Techniques*, 1998). *See*, *also*, *Current Protocols in Immunology*; Weir, *Handbook of Experimental Immunology*, Blackwell Scientific, Boston, MA (1986); Mishell and Shigii (eds.) *Selected Methods in Cellular Immunology*, Freeman Publishing, San Francisco, CA (1979); Green and Reed, *Science* 281:1309 (1998) and references cited therein.

"MHC-peptide tetramer staining" refers to an assay used to detect antigen-specific T cells, which features a tetramer of MHC molecules, each comprising an identical peptide having an amino acid sequence that is cognate (*e.g.*, identical or related to) at least one antigen (*e.g.*, $BRAF^{V600E}$), wherein the complex is capable of binding T cell receptors specific for the cognate antigen. Each of the MHC molecules may be tagged with a biotin molecule. Biotinylated MHC/peptides are tetramerized by

the addition of streptavidin, which can be fluorescently labeled. The tetramer may be detected by flow cytometry via the fluorescent label. In certain embodiments, an MHC-peptide tetramer assay is used to detect or select enhanced affinity TCRs of the instant disclosure.

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

In further aspects, the present disclosure provides compositions comprising a binding protein according to the present disclosure and a pharmaceutically acceptable carrier, diluent, or excipient. 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.

Antigen presentation by immune cells (*e.g.*, dendritic cells, phagocytes, and B cells) is determined in part by the HLA complexes present on the cells. Without wishing to be bound by theory, it is believed that HLA proteins encoded by different HLA alleles can vary in their ability to present particular antigen peptides and interact with immune cell proteins (*e.g.*, TCRs). For example, a given antigen peptide may be presented by HLA-DQ complexes, but not HLA-DR complexes, or vice versa. In certain embodiments, a binding protein according to the present disclosure is capable of

recognizing a BRAFV^{600E} peptide complexed with HLA-DQ. In certain embodiments, the HLA complex comprises HLA-DQB1*0301, *0302, or *0303. In certain embodiments, the HLA complex comprises HLA-DQB1*0302. In further embodiments, the HLA complex comprises HLA-DQA1*03.

Peptide antigens targeted by binding proteins according to the present disclosure can also vary on size depending on, for example, the type of HLA molecule presenting the antigen. Generally, HLA Class I complexes present peptides that are about 8-10 amino acids length, while HLA Class II complexes present peptides that are about 15-24 amino acids in length, though the peptides may be shorter or longer than these general lengths. Accordingly, in certain embodiments, a BRAF^{V600E} peptide specifically bound by a binding protein of the present disclosure comprises from about 7 to about 27 amino acids, from about 10 to about 25 amino acids, or from about 12 to about 20 amino acids, or from about 15 to about 19 amino acids. In particular embodiments, the BRAF^{V600E} peptide comprises the amino acid sequence set forth in SEQ ID NO:38 or 39.

Polynucleotides Encoding BRAF^{V600E}-Specific Binding Proteins and Related Vectors

In yet further aspects, isolated polynucleotides and expression vectors that encode binding proteins according to the present disclosure are provided. Construction of an expression vector that is used for genetically engineering and producing a binding protein or TCR specific for a BRAF^{V600E} peptide of interest can be accomplished by using any suitable molecular biology engineering techniques known in the art. 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 polynucleotides that encode the binding proteins provided herein, such as binding proteins (*e.g.*, TCRs or CARs) specific for a BRAF^{V600E} peptide:HLA complex. A nucleic acid may be a single- or a double-stranded DNA, cDNA or RNA in any form, and may include a positive and a negative strand of the nucleic acid which complement each other, including anti-sense DNA,

cDNA and RNA. Also included are siRNA, microRNA, RNA—DNA hybrids, ribozymes, and other various naturally occurring or synthetic forms of DNA or RNA. It will be appreciated that a polynucleotide of the present disclosure can vary (*i.e.*, comprise a different nucleotide sequence) as compared to a reference polynucleotide sequence disclosed herein and still encode a same amino acid or polypeptide, due to, for example, the degeneracy of the genetic code. In certain embodiments, a polynucleotide encoding, for example, a binding protein or a portion thereof, a self-cleaving peptide, a linker peptide; or a binding protein-encoding construct, may have at least about 80% (*e.g.*, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or at least about 99.9%) identity to a polynucleotide according to any one of SEQ ID NOs:8-24; 27; 28; 44-48; 62-68; and 73-77.

In any of the aforementioned embodiments, a polynucleotide encoding a binding protein of the present disclosure is codon optimized for efficient expression in a target host cell.

Certain embodiments include polynucleotides of this disclosure contained in a vector. An exemplary vector may comprise a polynucleotide capable of transporting another polynucleotide to which it has been linked, or which is capable of replication in a host organism. Some examples of vectors include plasmids, viral vectors, cosmids, and others. Some vectors may be capable of autonomous replication in a host cell into which they are introduced (*e.g.* bacterial vectors having a bacterial origin of replication and episomal mammalian vectors), whereas other vectors may be integrated into the genome of a host cell or promote integration of the polynucleotide insert upon introduction into the host cell and thereby replicate along with the host genome (*e.g.*, lentiviral vector, retroviral vector). Additionally, some vectors are capable of directing the expression of genes to which they are operatively linked (these vectors may be referred to as "expression vectors"). According to related embodiments, it is further understood that, if one or more agents (*e.g.*, polynucleotides encoding binding proteins or recombinant TCRs specific for $BRAF^{V600E}$, or variants thereof, as described herein) are co-administered to a subject, that each agent may reside in separate or the same

vectors, and multiple vectors (each containing a different agent or the same agent) may be introduced to a cell or cell population or administered to a subject.

In certain embodiments, polynucleotides encoding binding proteins specific for a $BRAF^{V600E}$ peptide:HLA complex may be operatively linked to certain elements of a vector. For example, polynucleotide sequences that are needed to effect the expression and processing of coding sequences to which they are ligated may be operatively linked. Expression control sequences may include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequences); sequences that enhance protein stability; and possibly sequences that enhance protein secretion. Expression control sequences may be operatively linked if they are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. In certain embodiments, polynucleotides encoding binding proteins of the instant disclosure are contained in an expression vector that is a viral vector, such as a lentiviral vector or a γ-retroviral vector.

In certain embodiments, expression vectors are provided comprising a polynucleotide encoding a binding protein of the present disclosure, wherein the polynucleotide is operably linked to an expression control sequence (*e.g.*, a promoter). In certain embodiments, the vector is capable of delivering the polynucleotide to a host cell. In certain embodiments, the host cell is a hematopoietic progenitor cell or a human immune system cell. In further embodiments, the immune system cell is a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a natural killer cell, a dendritic cell, or any combination thereof. In certain embodiments, the immune system cell is a CD4+ T cell. In certain embodiments, the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

In any of the embodiments herein, the vector is a viral vector. In certain embodiments, the viral vector is a lentiviral vector or a γ-retroviral vector.

Host Cells

In still further aspects, host cells are provided that comprise a heterologous polynucleotide according to the present disclosure, wherein the host cell expresses on its cell surface a binding protein encoded by the heterologous polynucleotide (*i.e.*, expresses a binding protein according to the present disclosure). In particular embodiments, an 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). In certain embodiments, a host cell (*e.g.*, a T cell, NK cell, or NK-T cell) lacks a CD8 co-receptor or a CD4 co-receptor and the encoded binding protein is capable of binding a $BRAF^{V600E}$ antigen:HLA complex in the absence of a CD4 or CD8 co-receptor. 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 γδ T cell, a natural killer cell, a natural killer T cell, a dendritic cell, or any combination thereof. In some embodiments, the encoded binding protein comprises a MHCII-restricted TCR binding domain and the host cell (*e.g.*, a $CD8^+$ T cell) comprises a polynucleotide encoding a heterologous $CD4^+$ co-receptor.

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, a stem cell memory T cell, or any combination thereof. In certain embodiments, the T cell is a $CD4^+$ T cell, a $CD8^+$ T cell, or both. The expression vectors introduced into the host cells may 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).

A host cell may include any individual cell or cell culture which may receive a vector or the incorporation of nucleic acids or express proteins. 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).

Accordingly, in one aspect, a host cell is provided that comprises a heterologous polynucleotide or an expression vector according to the present disclosure, wherein the host cell expresses on its cell surface a binding protein encoded by the heterologous polynucleotide. In certain embodiments, the portion of the heterologous polynucleotide that encodes the V_α domain is at least about 80% identical to the polynucleotide sequence set forth in any one of SEQ ID NOS:18-21. In certain embodiments, the portion of the heterologous polynucleotide that encodes the V_β domain is at least about 80% identical to the polynucleotide sequence set forth in any one of SEQ ID NOS:22-24.

In certain embodiments, the portion of the heterologous polynucleotide that encodes the V_α domain comprises or consists of the polynucleotide sequence set forth in any one of SEQ ID NOS:18-21. In certain embodiments, the portion of the heterologous polynucleotide that encodes the V_β domain comprises or consists of the polynucleotide sequence set forth in any one of SEQ ID NOS:22-24.

In certain embodiments, the portion of the heterologous polynucleotide that encodes the V_α domain comprises or consists of the polynucleotide sequence set forth in any one of SEQ ID NOS:18-21, and the portion of the heterologous polynucleotide that encodes the V_β domain comprises or consists of the polynucleotide sequence set forth in any one of SEQ ID NOS:22-24.

In certain embodiments, the portion of the heterologous polynucleotide that encodes the V_α domain is linked to a portion that encodes a TCR α-chain constant domain, wherein the portion that encodes the α-chain constant domain comprises or consists of a sequence that is at least about 80% identical to the polynucleotide sequence set forth in SEQ ID NO:27.

In certain embodiments, the portion of the heterologous polynucleotide that encodes the V_β domain is linked to a portion that encodes a TCR β-chain constant domain, wherein the portion that encodes the β-chain constant domain comprises or consists of a sequence that is at least about 80% identical to the polynucleotide sequence set forth in SEQ ID NO:28.

In particular embodiments, the portion of the polynucleotide that encodes the V_α domain comprises or consists of SEQ ID NO:18 and the portion that encodes the V_β domain comprises or consists of SEQ ID NO:22.

In other embodiments, the portion of the polynucleotide that encodes the V_α domain comprises or consists of SEQ ID NO: 19 and the portion that encodes the V_β domain comprises or consists of SEQ ID NO:23.

In other embodiments, the portion of the polynucleotide that encodes the V_α domain comprises or consists of SEQ ID NO:20 and the portion that encodes the V_β domain comprises or consists of SEQ ID NO:23.

In still other embodiments, the portion of the polynucleotide that encodes the V_α domain comprises or consists of SEQ ID NO: 21 and the portion that encodes the V_β domain comprises or consists of SEQ ID NO:24.

In any of the herein described embodiments, a host cell (*e.g.*, a T cell) expressing a $BRAF^{V600E}$-specific binding protein of the present disclosure is capable of producing an interferon when co-cultured with an antigen-presenting cell presenting or expressing a $BRAF^{V600E}$–containing antigen. In certain embodiments, the produced interferon comprises interferon-gamma (IFN-γ). In some embodiments, the target cell has been pulsed with a peptide or polypeptide comprising or consisting of the $BRAF^{V600E}$–containing antigen. In some embodiments, the target cell has been transfected with a polynucleotide (*e.g.*, DNA, cDNA, or mRNA) encoding a polypeptide or peptide comprising or consisting of the $BRAF^{V600E}$–containing antigen. In certain embodiments, a host cell of the present disclosure produces IFN-γ when cultured with an antigen-presenting cell that has been pulsed with a $BRAF^{V600E}$–containing antigen at a concentration of about 0.005 μg/mL to about 10 μg/mL antigen. In further embodiments, the host cell produces at least about 1,000 pg/mL IFN-γ when

cultured with an antigen-presenting cell that has been pulsed with a $BRAF^{V600E}$–containing antigen at a concentration of at least about 0.1μg/mL antigen. In some embodiments, the host cell produces at least about 1,000 pg/mL IFN-γ when cultured with an antigen-presenting cell pulsed with a $BRAF^{V600E}$–containing antigen at a concentration of at least about 0.1, 0.2, 0.3, 0.4, or 0.5 μg/mL antigen. In some embodiments, the host cell produces from about 1,000 pg/mL to about 10,000 pg/mL IFN-γ when cultured with an antigen-presenting cell pulsed with a $BRAF^{V600E}$–containing antigen at a concentration of about 0.5 μg/mL antigen to about 10 μg/mL antigen. In some embodiments, a target cell comprises a B cell. In further embodiments, the B cell expresses an HLA-DQ allele. In still further embodiments, the B cell is of B-LCL line 1331.

In certain embodiments, a portion of the polynucleotide encodes a self-cleaving peptide and is disposed between a TCR α-chain-encoding portion and a TCR β-chain-encoding portion. Self-cleaving peptides useful for expression of separable polypeptides by a single vector are known in the art and include, for example, Porcine teschovirus-1 2A (P2A) peptide, Thoseaasigna virus 2A (T2A) peptide, Equine rhinitis A virus (ERAV) 2A (E2A) peptide, and Foot-and-Mouth disease virus 2A (F2A) peptide. Accordingly, in certain embodiments, the portion of the heterologous polynucleotide that encodes the self-cleaving peptide comprises or consists of the polynucleotide sequence set forth in any one of SEQ ID NOS:44-48. In further embodiments, the encoded self-cleaving peptide comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOS:49-52.

In certain embodiments, the host cell is a hematopoietic progenitor cell or a human immune system cell. In certain embodiments, the immune system cell is a $CD4^+$ T cell, a $CD8^+$ T cell, a $CD4^-CD8^-$ double negative T cell, a γδ T cell, a natural killer cell, a dendritic cell, or any combination thereof. In certain embodiments, the immune system cell is a T cell. In particular embodiments, T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof. In further embodiments, the T cell is a CD4+ T cell.

In certain embodiments, the binding protein or TCR expressed by the T cell is capable of more efficiently associating with a CD3 protein, a CD4 protein, or both, as compared to endogenous TCR. In certain embodiments, the binding protein or TCR higher surface expression on a T cell as compared to endogenous TCR.

In any of the foregoing embodiments, a host cell that comprises a heterologous polynucleotide encoding a $BRAF^{V600E}$-specific binding protein is an immune cell which is modified to reduce or eliminate expression of one or more endogenous genes that encode a polypeptide product selected from PD-1, LAG-3, CTLA4, TIM3, TIGIT, an HLA molecule, a TCR molecule, or any component or combination thereof. Without wishing to be bound by theory, certain endogenously expressed immune cell proteins may downregulate the immune activity of a modified immune host cell (*e.g.*, PD-1, LAG-3, CTLA4, TIGIT), or may compete with a heterologous binding protein of the present disclosure for expression by the host cell, association with TCR complex components (*e.g.*, CD3 proteins), or may interfere with the binding activity of a heterologously expressed binding protein of the present disclosure (*e.g.*, an endogenous TCR that binds to a non-$BRAF^{V600E}$ antigen or a non-$BRAF^{V600E}$ antigen:HLA complex and interferes with binding of a presently disclosed binding protein to a $BRAF^{V600E}$ antigen) and interferes with the immune host cell binding a target cell that expresses $BRAF^{V600E}$ antigen), or any combination thereof. Further, endogenous proteins (*e.g.*, immune host cell proteins, such as an HLA) expressed on a donor immune cell to be used in a cell transfer therapy may be recognized as foreign by an allogeneic recipient, which may result in elimination or suppression of the donor immune cell by the allogeneic recipient.

Accordingly, decreasing or eliminating expression or activity of such endogenous genes or proteins can improve the activity, tolerance, and persistence of the host cells in an autologous or allogeneic host setting, and allows universal administration of the cells (*e.g.*, to any recipient regardless of HLA type). In certain embodiments, a modified host immune cell is a donor cell (*e.g.*, allogeneic) or an autologous cell. In certain embodiments, a modified immune 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, an HLA component (e.g., a gene that encodes an α1 macroglobulin, an α2 macroglobulin, an α3 macroglobulin, a β1 microglobulin, or a β2 microglobulin), or a TCR component (*e.g.*, a gene that encodes a TCR variable region or a TCR constant region) (*see*, *e.g.*, Torikai *et al.*, *Nature Sci. Rep. 6*:21757 (2016); Torikai *et al.*, *Blood 119*(24):5697 (2012); and Torikai *et al.*, *Blood 122*(8):1341 (2013) the gene editing techniques, compositions, and adoptive cell therapies of which are herein incorporated by reference in their entirety; *e.g.*, SEQ ID NOs:142-149). As used herein, the term "chromosomal gene knockout" refers to a genetic alteration in a host cell that prevents production, by the host cell, of a functionally active endogenous polypeptide product. Alterations resulting in a chromosomal gene knockout can include, for example, introduced nonsense mutations (including the formation of premature stop codons), missense mutations, gene deletion, and strand breaks, as well as the heterologous expression of inhibitory nucleic acid molecules that inhibit endogenous gene expression in the 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.

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 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 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 direct site-specific double-strand breaks (DSB) in the genome of T cells. Non-homologous end joining (NHEJ) ligates DNA from both sides of a double-strand break in which there is little or no sequence overlap for annealing, thereby introducing errors that knock out gene expression. Alternatively, homology directed repair can introduce a transgene at the site of DSB providing homologous flanking sequences are present in the transgene. In certain embodiments, a gene knockout comprises an insertion, a deletion, a mutation or a combination thereof, and made using a TALEN molecule.

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

to target a desired sequence (Xie *et al.*, *PLOS One 9*:e100448, 2014; U.S. Pat. Appl. Pub. No. US 2014/0068797, U.S. Pat. Appl. Pub. No. US 2014/0186843; U.S. Pat. No. 8,697,359, and PCT Publication No. WO 2015/071474; 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.

In some embodiments, a gene knockout comprises a CRISPR-mediated gene knockout of a TCR α-chain constant region locus (Cα), a TCR β-chain constant region locus (Cβ), or both. In certain embodiments, a gRNA sequence targeting a TCR Cα locus comprises the nucleotide sequence AGAGTCTCTCAGCTGGTACA (SEQ ID NO:136). In certain embodiments, a gRNA sequence targeting a TCR Cα locus comprises the nucleotide sequence TGTGCTAGACATGAGGTCTA (SEQ ID NO:137). In certain embodiments, a gRNA sequence targeting a TCR Cβ locus comprises the nucleotide sequence GCAGTATCTGGAGTCATTGA (SEQ ID NO:138). In certain embodiments, a gRNA sequence targeting a TCR Cβ locus comprises the nucleotide sequence GGAGAATGACGAGTGGACCC (SEQ ID NO:139). In some embodiments, a gene knockout comprises a CRISPR-mediated gene knockout of a human β2M locus. In certain embodiments, a gRNA sequence targeting a human β2M comprises the nucleotide sequence CGCGAGCACAGCTAAGGCCA (SEQ ID NO:140). In some embodiments, a gene knockout comprises a CRISPR-mediated gene knockout of a PD-1 locus. In certain embodiments, a gRNA sequence targeting a PD-1 comprises the nucleotide sequence GGCCAGGATGGTTCTTAGGT (SEQ ID NO:141)

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, 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, such as a TCRα chain, TCRβ chain or both, wherein the knocked-in TCR produced by the cell is specific for a $BRAF^{V600E}$ antigen or peptide.

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 (*i.e.*, of PD-1, TIM3, LAG3, CTLA4, TIGIT, an HLA component, or a TCR component, or any combination thereof) in the host immune 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 the knockout.

Methods of Treatment

In some aspects, methods of the instant disclosure are for treating a hyperproliferative disorder, wherein the methods comprise administering to human subject in need thereof a composition comprising a binding protein specific for human $BRAF^{V600E}$ or a host cell according to the present disclosure.

In certain aspects, the instant disclosure is directed to methods for treating a hyperproliferative disorder or a condition characterized by $BRAF^{V600E}$ expression by administering to human subject in need thereof a composition comprising a binding protein or a host cell expressing a binding protein specific for a $BRAF^{V600E}$ peptide:HLA complex according to any the aforementioned binding proteins.

The presence of a hyperproliferative disorder or malignant condition in a subject refers to the presence of dysplastic, cancerous and/or transformed cells in the subject, including, for example neoplastic, tumor, non-contact inhibited or oncogenically transformed cells, or the like (*e.g.,* solid cancers; hematologic cancers including lymphomas and leukemias, such as acute myeloid leukemia, chronic myeloid leukemia, etc. such as renal, gastric, ovarian, and colorectal cancers), which are known in the art and for which criteria for diagnosis and classification are established (*e.g.,* Hanahan and Weinberg, *Cell* 144:646, 2011; Hanahan and Weinberg, *Cell* 100:57, 2000; Cavallo *et al.*, *Canc. Immunol. Immunother.* 60:319, 2011; Kyrigideis *et al.*, *J. Carcinog.* 9:3, 2010). In particular, for example, hairy cell leukemia, melanoma, non-small cell lung cancer, colorectal cancer, papillary cancer, and thyroid cancer, such as poorly

differentiated thyroid cancer. Accordingly, in further embodiments, there are provided methods for treating a hyperproliferative disorder or other condition associated with $BRAF^{V600E}$ expression, including hairy cell leukemia, melanoma, thyroid cancer such as poorly differentiated thyroid cancer, non-small cell lung cancer, colorectal cancer, papillary cancer, non-Hodgkin lymphoma, adenocarcinoma of the lung, and brain tumors including glioblastoma and pilocytic astrocytomas.

As used herein, the terms, "treat" and "treatment," refer to medical management of a disease, disorder, or condition of a subject (*i.e.,* patient, host, who may be a human or non-human animal) (*see, e.g.,* Stedman's Medical Dictionary). In general, an appropriate dose and treatment regimen provide one or more of a binding protein or a $BRAF^{V600E}$ peptide:HLA complex or a host cell expressing the same, and optionally an adjunctive therapy (*e.g.*, a cytokine such as IL-2, IL-15, IL-21 or any combination thereof), in an amount sufficient to provide therapeutic or prophylactic benefit. Therapeutic or prophylactic benefit resulting from therapeutic treatment or prophylactic or preventative methods include, for example an improved clinical outcome, wherein the object is to prevent or retard or otherwise reduce (*e.g.,* decrease in a statistically significant manner relative to an untreated control) an undesired physiological change or disorder, or to prevent, retard or otherwise reduce the expansion or severity of such a disease or disorder. Beneficial or desired clinical results from treating a subject include abatement, lessening, or alleviation of symptoms that result from or are associated the disease or disorder to be treated; decreased occurrence of symptoms; improved quality of life; longer disease-free status (*i.e.,* decreasing the likelihood or the propensity that a subject will present symptoms on the basis of which a diagnosis of a disease is made); diminishment of extent of disease; stabilized (*i.e.,* not worsening) state of disease; delay or slowing of disease progression; amelioration or palliation of the disease state; and remission (whether partial or total), whether detectable or undetectable; or overall survival.

"Treatment" can also mean prolonging survival when compared to expected survival if a subject were not receiving treatment. Subjects in need of the methods and compositions described herein include those who already have the disease or disorder,

as well as subjects prone to have or at risk of developing the disease or disorder. Subjects in need of prophylactic treatment include subjects in whom the disease, condition, or disorder is to be prevented (*i.e.,* decreasing the likelihood of occurrence or recurrence of the disease or disorder). The clinical benefit provided by the compositions (and preparations comprising the compositions) and methods described herein can be evaluated by design and execution of *in vitro* assays, preclinical studies, and clinical studies in subjects to whom administration of the compositions is intended to benefit, as described in the examples.

Cells expressing the binding protein 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, in the context of adoptive cell therapy, is an amount of host cells (expressing a binding protein according to the present disclosure) 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 expressing $BRAF^{V600E}$ (*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 10^7 cells/m^2, about 5×10^7 cells/m^2, about 10^8 cells/m^2, about 5×10^8 cells/m^2, about 10^9 cells/m^2, about 5×10^9 cells/m^2, about 10^{10} cells/m^2, about 5×10^{10} cells/m^2, or about 10^{11} cells/m^2.

In any of the presently disclosed embodiments, a unit dose can comprise T cells, *e.g.*, $CD4^+$ T cells, $CD8^+$ T cells, or both, wherein the T cells can comprise bulk T cells, naïve T cells, stem cell memory T cells, central memory T cells, or effector memory T

cells. In certain embodiments, a unit dose comprises $BRAF^{V600E}$-specific $CD4^+$ T cells and does not comprise $CD8^+$ T cells. In other embodiments, a unit dose comprises $BRAF^{V600E}$-specific $CD8^+$ T cells, which may be engineered to express a heterologous $CD4^+$ co-receptor, and optionally does not comprise $CD4^+$ T cells. In certain embodiments, a unit dose comprises $BRAF^{V600E}$-specific $CD4^+$ T host cells of the present disclosure and further comprises $CD4^+$ T cells or $CD8^+$ T cells (*e.g.*, allogeneic or autologous, modified (*e.g.*, to express a heterologous protein such as a TCR, a CAR, a CD4 co-receptor, a CD8 co-receptor, or any combination thereof) or unmodified) that have binding specificity for one or more other antigens or antigen-HLA complexes, such as, for example: a $BRAF^{V600E}$ antigen, a BRAF antigen that does not comprise a V600E mutation; a $BRAF^{V600E}$-containing antigen that associates with a different HLA than does the binding protein of a presently disclosed host cell in the unit dose; or a different antigen that is associated with hyperproliferative disease or disorder; *e.g.*, NY-ESO-1, SSX-2, Tyrosinase, TMG1-4, GP100, MAGE-A3, MART1, ROR1, EGFR, EGFRvIII, EGP-2, EGP-40, GD2, GD3, HPV E6, HPV E7, Her2, L1-CAM, Lewis A, Lewis Y, MUC1, MUC16, PSCA, PSMA, CD19, CD20, CD22, CD56, CD23, CD24, CD30, CD33, CD37, CD44v7/8, CD38, CD56, CD123, CA125, c-MET, FcRH5, WT-1, folate receptor α, VEGF-α, VEGFR1, VEGFR2, IL-13Rα2, IL-11Rα, MAGE-A1, PSA, ephrin A2, ephrin B2, an NKG2D, NY-ESO-1, TAG-72, mesothelin, NY-ESO, 5T4, BCMA, FAP, Carbonic anhydrase 9, ERBB2, or CEA, or any combination thereof).

In certain embodiments, a unit dose comprises (i) a composition comprising at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% engineered $CD4^+$ T cells, combined with (ii) a composition comprising at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% engineered $CD8^+$ T cells, in about a 1:1 ratio, wherein 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 then about 1% the population of naïve T cells present in a unit dose as compared to a patient sample having a comparable number of PBMCs).

In some embodiments, a unit dose comprises (i) a composition comprising at least about 50% engineered $CD4^+$ T cells, combined with (ii) a composition comprising at least about 50% engineered $CD8^+$ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In further embodiments, a unit dose comprises (i) a composition comprising at least about 60% engineered $CD4^+$ T cells, combined with (ii) a composition comprising at least about 60% 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 still further embodiments, a 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 unit dose comprises (i) a composition comprising at least about 80% engineered $CD4^+$ T cells, combined with (ii) a composition comprising at least about 80% 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 unit dose comprises (i) a composition comprising at least about 85% engineered $CD4^+$ T cells, combined with (ii) a composition comprising at least about 85% 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 unit dose comprises (i) a composition comprising at least about 90% engineered $CD4^+$ T cells, combined with (ii) a composition comprising at least about 90% 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 any of the embodiments described herein, a unit dose comprises equal, or approximately equal numbers of engineered $CD45RA^- CD3^+ CD8^+$ and engineered $CD45RA^- CD3^+ CD4^+ T_M$ cells.

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 $BRAF^{V600E}$ expression includes any disorder or condition in which a $BRAF^{V600E}$-driven cellular or molecular event is present, and typically manifests in overgrowth of diseased cells relative to normal cells. Some conditions associated with $BRAF^{V600E}$ expression 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 $BRAF^{V600E}$ expression include hyperproliferative disorders, which refer to states of activated and/or proliferating cells (which may also be transcriptionally overactive) in a subject including tumors, neoplasms, cancer, malignancy, etc. In addition to activated or proliferating cells, the hyperproliferative disorder may also include an aberration or dysregulation of cell death processes, whether by necrosis or apoptosis. Such aberration of cell death processes may be associated with a variety of conditions, including cancer (including primary, secondary malignancies as well as metastasis), or other conditions.

According to certain embodiments, a cancer that is characterized by $BRAF^{V600E}$ expression may be treated through the use of compositions and methods disclosed herein. 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 $BRAF^{V600E}$ expression.

Certain methods of treatment or prevention contemplated herein include administering a host cell (which may be autologous, allogeneic or syngeneic) comprising a desired polynucleotide 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, $BRAF^{V600E}$ -targeted T-cell composition to a subject as an adoptive immunotherapy. In certain embodiments, the host cell is a hematopoietic progenitor cell or a human immune cell. In certain embodiments, the immune system cell is a $CD4^+$ T cell, a $CD8^+$ T cell, a $CD4^-$ $CD8^-$ double-negative T cell, a γδ T cell, a natural killer cell, a natural killer T cell, a dendritic cell, or any combination thereof. In certain embodiments, the immune system cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof. In particular embodiments, the cell is a $CD4^+$ T cell.

As used herein, administration of a composition or therapy 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.*, $BRAF^{V600E}$-specific recombinant (*i.e.*, 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).

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 (*e.g.*, IL-2, IL-15, IL-21) is administered sequentially, provided that the subject was administered the recombinant host cell at least three or four times before cytokine administration. In certain embodiments, the cytokine is administered concurrently with the host cell. In certain embodiments, the cytokine is administered subcutaneously.

In still further embodiments, the subject being treated is further receiving immunosuppressive therapy, such as calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof. In yet further embodiments, the subject being treated has received a non-myeloablative or a myeloablative hematopoietic cell transplant, wherein the treatment may be administered at least two to at least three months after the non-myeloablative hematopoietic cell transplant.

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

The level of a CTL immune response may be determined by any one of numerous immunological methods described herein and routinely practiced in the art. The level of a CTL immune response may be determined prior to and following administration of any one of the herein described $BRAF^{V600E}$-specific binding proteins expressed by, for example, a T cell. Cytotoxicity assays for determining CTL activity may be performed using any one of several techniques and methods routinely practiced in the art (*see, e.g.,* Henkart et al., "Cytotoxic T-Lymphocytes" *in Fundamental Immunology*, Paul (ed.) (2003 Lippincott Williams & Wilkins, Philadelphia, PA), pages 1127-50, and references cited therein).

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

A biological sample may be obtained from a subject for determining the presence and level of an immune response to a $BRAF^{V600E}$-containing antigen peptide as described herein. A "biological sample" as used herein may be a blood sample (from which serum or plasma may be prepared), biopsy specimen, body fluids (*e.g.,* lung lavage, ascites, mucosal washings, synovial fluid), bone marrow, lymph nodes, tissue explant, organ culture, or any other tissue or cell preparation from the subject or a biological source. Biological samples may also be obtained from the subject prior to receiving any immunogenic composition, which biological sample is useful as a control for establishing baseline (*i.e.,* pre-immunization) data.

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/m^2 to about 10^{11} cells/m^2. 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.

In still further aspects, unit dose forms comprising host cells according to the present disclosure are provided.

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 $BRAF^{V600E}$-specific binding protein (or an engineered host cell expressing the same) with (concurrently, simultaneously, or sequentially) an immune checkpoint inhibitor. In some embodiments, a combination therapy comprises administering a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) with an agonist of a stimulatory immune checkpoint agent. In further embodiments, a combination therapy comprises administering a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) with a secondary therapy, such as chemotherapeutic agent, a radiation therapy, a surgery, an antibody, or any combination thereof.

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

An immune suppression agent inhibitor (also referred to as an immune checkpoint inhibitor) may be a compound, an antibody, an antibody fragment or fusion polypeptide (*e.g.,* Fc fusion, such as CTLA4-Fc or LAG3-Fc), an antisense molecule, a ribozyme or RNAi molecule, or a low molecular weight organic molecule. In any of the embodiments disclosed herein, a method may comprise administering a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) with one or more inhibitor of any one of the following immune suppression components, singly or in any combination.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein is used in combination with a PD-1 inhibitor, for example a PD-1-specific antibody or binding fragment thereof, such as pidilizumab, nivolumab (Keytruda, formerly MDX-1106), pembrolizumab (Opdivo, formerly MK-3475), MEDI0680 (formerly AMP-514), AMP-224, BMS-936558 or any combination thereof. In further embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with a PD-L1 specific antibody or binding fragment thereof, such as BMS-936559, durvalumab (MEDI4736), atezolizumab (RG7446), avelumab (MSB0010718C), MPDL3280A, or any combination thereof.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with a LAG3 inhibitor, such as LAG525, IMP321, IMP701, 9H12, BMS-986016, or any combination thereof.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein is used in combination with an inhibitor of CTLA4. In particular embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with a CTLA4 specific antibody or binding fragment thereof, such as ipilimumab, tremelimumab, CTLA4-Ig fusion proteins (*e.g.*, abatacept, belatacept), or any combination thereof.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with a B7-H3 specific antibody or binding fragment thereof, such as enoblituzumab (MGA271), 376.96, or both. A B7-H4 antibody binding fragment may be a scFv or fusion protein thereof, as described in, for example, *Dangaj et al., Cancer Res. 73*:4820, 2013, as well as those described in U.S. Patent No. 9,574,000 and PCT Patent Publication Nos. WO/201640724A1 and WO 2013/025779A1.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of CD244.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of BLTA, HVEM, CD160, or any combination thereof. Anti CD-160 antibodies are described in, for example, PCT Publication No. WO 2010/084158.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of TIM3.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of Gal9.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of adenosine signaling, such as a decoy adenosine receptor.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of A2aR.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of KIR, such as lirilumab (BMS-986015).

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of an inhibitory cytokine (typically, a cytokine other than TGFβ) or Treg development or activity.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an IDO inhibitor, such as levo-1-methyl tryptophan, epacadostat (INCB024360; Liu *et al.*, *Blood 115*:3520-30, 2010), ebselen (Terentis *et al.* , *Biochem. 49*:591-600, 2010), indoximod, NLG919 (Mautino *et al.*, American Association for Cancer Research 104th Annual Meeting 2013; Apr 6-10, 2013), 1-methyl-tryptophan (1-MT)-tira-pazamine, or any combination thereof.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an arginase inhibitor, such as N(omega)-Nitro-L-arginine methyl ester (L-NAME), N-omega-hydroxy-nor-l-arginine (nor-NOHA), L-NOHA, 2(S)-amino-6-boronohexanoic acid (ABH), S-(2-boronoethyl)-L-cysteine (BEC), or any combination thereof.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of VISTA, such as CA-170 (Curis, Lexington, Mass.).

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of TIGIT such as, for example, COM902 (Compugen, Toronto, Ontario Canada), an inhibitor of CD155, such as, for example, COM701 (Compugen), or both.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) 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 $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with a LAIR1 inhibitor.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) is used in combination with an inhibitor of CEACAM-1, CEACAM-3, CEACAM-5, or any combination thereof.

In certain embodiments, a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) 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 $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) 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 $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) 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 $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) 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 $BRAF^{V600E}$-specific binding protein 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 or non-inflamed solid tumor in a subject are well-known to those of ordinary skill in the art.

In certain embodiments, a combination therapy method comprises administering a $BRAF^{V600E}$-specific binding protein of the present disclosure (or an engineered host cell expressing the same) and further administering a chemotherapeutic agent. A chemotherapeutic agent includes, but is not limited to, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such

as an intercalating agent), and a DNA repair inhibitor. Illustrative chemotherapeutic agents include, without limitation, the following groups: anti-metabolites/anti-cancer agents, such as pyrimidine analogs (5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine) and purine analogs, folate antagonists and related inhibitors (mercaptopurine, thioguanine, pentostatin and 2- chlorodeoxyadenosine (cladribine)); antiproliferative/antimitotic agents including natural products such as vinca alkaloids (vinblastine, vincristine, and vinorelbine), microtubule disruptors such as taxane (paclitaxel, docetaxel), vincristin, vinblastin, nocodazole, epothilones and navelbine, epidipodophyllotoxins (etoposide, teniposide), DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, Cytoxan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylmelamineoxaliplatin, iphosphamide, melphalan, merchlorehtamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, temozolamide, teniposide, triethylenethiophosphoramide and etoposide (VP 16)); antibiotics such as dactinomycin (actinomycin D), daunorubicin, doxorubicin (adriamycin), idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin; enzymes (L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates -busulfan, nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes— dacarbazinine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate); platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, nilutamide) and aromatase inhibitors (letrozole, anastrozole); anticoagulants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine, clopidogrel,

abciximab; antimigratory agents; antisecretory agents (breveldin); immunosuppressives (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); anti-angiogenic compounds (TNP470, genistein) and growth factor inhibitors (vascular endothelial growth factor (VEGF) inhibitors, fibroblast growth factor (FGF) inhibitors); angiotensin receptor blocker; nitric oxide donors; anti-sense oligonucleotides; antibodies (trastuzumab, rituximab); chimeric antigen receptors; cell cycle inhibitors and differentiation inducers (tretinoin); mTOR inhibitors, topoisomerase inhibitors (doxorubicin (adriamycin), amsacrine, camptothecin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin, irinotecan (CPT-11) and mitoxantrone, topotecan, irinotecan), corticosteroids (cortisone, dexamethasone, hydrocortisone, methylpednisolone, prednisone, and prenisolone); growth factor signal transduction kinase inhibitors; mitochondrial dysfunction inducers, toxins such as Cholera toxin, ricin, Pseudomonas exotoxin, Bordetella pertussis adenylate cyclase toxin, or diphtheria toxin, and caspase activators; and chromatin disruptors.

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

Another cancer therapy approach involves reducing expression of oncogenes and other genes needed for growth, maintenance, proliferation, and immune evasion by cancer cells. RNA interference, and in particular the use of microRNAs (miRNAs) and small inhibitory RNAs (siRNAs) provides an approach for knocking down expression of cancer genes (*see, e.g.*, Larsson *et al., Cancer Treat. Rev. 16*(55):128-135, 2017), which can be used in combination with the binding proteins or cells expressing the same of this disclosure.

In any of the embodiments disclosed herein, any of the therapeutic agents (*e.*g, a $BRAF^{V600E}$-specific binding protein or engineered host cell, an inhibitor of an immune

suppression component, an agonist of a stimulatory immune checkpoint molecule, an antitumor lymphocyte, a chemotherapeutic agent, a radiation therapy, a surgery, a cytokine, or an inhibitory RNA) may be administered once or more than once to the subject over the course of a treatment, and, in combinations, may be administered to the subject in any order or any combination. An appropriate dose, suitable duration, and frequency of administration of a therapeutic agent will be determined by such factors as a condition of the patient; size, type, spread, growth, and severity of the tumor or cancer; particular form of the active ingredient; and the method of administration.

EXAMPLES

EXAMPLE 1

IDENTIFICATION OF $CD4^+$ $BRAF^{V600E}$-SPECIFIC T CELLS IN A MELANOMA PATIENT

A 52-year-old man presented with stage IIIC, $BRAF^{V600E}$-mutated melanoma originating on the left foot and was treated with wide excision, lymph node dissection and adjuvant ipilimumab. Shortly before completing one year of ipilimumab, he required resection of three in-transit metastases in his left leg, and an additional in-transit metastasis 3 months later. He subsequently progressed with a 3cm left iliac nodal metastasis and soft tissue nodular FDG avid lesion in the left thigh (Figure 1A). The iliac node was resected for whole exome sequencing and expansion of TIL, and the patient subsequently received TIL infusion following lymphodepleting chemotherapy. The left thigh lesion resolved and the patient remains free of disease 27 months after therapy.

Whole exome and RNA sequencing of purified tumor cells and normal tissue identified only 20 nonsynonymous missense mutations, shown in Table 1: (columns 2 and 3 from left); columns 4-6 from left: 27-mer peptides encompassing the encoded nonsynonymous mutations and the presence of the mutation in DNA or RNA-Seq (columns 4-6 from left); (columns 7 and 8 from left) engineered 20-mer peptides comprising the mutation; and RNA-seq expression in units of transcripts per million (TPM).

Table 1. Nonsynonymous Mutations in Purified Tumor Cells and Normal Tissue from a Melanoma Patient

symbol	sub.nt	sub.aa	27-mer aa sequence	Mut'n in DNA	Mut'n in RNA	20-mer peptide 1	20-mer peptide 2	TPM in RNA
AP1M1	AP1M1.A>C	p.I295L	VIEKHSHSRIEYMLKAKSQFKRRSTAN (SEQ ID NO: 79)	yes	yes	VIEKHSHSRIEYMLKAKSQF (SEQ ID NO: 80)	SRIEYMLKAKSQFKRRSTAN SEQ ID NO: 81)	61.33
BRAF	BRAF.A>T	p.V600E	DLTVKIGDFGLATEKSRWSGSHQFEQL (SEQ ID NO: 37)	yes	yes	DLTVKIGDFGLATEKSRWSG (SEQ ID NO: 38)	DFGLATEKSRWSGSHQFEQL (SEQ ID NO: 39)	10.83
DCAF6	DCAF6.G>A	p.A419T	EQFLQPSTSSTMSTQAHSTSSPTESPH (SEQ ID NO: 82)	yes	yes	EQFLQPSTSSTMSTQAHSTS (SEQ ID NO: 83)	TSSTMSTQAHSTSSPTESPH (SEQ ID NO: 84)	41.11
GTF2H4	GTF2H4.C>T	p.T319M	FIVVETNYRLYAYMESELQIALIALFS (SEQ ID NO: 85)	yes	yes	FIVVETNYRLYAYMESELQI (SEQ ID NO: 86)	YRLYAYMESELQIALIALFS (SEQ ID NO: 87)	49.95
NBPF12	NBPF12.A>G	p.E2471G	DSCQPYRSSFYALGEKHVGFSLDVGEI (SEQ ID NO: 88)	yes	yes	DSCQPYRSSFYALGEKHVGF (SEQ ID NO: 89)	SSFYALGEKHVGFSLDVGEI (SEQ ID NO: 90)	12.5
ORC3	ORC3.A>C	p.I236L	ESFATKVLQDFIILSSQHLHEFPLILI (SEQ ID NO: 91)	yes	yes	ESFATKVLQDFIILSSQHLH (SEQ ID NO: 92)	LQDFIILSSQHLHEFPLILI (SEQ ID NO: 93)	19.92
ROR1	ROR1.A>G	p.N53S	LVPTSSWNISSELSKDSYLTLDEPMNN (SEQ ID NO: 94)	yes	yes	LVPTSSWNISSELSKDSYLT (SEQ ID NO: 95)	NISSELSKDSYLTLDEPMNN (SEQ ID NO: 96)	4.69
SF3B1	ZNF700.T>G	p.T358A	QMGGSTPVLTPGKAPIGTPAMNMATPT (SEQ ID NO: 97)	yes	yes	QMGGSTPVLTPGKAPIGTPA (SEQ ID NO: 98)	VLTPGKAPIGTPAMNMATPT (SEQ ID NO: 99)	105.2

symbol	sub.nt	sub.aa	27-mer aa sequence	Mut'n in DNA	Mut'n in RNA	20-mer peptide 1	20-mer peptide 2	TPM in RNA
UNKL	UNKL.C>T	p.V154I	AHGPLDLRPPVCDIRELQAQEALQNGQ (SEQ ID NO: 100)	yes	yes	AHGPLDLRPPVCDIRELQAQ (SEQ ID NO: 101)	RPPVCDIRELQAQEALQNGQ (SEQ ID NO: 102)	6.72
ZNF700	ZNF700.T>G	p.F287L	GEKPYECSKCDKALHSSSSYHRHERSH (SEQ ID NO: 103)	yes	yes	GEKPYECSKCDKALHSSSSY (SEQ ID NO: 104)	SKCDKALHSSSSYHRHERSH (SEQ ID NO: 105)	8.69
NVL	NVL.T>G	p.T370P	APCIIFIDEIDAIPPKREVASKDMERR (SEQ ID NO: 106)	yes	yes	APCIIFIDEIDAIPPKREVA (SEQ ID NO: 107)	DEIDAIPPKREVASKDMERR (SEQ ID NO: 108)	18.42
MATN1	MATN1.T>G	p.T153P	SRSPDISKVVIVVPDGRPQDSVQDVSA (SEQ ID NO: 109)	yes	yes	SRSPDISKVVIVVPDGRPQD (SEQ ID NO: 110)	KVVIVVPDGRPQDSVQDVSA (SEQ ID NO: 111)	0.14
CTNNA2	CTNNA2.A>C	p.N351H	VRQALQDLLSEYMHNTGRKEKGDPLNI (SEQ ID NO: 112)	yes	no	VRQALQDLLSEYMHNTGRKE (SEQ ID NO: 113)	LLSEYMHNTGRKEKGDPLNI (SEQ ID NO: 114)	2.36
GET4	GET4.T>G	p.L65R	RYMSQSKHTEARERMYSGALLFFSHGQ (SEQ ID NO: 115)	yes	no	RYMSQSKHTEARERMYSGAL (SEQ ID NO: 116)	HTEARERMYSGALLFFSHGQ (SEQ ID NO: 117)	26.97
NTNG1	NTNG1.G>A	p.V271I	TVTDLRIRLLRPAIGEIFVDELHLARY (SEQ ID NO: 118)	yes	yes	TVTDLRIRLLRPAIGEIFVD (SEQ ID NO: 119)	RLLRPAIGEIFVDELHLARY (SEQ ID NO: 120)	0.73
SPTBN5	SPTBN5.G>A	p.T3127I	TLLLDAWLTTKAAIAESQDYGQDLEGV (SEQ ID NO: 121)	yes	yes	TLLLDAWLTTKAAIAESQDY (SEQ ID NO: 122)	LTTKAAIAESQDYGQDLEGV (SEQ ID NO: 123)	1.11
DPP6	DPP6.C>T	p.S113L	LLVILVICSLIVTLVILLTPAEDNSLS (SEQ ID NO: 124)	yes	no	LLVILVICSLIVTLVILLTP (SEQ ID NO: 125)	CSLIVTLVILLTPAEDNSLS (SEQ ID NO: 126)	0.03
HIAT1	HIAT1.G>T	p.G93C	VKGLLSFLSAPLICALSDV	yes	no	VKGLLSFLSAPLICA	LSAPLICALSDVWGR	46.71

symbol	sub.nt	sub.aa	27-mer aa sequence	Mut'n in DNA	Mut'n in RNA	20-mer peptide 1	20-mer peptide 2	TPM in RNA
			WGRKSFLL (SEQ ID NO: 127)			LSDVW (SEQ ID NO: 128)	KSFLL (SEQ ID NO: 129)	
ITGA4	ITGA4.G>T	p.V359F	GSGAVMNA METNLFGSD KYAARFGES I (SEQ ID NO: 130)	yes	no	GSGAVM NAMETNL FGSDKYA (SEQ ID NO: 131)	AMETNLF GSDKYAA RFGESI (SEQ ID NO: 132)	4.59
MYO1A	MYO1A.C>T	p.V1017I	SVRFKENSV AVKVIQGPA GGDNSKLRY (SEQ ID NO: 133)	yes	no	SVRFKEN SVAVKVI QGPAGG (SEQ ID NO: 134)	SVAVKVI QGPAGGD NSKLRY (SEQ ID NO: 135)	0.05

Also determined, but not shown in Table 1, were chromosomal positions (using GRCh37/hg19 reference assembly) and the variant allele frequency (VAF) for each mutation. The VAF for the $BRAF^{V600E}$ mutation was 35.4%.

T cell responses to these potential neoantigens were evaluated by stimulating peripheral blood mononuclear cells (PBMC) obtained from the patient after TIL therapy with a pool of peptides flanking each of the 20 mutations. No $CD8^+$ T cell responses to the candidate neoantigens were detected; however, a $CD4^+$ T cell response specific for 20-mer peptides encompassing $BRAF^{V600E}$ was identified. The $BRAF^{V600E}$-reactive T cells were purified by IFN-γ capture and shown to recognize autologous B cells pulsed with mutant but not wildtype BRAF peptide, confirming specificity for the mutant peptide (Figure 1B). To determine whether $BRAF^{V600E}$ is processed and presented by class II MHC^+ APC, autologous B cells were transfected with mRNA encoding wildtype or mutant BRAF sequences targeted to the endosome. The T cells recognized B cells expressing mutant, but not wildtype BRAF (Figure 1C).

Recognition was blocked by anti-HLA-DQ but not anti-class I or anti HLA-DR antibodies, identifying HLA-DQ as the likely restricting allele (Figure 1D). Analysis of multiple B cell lines of known genotype suggested restriction by HLA-DQA1*03 paired with HLA-DQB1*03, with weak recognition of DQB1*0301 and stronger recognition of DQB1*0302 and DQB1*0303 (Figure 1F and Table 2).

Table 2. IFN-γ production by BRAF ^{V600E} -specific CD4+ T cells following incubation with allogeneic B-LCL cell lines expressing different class II alleles and in the presence or absence of antigen.

Cell line	BRAF V600E Peptide	Mean IFN-G pg/ml	HLA DRB1	HLA DQB1
1331	-	10	404	302
	+	41791		
CFS	-	10	0401, 0101	0301, 0501
	+	1547		
DEM	-	23	0401, 1602	0302, 0502
	+	29873		
DEU	-	6	401	301
	+	10359		
FAL	-	9	0403, 0801	03BG, 0402
	+	31620		
BM14	-	536	401	302
	+	42832		
DMB	-	38	0101, 1501	0501, 0602
	+	7		
DLM	-	26	0403, 0801	03BG, 0402
	+	39388		
AMM	-	6	0802, 1501	0402, 06WG
	+	9		
CLC	-	9	0301, 1104	02AB, 0301
	+	6		
BP	-	35	1601, 1101	0502, 0301
	+	36		
JWP	-	12	0701, 0701	02AB, 0303
	+	359		
DAH2	-	17	09, 1501	0303, 06W6
	+	49784		
VRM	-	6	0701, 10	0303, 0501
	+	188		

The complete patient HLA typing was as follows: A*11:01:01/A*24:02:01; B*15:01:01/B*40:01:02; C*03:03:01/C*03:04:01; DPA1*01:03:01/DPA1*01:03:01; DPB1*04:01:01/DPB1*04:01:01; DQA1*03:01:01/DQA1*03:02; DQB1*03:02:01/DQB1*03:03:02; DRB1*04:03:01/DRB1*09:01:02; DRB4*01:03:01/DRB4*01:03:02.

B-LCL transfected with HLA-DQA1*03 or DQB1*0302, but not the closely linked HLA-DRB1*04, were recognized by $BRAF^{V600E}$-specific $CD4^+$ T cells when pulsed with the mutant peptide, confirming the HLA restriction (Figure 1E).

Recognition of three melanoma cell lines with an HLA-DQB1*0302 and $BRAF^{V600E}$ genotype was tested. One tumor cell line that expressed the greatest amount of HLA-DQ was recognized by the $BRAF^{V600E}$-specific $CD4^+$ T cells demonstrating that the epitope can be presented directly by tumor cells (Figures 1G-1I). Tumor-specific $CD4^+$ T cells can have anti-tumor activity through direct cell killing and cytokine release (*see*, *e.g.*, Quezada *et al.*, *J. Exp. Med. 207*(3):637-650 (2010); Manici *et al.*, *J. Exp. Med. 189*(5):871-876 (1999)), but a major role is to support the development and function of $CD8^+$ T cells by licensing APC and producing cytokines (*see*, *e.g.*, Sun and Bevan, *Science 300*(5617):339-342 (2003); Williams *et al.*, *Nature 441*(7095):890-893 (2006)). Although adoptively transferred TIL contained $BRAF^{V600E}$-specific $CD4^+$ T cells, $CD8^+$ T cells were the prevalent population in TIL (Table 3). Briefly, the final TIL product infused into the patient was analyzed by flow cytometry for phenotype and, following stimulation with PMA/Ionomycin, was stained intracellularly for cytokines. Percentages shown in Table 3 are percentages of CD45+ cells (top) or CD4 or CD8 cells (bottom)

Table 3. Phenotype of Final TIL product

% of live	% of CD45					
CD45	CD3 T	Γδ T	NKT	CD8T	CD4T	Treg
99.9	99.7	0.04	0.014	93.9	3.4	0.52
% of CD45						
CD8P1	CD8 TIM3	CD8 TM	CD8 naïve	CD8	CD8EM	

				EMRA		
51.6	93.4	0.002	0.003	36.5	58.6	
% of CD8 or CD4						
CD8 IFN-γ+	CD4 IL17+	CD4 IL22	CD4 IFN-γ+			
99.3	3.6	0.42	98.8			

Moreover, a majority of IFN- γ produced by stimulation of multiple independent TIL cultures with autologous tumor was blocked by a HLA class I blocking antibody (Table 4).

Table 4. Tumor specificity and class I blocking of initial TIL cultures

	Pool T	**Pool R**	**Pool B**	**Pool A**	**Pool S**	**Pool 1**	**Pool 2**	**Pool 3**	**Pool 4**	**Pool 5**	**Pool 6**	**Frag. 12**
Tumor	2179.50	752.12	2993.64	4427.63	2313.73	3409.99	7213.00	3722.50	3866.23	2914.58	3916.23	2.44
Tumor + Class I Block	94.32	11.95	67.49	38.78	67.88	75.74	394.68	258.88	86.28	369.32	513.78	2.44
Media only	2.44	52.71	67.56	2.44	2.44	40.87	16.52	131.40	2.44	2.44	21.22	2.44
PMA/ Iono	2010.14	360.33	2563.22	2929.14	1203.62	2038.37	1565.83	1454.78	2757.56	2012.09	2155.09	2.44
% Blocking	96%	98%	98%	99%	97%	98%	95%	93%	98%	87%	87%	0%

No IFN- γ production was observed when TIL were cultured with autologous B cells pulsed with pools of peptides that included all of the 20 non-synonymous mutations identified by tumor exome sequencing (Figure 2A), but IFN-γ was produced after co-culture with B cells pulsed with peptides from lineage-restricted self-antigens (tyrosinase, Mart-1, TRP2) and a cancer testes antigen (Mage A3), which are known targets of T cells in melanoma (*see, e.g.*, Gros *et al. Nat. Med.* 2016) (Figure 2F), or transfected with tandem minigenes encompassing 29 non-synonymous mutations or the coding sequences of Tyrosinase, Mage-A3, Mart1, SSX2, and GP100 (Figures 2B-2E).

Thus, the patient TIL contained $BRAF^{V600E}$-specific $CD4^+$ T cells and a diverse $CD8^+$ T cell response to self-antigens.

Clinical Protocol

The patient was enrolled for TIL generation under an FDA-approved IND and a clinical protocol approved by the Institutional Review Board of Fred Hutchinson Cancer Research Center (FHCRC 2643; NCT01807182). Patients with stage IV melanoma, or stage III unlikely to be cured by surgery, >18 years of age, with an ECOG </=1, with a site of metastatic disease that could be safely resected or biopsied, were eligible. TIL were expanded from tumor fragments in 6,000 IU/ml recombinant IL-2 (Proleukin; Novartis), using methodologies developed at the Surgery Branch of the National Cancer Institute (*e.g.*, Dudley *et al.*, *J. Immunother. 24*(4):363-373 (2002)). TIL cultures were selected based on cell growth and autologous tumor reactivity as determined by IFN-γ secretion following co-culture with autologous tumor cells. The TIL were cryopreserved until needed for use, then thawed and further expanded using a rapid expansion protocol, as previously-described (Riddell and Greenberg, *J. Immunological Methods 128*(2):189-201 (1990)). The expanded TIL were administered to the patient following a lymphodepleting chemotherapy regimen of cyclophosphamide 60mg/kg/day x 2 days, then fludarabine 25 mg/m^2/day x 5 days. Within 24 hours of the TIL infusion, the patient received high-dose IL-2 at 600,000 IU/kg IV every 8 hours, for a total of 9 doses. Tumor responses were assessed using RECIST version 1.1 with CT and MRI at weeks 6, 12, and 24, then every 3-6 months, at the discretion of the primary provider.

Nucleic Acid Preparation for Exome Capture and RNA sequencing

Post-treatment blood was used to isolate non-tumor DNA. A single-cell suspension derived from the iliac nodal tumor recurrence was flow sorted (propidium iodide negative and CD45 negative) to deplete abundant infiltrating lymphocytes and enrich for neoplastic cells. Normal tissue and sorted tumor cells were processed with the Qiagen DNA/RNA AllPrep Micro kit to isolate DNA for exome capture, with RNA reserved for subsequent RNA-seq profiling. Genomic DNA concentration was

quantified on an Invitrogen Qubit® 2.0 Fluorometer (Life Technologies-Invitrogen, Carlsbad, CA, USA) and Trinean DropSense96 spectrophotometer (Caliper Life Sciences, Hopkinton, MA).

Whole Exome Sequencing

Exome sequencing libraries were prepared using the Agilent SureSelectXT Reagent Kit and exon targets isolated using the Agilent All Human Exon v6 (Agilent Technologies, Santa Clara, CA, USA). 200 ng of genomic DNA was fragmented using a Covaris LE220 focused-ultrasonicator (Covaris, Inc., Woburn, MA, USA) and libraries prepared and captured on a Sciclone NGSx Workstation (PerkinElmer, Waltham, MA, USA). Library size distributions were validated using an Agilent 2200 TapeStation. Additional library QC, blending of pooled indexed libraries, and cluster optimization was performed using Life Technologies' Invitrogen Qubit® 2.0 Fluorometer.

The resulting libraries were sequenced on an Illumina HiSeq 2500 using a paired-end 100bp (PE100) strategy. Image analysis and base calling was performed using Illumina's Real Time Analysis v1.18 software, followed by "demultiplexing" of indexed reads and generation of FASTQ files using Illumina's bcl2fastq Conversion Software v1.8.4 (http://support.illumina.com/downloads/bcl2fastq_conversion_software_184.html). Read pairs passing standard Illumina quality filters were retained for further analysis, yielding 77M read pairs for the tumor and 89M read pairs for the normal. Paired reads were aligned to the human genome reference (GRCh37/hg19) with the BWA-MEM short-read aligner (*see*, *e.g.*, Li, H., *arXiv preprint arXiv*:1303.3997 (2013); Li and Rudbin, *Bioinfomatics 25*(14):1754-1760). The resulting alignment files, in standard BAM format, were processed by Picard 2.0.1 and GATK 3.5[37] for quality score recalibration, indel realignment, and duplicate removal according to recommended best practices (*see*, Auwera *et al.*, *Current Protocols in Bioinformatics* pp. 11.10.1-11.10.33 (2013)).

Three independent software packages were used to call somatic mutations from the analysis-ready tumor and normal BAM files: MuTect 1.1.7[39], Strelka 1.0.14[40],

and VarScan.v2.4.1 (Koboldt *et al.*, *Genome Res.* *22*(3):568-576 (2012)). Variant calls from all tools, in VCF format, were annotated with Oncotator (Ramos *et al.*, *Human Mutation* *36*(4):E2423-E2429 (2015)). Missense somatic variants were combined and annotated further, including wild-type and variant peptide sequences, to form an integrated summary from which candidate peptides were chosen for synthesis.

RNA-Seq Data Processing

To rank candidate peptides by observed expression level, RNA-seq was performed on flow-sorted tumor cells from the same single cell suspension. RNA-seq libraries were prepared from total RNA using the TruSeq RNA Sample Prep v2 Kit (Illumina, Inc., San Diego, CA, USA) and a Sciclone NGSx Workstation (PerkinElmer, Waltham, MA, USA). Library size distributions were validated using an Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA). Additional library QC, blending of pooled indexed libraries, and cluster optimization was performed using Life Technologies' Invitrogen Qubit® 2.0 Fluorometer (Life Technologies-Invitrogen, Carlsbad, CA, USA). The library was sequenced on an Illumina HiSeq 2500 to generate 133M 50nt paired reads (PE50). Reads were aligned to a RefSeq derived reference transcriptome with RSEM 1.2.19 (*see* Li and Dewer, *BMC Bioinformatics* *12*(1):323 (2011)). Gene-level expression values from RSEM, in TPM units, were added to the summary of missense somatic variants.

T Cell Culture

Initial stimulations were performed with overlapping 20-mer crude peptides obtained from Elim Biopharma, with 2 peptides spanning each mutation with the mutated residue at position +7 or +13 of the 20 amino acid sequence. Subsequent experiments were performed with >80% purity 21 mer peptides with V600 (wildtype) or E600 (mutant) at position +11. Cryopreserved PBMC were thawed and rested overnight in CTL (RPMI media with L-glutamine and HEPES (Gibco) supplemented with 10% human serum (produced in house), 50 μM beta-mercaptoethanol, penicillin and streptomycin, 4 mM L-glutamine and 2ng/ml recombinant human IL-7 (Peprotech). The following morning, PBMC were washed and stimulated at 10e6 cells in 5 ml CTL

per well of a 6 well plate with a pool of 1μg/ml of each peptide without cytokines. Recombinant IL-2 (Peprotech) was added to a final concentration of 10 U/ml on day +3, and half media changes with supplemental IL-2 were performed on days +3, +6, and +9. On day +13, cells were used in an ELISA and cytokine staining assays.

Antigen-specific T cell enrichment was carried out by staining live cells for secreted IFN-γ using the IFN- γ secretion assay APC (Milltenyi) following the manufacturer's instructions, and using autologous B cells as antigen presenting cells pulsed with 10 μg/ml 21-mer BRAF mutant peptide. $CD4^+$ IFN- γ secreting cells were sorted on a FACS Aria2. Sorted cells were rested in CTL supplemented with 10 ng/ml human IL-15 for 5 days, then expanded using a rapid expansion protocol described previously (Riddell and Greenberg, *supra*) . Antigen-specific T cells were further enriched by sorting for Vβ3.1 positive, $CD4^+$ cells by staining with anti-Vβ 3.1 (Thermo Scientific, cat. no. TCR2740), expanded, and cryopreserved at day 13 or 14 after expansion. Cryopreserved cells were thawed and rested overnight in CTL supplemented with 10 U/ml IL-2 prior to assays.

Antigen-Presenting Cells

Autologous B cells were isolated from fresh or thawed PBMC using magnetic beads coated with antibodies recognizing CD19 (Miltenyi, cat. no. 130-050-301) and magnetic positive selection according to the manufacturer's instructions (Miltenyi, cat. no. 130-042-401). Primary B cells were incubated in a 1:1 ratio with NIH 3T3 cells expressing humanCD40L for 7 days in B cell medium supplemented with 200U/ml human IL-4 (Peprotech) as described (*see* Tran *et al.*, *Science 344*(3184):641-645 (2014)). B cells were subsequently harvested and restimulated with 3T3 CD40L and fresh medium every 3 days. B cells were used in assays at day +3 of stimulation 2 or 3.

Cytokine Release Assays

In ELISA assays, 50,000 effector T cells were incubated in 96 well round bottom plates with 100,000 B cells or B-LCL lines and 10 μg/ml or specific concentrations of peptides in RPMI (Gibco) supplemented with 5% heat inactivated fetal bovine serum. IFN-γ in supernatants was quantitated using the ready set go human

IFN-γ ELISA kit (ebiosciences) in technical triplicate. HLA blocking experiments were carried out with 20 μg/ml antibody anti class I (Biolegend, cat. no. 311411) anti-HLA DR (clone L243, cat. no. 307611) and HLA-DQ (Abcam, clone spv-l3, cat. no. ab23632) added 1 hour prior to adding peptide. For elispot assays, 50,000 tumor infiltrating lymphocytes were incubated with 200,000 autologous B cells pulsed with peptide pools at a final concentration of 10 μg/ml of each peptide in CTL medium using the human IFN-γ ELISpot-Pro kit (Mabtech) and developed using the manufacturer's instructions.

HLA Identification

LCL cell lines 1331, DUCAF, VAVY, BM14, DEM and DEU were utilized. For co-culture assays, LCL cell lines were pulsed with 10 μg/ml of BRAF mutant peptide or DMSO control for 4 hours and then washed 3 times with PBS prior to ELISA assay. For identification of specific class II alleles, codon optimized linear DNA fragments encoding HLA-DRB1 0404 protein or the HLA-DQB1 0302 protein linked by a T2A skip sequence to HLA-DQA1 0301 protein were synthesized using Genestrings™ (Life Sciences) and cloned into the vector MP71 (Engels *et al.*, *Hum. Gene Ther. 14*(12):1155-1168 (2003)) linearized with NotI and EcoRI (Thermo Fisher) using the NEBuilder cloning kit (New England Biolabs) and sequence verified. Retroviral transduction was performed as described by Sommermeyer *et al.*, (*Leukemia*, 2015))into the VAVY cell line homozygous for HLA DRB1 0301, DQA1 0501, and DQB1 0201 (Research cell bank). Cells positive for DRB1 0404 were sorted on a FACSAria2 sorter using the antibody DRB1-PE (Biolegend, cat. no. 362303) and cells positive for DQB1 03 DQA1 03 were sorted using the anti DQ antibody clone HLADQ1-FITC (Biolegend, cat. no. 318104). Assays were performed with and without pulsing with $BRAF^{V600E}$ peptide. ELISA experiments were performed in technical duplicate or triplicate as indicated and are representative of two independent experiments.

EXAMPLE 2

IDENTIFICATION OF TCR GENE USAGE BY TIL

Deep sequencing was performed to identify TCR gene usage in $BRAF^{V600E}$-specific T cells and other T cells in the TIL. Three TCR Vβ clonotypes showed marked expansion after stimulation of post-treatment PBMC with $BRAF^{V600E}$ peptide, and these sequences were further enriched after IFN-γ capture, indicating their specificity for the mutant peptide (Figure 2G). TCR Vβ sequencing of tumor, TIL, and PBMC obtained prior to TIL infusion identified all 3 TCR Vβ clones in the tumor, and 2 of 3 in TIL. All 3 TCR Vb sequences were below the level of detection in pre-treatment PBMC indicating enrichment at the tumor site (Figure 2H). A total of 34 common Vβ sequences collectively made up >50% of the TIL product (Figure 2I). Only 5 of these 34 clones were detected in the pretreatment blood, with 4 at very low frequency (Figure 2J). To assess TCR gene usage of CD8+ T cells recognizing each of the 4 lineage-specific or C/T antigens, IFN-γ capture was used to sort these cells from TIL and assess TCR Vβ usage. Seven different Vβ sequences in the sorted cells were identified and represented 4.7% of the T cells in the TIL product (Figures 2L-2O). These 7 clonotypes and one of the BRAF-specific clones were detected in blood obtained 10 and 24 months post-treatment (Figures 2J and 2K). RNA expression targeted to the endosome was carried out using the method described by Kreiter *et al.* (*J. Immunol. 180*(1):309-318 (2008)) where antigens are targeted to the endosome by fusion of the antigen to class I MHC sorting signals. The mRNA expression construct pJV57 was constructed by gene synthesis (Geneart, Life Sciences), which contained a T7 promoter fused to the N terminal 25 amino acids of the human HLA-B gene, followed by a BamHI restriction site, the coding sequence of enhanced GFP, an AgeI restriction site, the C terminal 55 amino acids of the human HLA-B gene, followed by the human beta globin untranslated region followed by a 30 nucleotide poly A tail followed by a SapI restriction site directing cleavage in the poly A tail. Construct pJV84 was cloned by ligating the following into AgeI/BamHI digested pJV57: annealed oligonucleotides (Ultramers, Integrated DNA Technologies) encoding BRAF amino acids 575-624 flanked by a 5' AgeI and 3' BamHI site containing the E600 substitution. Construct

pJV85 was made by ligating annealed oligonucleotides (Ultramers, Integrated DNA Technologies) encoding BRAF amino acids 575-624 flanked by a 5' AgeI and 3' BamHI site containing the wildtype V600 amino acid. pJV84 and pJV85 were then linearized with SapI (Thermo Fisher) and mRNA was in vitro transcribed using the Highscribe T7 ARCA mRNA kit (New England Biolabs) and purified by lithium precipitation according to the manufacturer's instructions. mRNA was electroporated into CD40L stimulated B cells 16 hours prior to co-culture experiments as described by Tran *et al.* (*supra*).

EXAMPLE 3

PHENOTYPIC CHARACTERIZATION OF TIL PRODUCT

Phenotypic analysis of BRAF-specific clones was performed following TIL treatment (Figures 3A-3C). $BRAF^{V600E}$-specific $CD4^+$ T cells showed an effector memory phenotype ($CD45RA^-CCR7^-CD27^-KLRG1^+$) and expressed low levels of PD-1 (Figures 3A, 3B). The majority of $BRAF^{V600E}$-specific cells expressed CXCR3 and CCR4. A fraction of the cells also expressed the skin-homing marker cutaneous lymphocyte-associated antigen (CLA). $BRAF^{V600E}$ peptide–activated cells produced IFN-γ, TNF-α, IL-4, and IL-21 (Figure 3C), sometimes in combination (data not shown). Taken together, these data suggest that circulating BRAF-specific CD4+ T cells after TIL infusion have a mixed Th1/Th2 phenotype, consistent with an established memory cellular immune response to mutated BRAF in melanoma.

EXAMPLE 4

CONSTRUCTION AND TESTING OF $BRAF^{V600E}$ TCRS

Durable remissions in melanoma after adoptive transfer of self-antigen reactive $CD8^+$ T cells alone are exceedingly rare (*see*, *e.g.*, Johnson *et al.*, *Blood 114*(3):535-546 (2009); Yee *et al.*, *PNAS 99*(25):16168-16173 (2002); Dudley *et al.*, *J. Immunother. 24*(4):363-373 (2001)). Without wishing to be bound by theory, it is believed that the $BRAF^{V600E}$-specific $CD4^+$ T cells provide direct antitumor effects and aid the persistence and function of self-antigen reactive $CD8^+$ T cells against a tumor that

contained few neoantigens. The HLA-DQA1*03/DQB1*03 restricting allele for the $BRAF^{V600E}$-specific $CD4^+$ T cells is present in 29% of individuals in the International Histocompatibility Workgroup database (Petersdorf, E., *personal communication, International Histocompatibility Working Group in Hematopoietic Cell Transplantation. 2017*), and isolation of the $BRAF^{V600E}$-specific TCR genes from this patient can facilitate adoptive therapy for patients with BRAF mutant tumors with TCR engineered T cells. TCR Vα sequencing on samples with varying levels of BRAF-reactive clones identified four (4) TCR Vα sequences that correlated in frequency with the three (3) TCR Vβ sequences (Figure 2G).

Four (4) TCRs from the three (3) TCRB alleles and four (4) TRCA alleles identified were constructed and tested for antigen-specific response (Figures 4A, 4B, and 5). The dominant TCR from the patient (pJV88) was expressed in $CD4^+$ T cells from two healthy donors and conferred specificity to cells expressing $BRAF^{V600E}$ but not wildtype BRAF sequences (Figure 2H). The TCR pJV90, which was made from the second most dominant Vβ clone from the patient and one of the possible alpha chains, showed some activity but also possibly nonspecific baseline activation.

TCR Vb and Va Sequencing

DNA from clinical samples was isolated using the Qiagen DNeasy or Qiamp micro DNA kits according to the manufacturer's instructions. TCRB sequencing was carried out using the human TCRB sequencing kit (Adaptive Biotechnology) following the manufacturer's instructions and sequenced using a MiSeq (Fred Hutchinson Cancer Research Center Genomics core) with data analysis carried out by Adaptive biotechnology software. TCRA sequencing was carried out using the human TCRA sequencing service (Adaptive Biotechnology).

T Cell Receptor Construction

TCR construction was in the vector PRRL (Jones *et al.*, *Hum. Gene Ther. 20*(6):630-640 (2009)), which was further modified by introducing six point mutations into the start codon and putative promoter region of the woodchuck hepatitis virus X protein as in Lim and Brown, *RNA Biology 13*(9):743-747 (2016)) with the beta chain

followed by a P2A translational skip sequence followed by the alpha chain with cysteines introduced to facilitate pairing (*see* Kuball *et al.*, *Blood 109*(6):2331-2338 (2007)). A codon-optimized DNA fragment containing the TRBV28 and CDR3 and TRBJ1-3 sequences followed by TCRB1 sequence with a cysteine substituted at residue 57 followed by a P2A skip sequence and the TRAV21 and CDR3 sequences followed by TRAJ43 and TRAC sequences was synthesized as a genestring (Life Sciences) and cloned using the NEBuilder cloning kit (New England Biolabs) into the vector PRRL-SIN linearized with PstI and AscI (Thermo Fisher) and sequence verified. One week after transduction, cells were sorted based on Vbeta3.1 expression using antibody clone 8F10 (Thermo Scientific, cat. no. TCR2740) and expanded via rapid expansion as described above. T cells were used in assays or cryopreserved on day 14 of the rapid expansion.

EXAMPLE 5

CRISPR-MEDIATED DELETION OF ENDOGENOUS TCR LEADS TO INCREASED EXPRESSION OF TRANSGENIC BRAF-SPECIFIC TCR

Following gene transfer of synthetic T cell receptor sequences into T cells, the transferred TCR alpha and beta chains may compete with endogenous TCR subunits for expression and signaling machinery. To investigate whether deletion of endogenous TCR increased expression of the transferred TCR, stimulated T cells were first transfected with CRISPR-Cas9 ribonucleoproteins with guide RNA sequences directing cleavage of the endogenous TCR alpha (guide rna AGAGTCTCTCAGCTGGTACA; SEQ ID NO:136) and TCR beta (guide rna: GGAGAATGACGAGTGGACCC; SEQ ID NO:139) constant region genes (from Ren *et al.*, *Clin. Cancer Res. 23*(9):2255-2266 (2017)). 12uM Cas9 protein (IDT) 20uM guide RNA (IDT) with electroporation enhancer (IDT) was assembled and 3ul was added to 2e6 primary human T cells 2 days following stimulation with antiCD3/antiCD28 Dynabeads (Thermo-Fisher) in nucleofection buffer P3 (Lonza), and nucleofected using an AMAXA 4D nucleofection electroporator (Lonza) using program EH-115. This combination led to >99% reduction in cells with TCR expression, as measured by staining of the CD3 component

of the TCR receptor complex (Fig. 6A). When gene deletion of the endogenous TCR was combined with gene transfer of the $BRAF^{V600E}$-specific TCR, increased expression of the transferred T cell receptor on the cell surface was observed, as measured by tetramer staining (Fig. 6B).

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including U.S. Provisional Patent Application No. 62/544,695, filed August 11, 2017, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

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

CLAIMS

What is claimed is:

1. A binding protein, comprising:

(a) a T cell receptor (TCR) α chain variable (Vα) domain having a CDR3 amino acid sequence set forth in any one of SEQ ID NOS:29-32, or a CDR3 amino acid sequence set forth in any one of SEQ ID NOS:29-32 with up to five amino acid substitutions, insertions, and/or deletions, and a TCR β chain variable (Vβ) domain;

(b) a Vα domain, and a Vβ domain having a CDR3 amino acid sequence as set forth in any one of SEQ ID NOS:33-35, or a CDR3 amino acid sequence set forth in any one of SEQ ID NOS:33-35 with up to five amino acid substitutions, insertions, and/or deletions; or

(c) a Vα domain of (a) and a Vβ domain of (b),

wherein the binding protein is capable of specifically binding to a HLA complex on a cell surface comprising a BRAF peptide containing a $BRAF^{V600E}$ mutation and does not bind a HLA complex on a cell surface comprising a BRAF peptide not containing the $BRAF^{V600E}$ mutation.

2. The binding protein according to claim 1, wherein:

(a) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 29 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:33;

(b) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 30 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:34;

(c) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 31 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:34;

(d) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 32 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:35;

(e) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 29 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:34;

(f) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 29 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:35;

(g) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 30 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:33;

(h) the Vα domain comprises the CDR3 amino acid of SEQ ID NO: 30 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:35;

(i) the Vα domain comprises the CDR3 amino acid of SEQ ID NO: 31 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:33;

(j) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO: 31 and the Vβ domain comprises the CDR3 amino acid sequence ofn SEQ ID NO:35;

(k) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO:32 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:33; or

(l) the Vα domain comprises the CDR3 amino acid sequence of SEQ ID NO:32 and the Vβ domain comprises the CDR3 amino acid sequence of SEQ ID NO:34.

3. The binding protein according to claim 1 or 2, wherein the binding protein comprises a Vα domain that is at least about 90% identical to the amino acid sequence set forth in any one of SEQ ID NOS:1-4, and comprises a Vβ domain that is at least about 90% identical to the amino acid sequence set forth in any one of SEQ ID NOS:5-7, provided that (a) at least three or four of the CDRs have no mutations and (b) the CDRs that do have mutations have only up to three amino acid substitutions, insertions, and/or deletions.

4. The binding protein according to any one of claims 1-3, wherein the Vα domain comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOS:1-4.

5. The binding protein according to any one of claims 1-4, wherein the Vβ domain comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOS:5-7.

6. The binding protein according to any one of claims 1-5, wherein the binding protein is a TCR, an antigen-binding fragment of a TCR, or a chimeric antigen receptor.

7. The binding protein according to claim 6, wherein the antigen-binding fragment of the TCR comprises a single chain TCR (scTCR).

8. The binding protein according to claim 6 or 7, wherein the binding protein is a chimeric antigen receptor.

9. The binding protein according to any one of claims 6-8, wherein the TCR, the antigen-binding fragment of the TCR, or chimeric antigen receptor is chimeric, humanized, or human.

10. The binding protein according to claim 6 or 9, wherein the binding protein is a TCR.

11. The binding protein according to claim 10, wherein the TCR comprises an α chain constant (Cα) domain having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:25.

12. The binding protein according to claim 11, wherein the Cα domain comprises the amino acid sequence of SEQ ID NO: 25.

13. The binding protein according to any one of claims 10-12, wherein the TCR comprises a β chain (Cβ) constant domain having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 26.

14. The binding protein according to claim 13, wherein the Cβ domain comprises the amino acid sequence of SEQ ID NO: 26.

15. The binding protein according to any one of claims 11-14, wherein the TCR α chain comprises the amino acid sequence set forth in any one of SEQ ID NOS: 55-58.

16. The binding protein according to any one of claims 11-15, wherein the TCR β chain comprises the amino acid sequence set forth in any one of SEQ ID NOS: 59-61.

17. The binding protein according to any one of claims 1-16, wherein the BRAFV600E peptide comprises from about 7 to about 27 amino acids, from about 10 to about 25 amino acids, or from about 12 to about 20 amino acids, or from about 15 to about 19 amino acids.

18. The binding protein according to any one of claims 1-17, wherein the BRAFV600E peptide comprises the amino acid sequence set forth in SEQ ID NO: 38 or 39.

19. The binding protein according to any one of claims 1-18, wherein the HLA complex comprises HLA-DQ.

20. The binding protein according to any one of claims 1-19, wherein the HLA complex comprises HLA-DQB1*0301, *0302, or *0303.

21. The binding protein according to claim 20, wherein the HLA complex comprises HLA-DQB1*0302.

22. The binding protein according to any one of claims 19-21, wherein the HLA complex comprises HLA-DQA1*03.

23. A composition comprising a binding protein according to any one of claims 1-22 and a pharmaceutically acceptable carrier, diluent, or excipient.

24. An isolated polynucleotide encoding a binding protein according to any one of claims 1-22.

25. The isolated polynucleotide according to claim 24, wherein the polynucleotide encoding a binding protein is codon optimized for expression in a host cell of interest.

26. An expression vector, comprising a polynucleotide according to claim 24 or 25 operably linked to an expression control sequence.

27. The expression vector according to claim 26, wherein the vector is capable of delivering the polynucleotide to a host cell.

28. The expression vector according to claim 27, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

29. The expression vector according to claim 28, wherein the immune system cell is a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a natural killer cell, a dendritic cell, or any combination thereof.

30. The expression vector according to claim 29, wherein the immune system cell is a CD4+ T cell.

31. The expression vector according to claim 29 or 30, wherein the T cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof.

32. The expression vector according to any one of claims 26-31, wherein the vector is a viral vector.

33. The expression vector according to claim 32, wherein the viral vector is a lentiviral vector or a γ-retroviral vector.

34. A host cell, comprising a heterologous polynucleotide according to claim 24 or 25 or an expression vector according to any one of claims 26-33, wherein the host cell expresses on its cell surface a binding protein encoded by the heterologous polynucleotide.

35. The host cell according to claim 34, wherein:

(a) the portion of the heterologous polynucleotide that encodes the Vα domain is at least about 80% identical to the polynucleotide sequence set forth in any one of SEQ ID NOS:18-21; and/or

(b) the portion of the heterologous polynucleotide that encodes the Vβ domain is at least about 80% identical to the polynucleotide sequence set forth in any one of SEQ ID NOS:22-24.

36. The host cell according to claim 34 or 35, wherein the portion of the heterologous polynucleotide that encodes the Vα domain comprises or consists of the polynucleotide sequence set forth in any one of SEQ ID NOS:18-21.

37. The host cell according to any one of claims 34-36, wherein the portion of the heterologous polynucleotide that encodes the Vβ domain comprises or consists of the polynucleotide sequence set forth in any one of SEQ ID NOS:22-24.

38. The host cell according to any one of claims 34-37, wherein the portion of the heterologous polynucleotide that encodes the Vα domain is linked to a portion that encodes a TCR α chain constant domain, wherein the portion that encodes the α chain constant domain comprises or consists of a sequence that is at least about 80% identical to the polynucleotide sequence of SEQ ID NO:27.

39. The host cell according to any one of claims 34-38, wherein the portion of the heterologous polynucleotide that encodes the Vβ domain is linked to a portion that encodes a TCR β chain constant domain, wherein the portion that encodes the β chain constant domain comprises or consists of a sequence that is at least about 80% identical to the polynucleotide sequence of SEQ ID NO:28.

40. The host cell according to any one of claims 34-39, wherein the portion that encodes the Vα domain comprises or consists of the polynucleotide sequence of SEQ ID NO:18 and the portion that encodes the Vβ domain comprises or consists of the polynucleotide sequence of SEQ ID NO:22.

41. The host cell according to any one of claims 34-39, wherein the portion that encodes the Vα domain comprises or consists of the polynucleotide sequence of SEQ ID NO: 19 and the portion that encodes the Vβ domain comprises or consists of the polynucleotide sequence of SEQ ID NO:23.

42. The host cell according to any one of claims 34-39, wherein the portion that encodes the Vα domain comprises or consists of the polynucleotide sequence of SEQ ID NO:20 and the portion that encodes the Vβ domain comprises or consists of the polynucleotide sequence of SEQ ID NO:23.

43. The host cell according to any one of claims 34-39, wherein the portion that encodes the Vα domain comprises or consists of the polynucleotide sequence of SEQ ID NO: 21 and the portion that encodes the Vβ domain comprises or consists of the polynucleotide sequence of SEQ ID NO:24.

44. The host cell according to any one of claims 34-43, wherein a portion of the polynucleotide encodes a self-cleaving peptide and is disposed between a TCR α chain-encoding portion and a TCR β chain-encoding portion.

45. The host cell according to claim 44, wherein the portion that encodes the self-cleaving peptide comprises or consists of the polynucleotide sequence set forth in any one of SEQ ID NOS:44-48.

46. The host cell according to claim 45, wherein the encoded self-cleaving peptide comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOS:49-52.

47. The host cell according to any one of claims 34-46, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

48. The host cell according to claim 47, wherein the immune system cell is a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a natural killer cell, a dendritic cell, or any combination thereof.

49. The host cell according to claim 47, wherein the immune system cell is a T cell.

50. The host cell according to claim 47, wherein the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

51. The host cell according to any one of claims 48-50, wherein the T cell is a CD4+ T cell.

52. The host cell according to claim 50 or 51, wherein the binding protein or TCR expressed by the T cell is capable of more efficiently associating with a CD3 protein as compared to endogenous TCR.

53. The host cell according to any one of claims 47-52, comprising a chromosomal gene knockout of a PD-1 gene; a LAG3 gene; a TIM3 gene; a CTLA4 gene; an HLA component gene; a TCR component gene, or any combination thereof.

54. The host cell according to claim 53, wherein the chromosomal gene knockout comprises a knockout of an HLA component gene selected from an α1 macroglobulin gene, an α2 macroglobulin gene, an α3 macroglobulin gene, a β1 microglobulin gene, or β2 microglobulin gene.

55. The host cell according to claim 53 or 54, wherein the chromosomal gene knockout comprises a knockout of a TCR component gene selected from a TCR α variable

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

56. The host cell according to any one of claims 48-55, wherein the binding protein or TCR higher surface expression on a T cell as compared to endogenous TCR.

57. A composition, comprising a host cell of any one of claims 34-56, and a pharmaceutically acceptable carrier, diluent, or excipient.

58. A unit dose, comprising an effective amount of (i) the host cell of any one of claims 34-52 or (ii) a composition according to claim 57, wherein the host cell is optionally at a dose of about 10^7 cells/m^2 to about 10^{11} cells/m^2.

59. The unit dose of claim 58, comprising (i) a composition comprising at least about 30% engineered CD4+ T cells, combined with (ii) a composition comprising at least about 30% engineered CD8+ T cells, in about a 1:1 ratio, wherein the unit dose contains substantially no naïve T cells.

60. A method for treating a hyperproliferative disorder, comprising administering to human subject in need thereof (i) a composition comprising a binding protein specific for a $BRAFV^{600E}$ peptide:HLA complex according to any one of claims 1-22; (ii) a host cell according to any one of claims 34-56; (iii) a composition according to claim 57; or (iv) a unit dose according to claim 58 or 59.

61. The method according to claim 60, wherein the hyperproliferative disorder is a cancer.

62. The method according to claim 61, wherein the cancer is selected from hairy cell leukemia, melanoma, thyroid cancer such as poorly differentiated thyroid cancer, non-small cell lung cancer, colorectal cancer, papillary cancer, non-Hodgkin lymphoma, glioblastoma, and pilocytic astrocytoma.

63. The method according to any one of claims 60-62, wherein the composition comprises the host cell according to any one of claims 34-56.

64. An adoptive immunotherapy method for treating a condition characterized by $BRAF^{V600E}$ expression in cells of a subject having a hyperproliferative disorder, comprising administering to the subject an effective amount of a host cell according to any one of claims 34-56, a composition according to claim 57, or a unit dose according to claim 58 or 59.

65. The method according to claim 64, wherein the host cell is modified *ex vivo*.

66. The method according to claim 64 or 65, wherein the host cell is an allogeneic cell, a syngeneic cell, or an autologous cell to the subject.

67. The method according to any one of claims 64-66, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

68. The method according to claim 67, wherein the immune system cell is a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a natural killer cell, a natural killer T cell, a dendritic cell, or any combination thereof.

69. The method according to claim 67 or 68, wherein the immune system cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof.

70. The method according to claim 68 or 69, wherein the cell is a CD4+ T cell.

71. The method according to any one of claims 64-70, wherein the hyperproliferative disorder is a cancer.

72. The method according to claim 71, wherein the cancer is selected from hairy cell leukemia, melanoma, thyroid cancers such as papillary thyroid cancer and poorly

differentiated thyroid cancer, non-small cell lung cancer, colorectal cancer, papillary cancer, non-Hodgkin lymphoma, glioblastoma, and pilocytic astrocytoma, breast cancer, ovarian cancer, Langerhans cell histiocytosis, or a sarcoma (*e.g.*, fibrosarcoma (fibroblastic sarcoma), Dermatofibrosarcoma protuberans (DFSP), osteosarcoma, rhabdomyosarcoma, Ewing's sarcoma, a gastrointestinal stromal tumor, Leiomyosarcoma; angiosarcoma (vascular sarcoma), Kaposi's sarcoma, liposarcoma, pleomorphic sarcoma, or synovial sarcoma).

73. The method according to any one of claims 64-72, wherein the host cell is administered parenterally.

74. The method according to any one of claims 64-73, wherein the method comprises administering a plurality of doses of the host cell to the subject.

75. The method according to claim 74, wherein the plurality of doses are administered at intervals between administrations of about two to about four weeks.

76. The method according to any one of claims 64-75, wherein the recombinant host cell is administered to the subject at a dose of about 10^7 cells/m^2 to about 10^{11} cells/m^2.

77. The method according to any one of claims 64-76, wherein the method further comprises administering a cytokine.

78. The method according to claim 77, wherein the cytokine is IL-2, IL-15, IL-21 or any combination thereof.

79. The method according to claim 78, wherein the cytokine is IL-2 and is administered concurrently or sequentially with the host cell.

80. The method according to claim 79, wherein the cytokine is administered sequentially, provided that the subject was administered the host cell at least three or four times before cytokine administration.

81. The method according to any one of claims 78-80, wherein the cytokine is IL-2 and is administered subcutaneously.

82. The method according to any one of claims 64-81, wherein the subject is further receiving an immunosuppressive therapy.

83. The method according to claim 82, wherein the immunosuppressive therapy is selected from calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof.

84. The method according to any one of claims 64-83, wherein the subject has received a non-myeloablative or a myeloablative hematopoietic cell transplant.

85. The method according to claim 84, wherein the subject is administered the host cell at least three months after the non-myeloablative hematopoietic cell transplant.

86. The method according to claim 85, wherein the subject is administered the host cell at least two months after the myeloablative hematopoietic cell transplant.

87. The method according to any one of claims 64-86, further comprising administering an inhibitor of an immune suppression agent to the subject.

88. The method according to claim 87, wherein the inhibitor of the immune suppression agent inhibits PD-1, PD-L1, PD-L2, LAG3, CTLA4, B7-H3, B7-H4, CD244/2B4, HVEM, BTLA, CD160, TIM3, GAL9, adenosine, A2aR, an immunosuppressive cytokine, IDO, arginase, VISTA, TIGIT, PVRIG, PVRL2, KIRs, LAIR1, CEACAM-1, CEACAM-3, CEACAM-5, CD160, Treg cells, or any combination thereof.

89. The method according to claim 87 or 88, wherein the inhibitor of the immune suppression agent is selected from the group consisting of an antibody or antigen binding

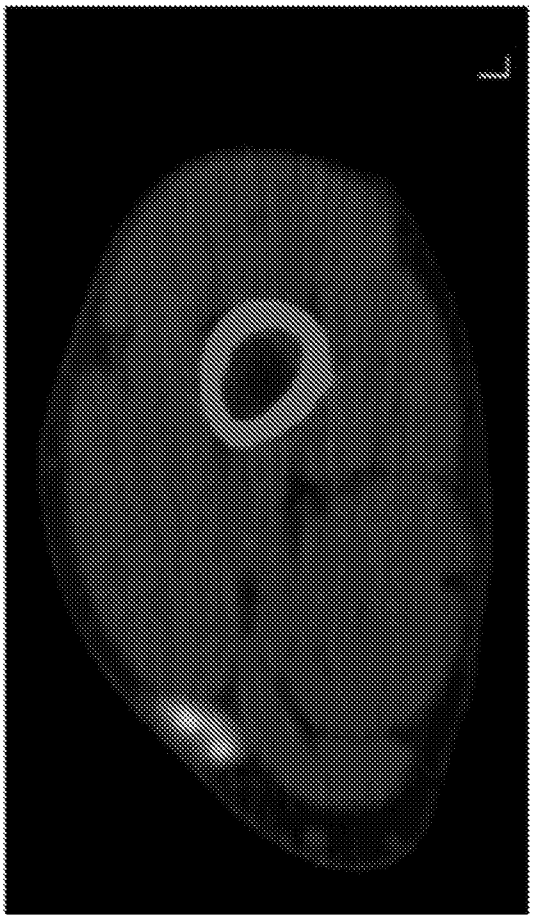
fragment thereof, a fusion protein, a small molecule, an RNAi molecule, a ribozyme, an aptamer, an antisense oligonucleotide, or any combination thereof.

90. The method according to claim 89, wherein the inhibitor of the immune suppression agent comprises pidilizumab, nivolumab, pembrolizumab, MEDI0680, AMP-224, BMS-936558 BMS-936559, durvalumab, atezolizumab, avelumab, MPDL3280A, LAG525, IMP321, IMP701, 9H12, BMS-986016, ipilimumab, tremelimumab, abatacept, belatacept, enoblituzumab, 376.96, an anti-B7-H4 antibody or antigen binding fragment, lirilumab, levo-1-methyl tryptophan, epacadostat, ebselen, indoximod, NLG919, 1-methyl-tryptophan (1-MT)-tira-pazamine, N(omega)-Nitro-L-arginine methyl ester (L-NAME), N-omega-hydroxy-nor-l-arginine (nor-NOHA), L-NOHA, 2(S)-amino-6-boronohexanoic acid (ABH), S-(2-boronoethyl)-L-cysteine (BEC), CA-170, COM902, COM701, or antigen binding fragments thereof, or any combination thereof.

91. The method according to any one of claims 64-90, further comprising administering to the subject a therapeutically effective amount of an agonist of a stimulatory immune checkpoint molecule.

92. The method according to claim 91, wherein the agonist is selected from urelumab, MEDI6469, MEDI6383, MEDI0562, lenalidomide, pomalidomide, CDX-1127, TGN1412, CD80, CD86, CP-870,893, rhuCD40L, SGN-40, IL-2, GSK3359609, mAb 88.2, JTX-2011, Icos 145-1, Icos 314-8, or any combination thereof.

93. The method according to any one of claims 64-92, further comprising administering to the subject one or more of: a therapeutic antibody, chemotherapy, radiation therapy, surgery, or any combination thereof.



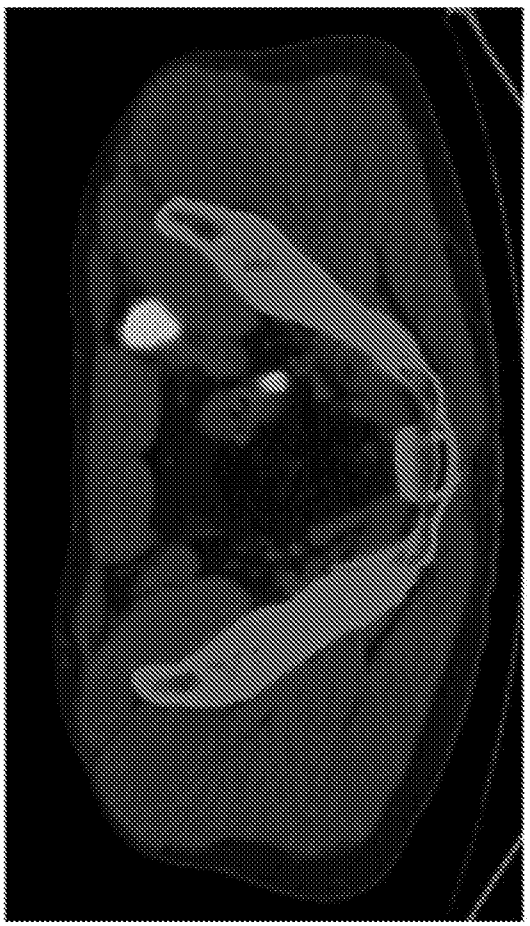


FIG. 1A

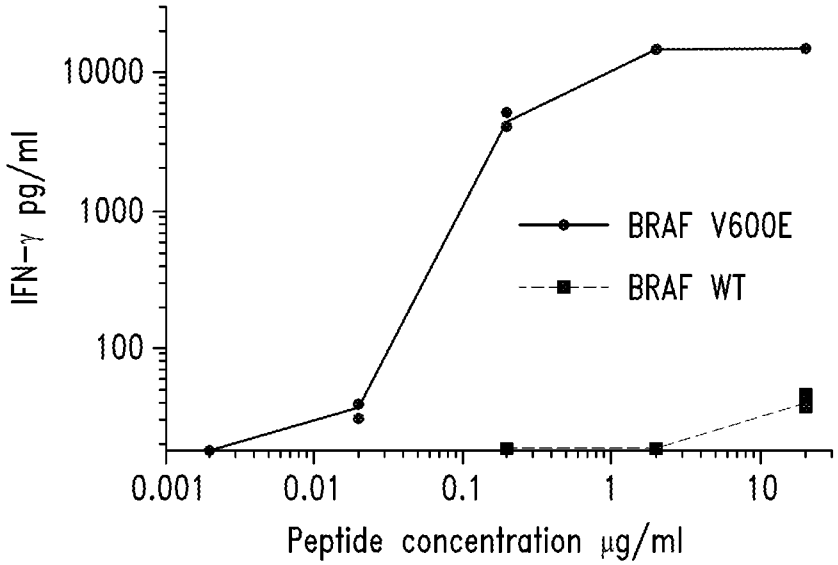

10000
1000
100
IFN-γ pg/ml
BRAF V600E
BRAF WT
0.001
0.01
0.1
1
10
Peptide concentration µg/ml

FIG. 1B

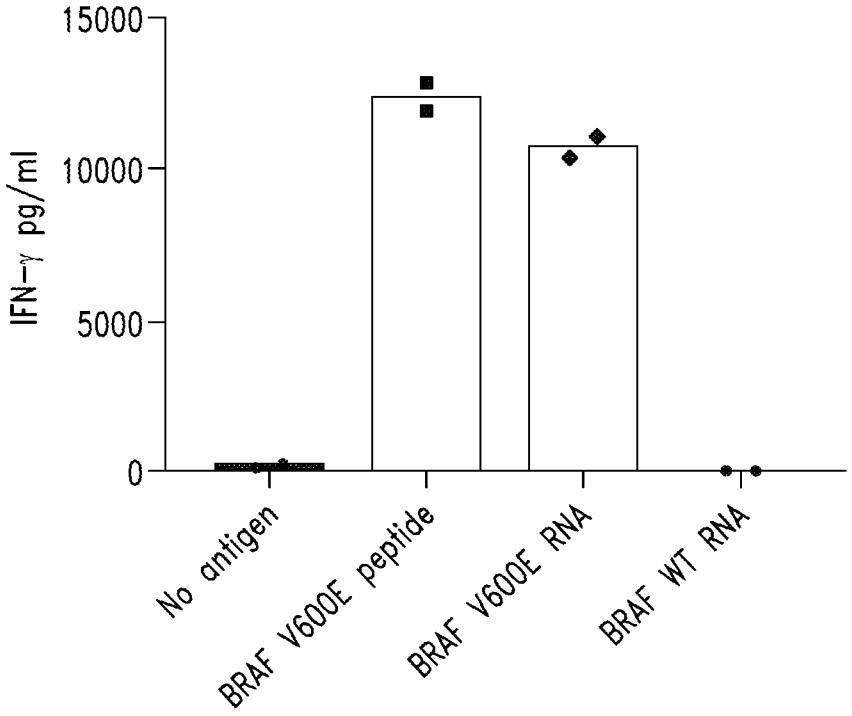

15000
10000
5000
0
IFN-γ pg/ml
No antigen
BRAF V600E peptide
BRAF V600E RNA
BRAF WT RNA

FIG. 1C

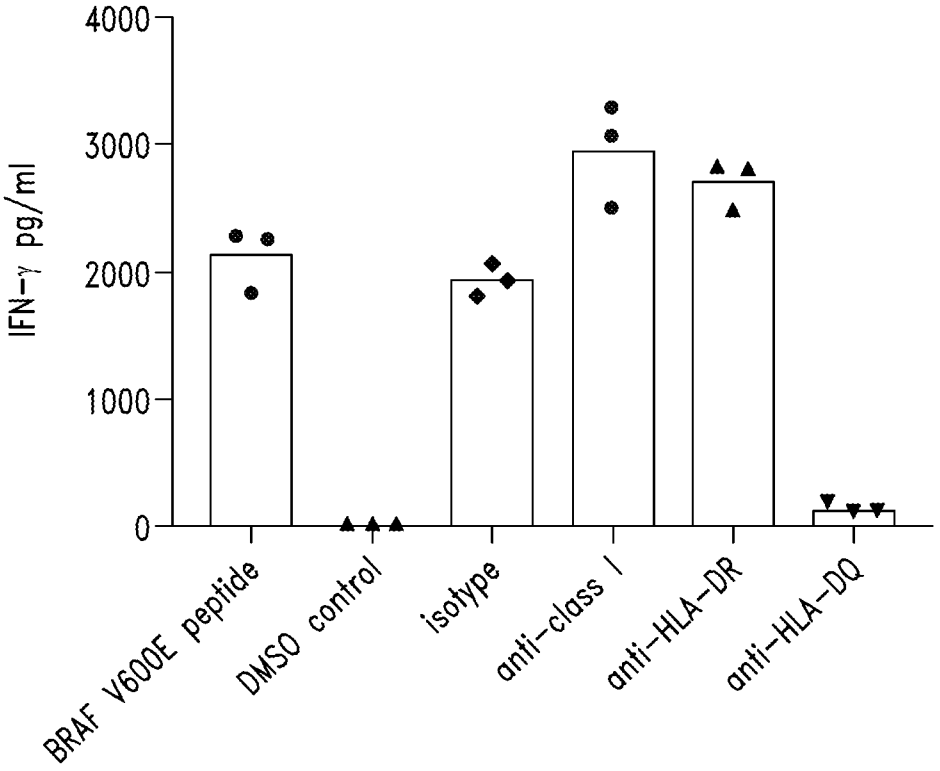

4000
3000
2000
1000
0
IFN-γ pg/ml
BRAF V600E peptide
DMSO control
isotype
anti-class I
anti-HLA-DR
anti-HLA-DQ

FIG. 1D

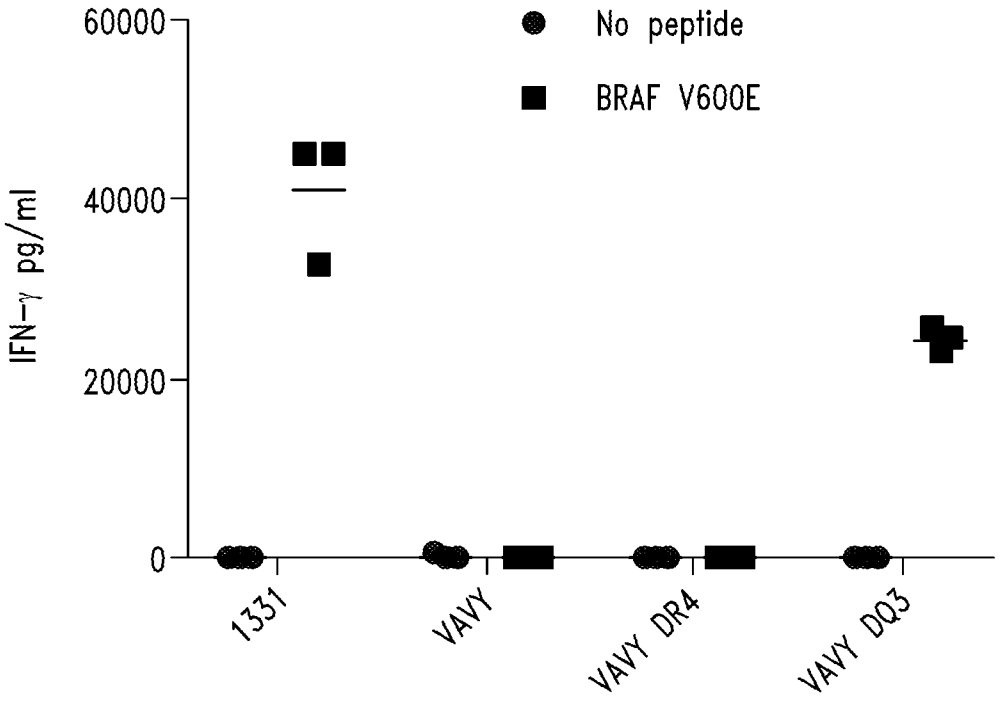

60000
40000
20000
0
IFN-γ pg/ml
No peptide
BRAF V600E
1331
VAVY
VAVY DR4
VAVY DQ3

FIG. 1E

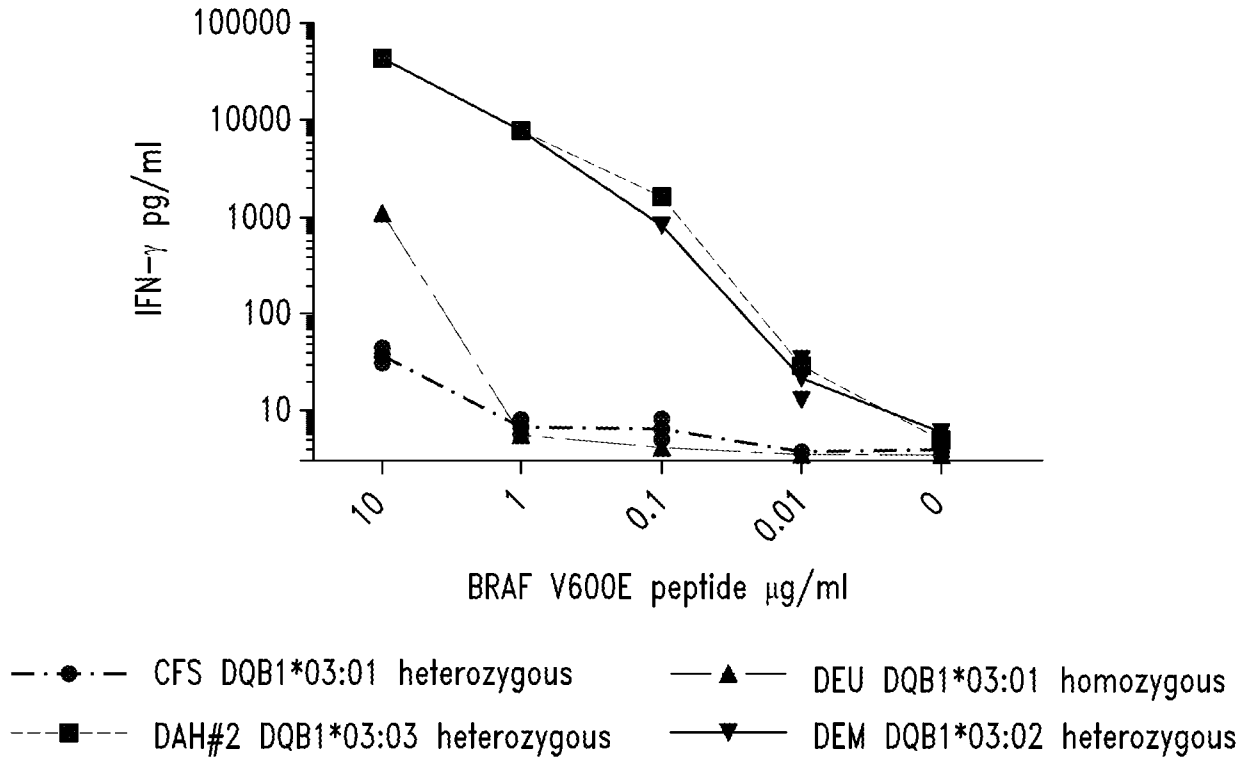

100000
10000
1000
100
10
IFN-γ pg/ml
10
1
0.1
0.01
0
BRAF V600E peptide µg/ml
CFS DQB1*03:01 heterozygous
DEU DQB1*03:01 homozygous
DAH#2 DQB1*03:03 heterozygous
DEM DQB1*03:02 heterozygous

FIG. 1F

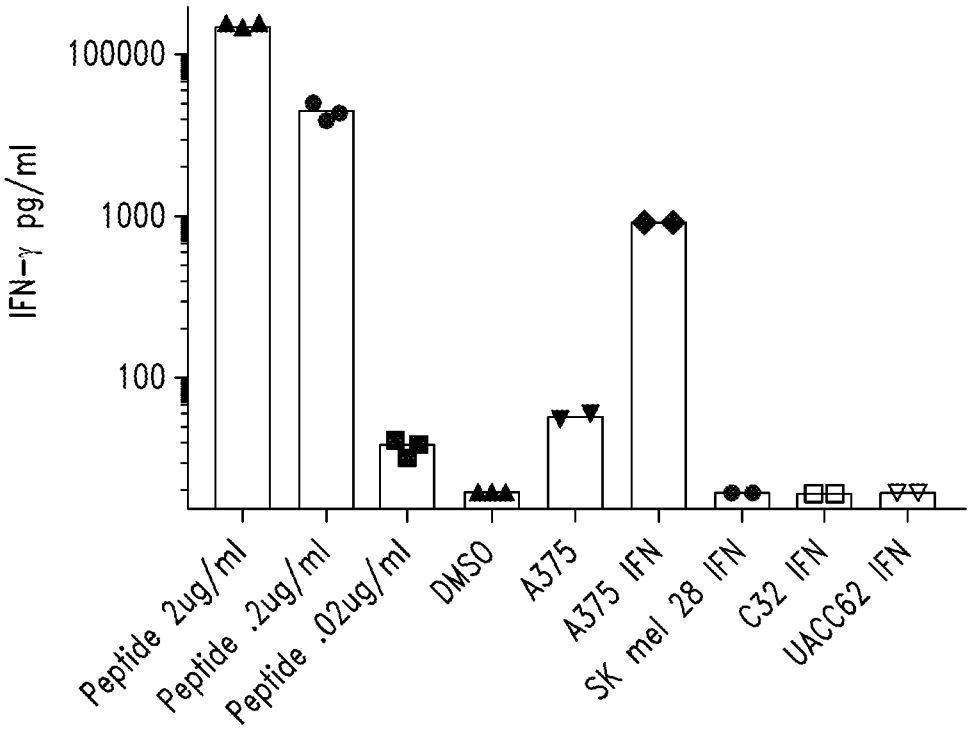

100000
1000
100
IFN-γ pg/ml
Peptide 2ug/ml
Peptide .2ug/ml
Peptide .02ug/ml
DMSO
A375
A375 IFN
SK mel 28 IFN
C32 IFN
UACC62 IFN

FIG. 1G

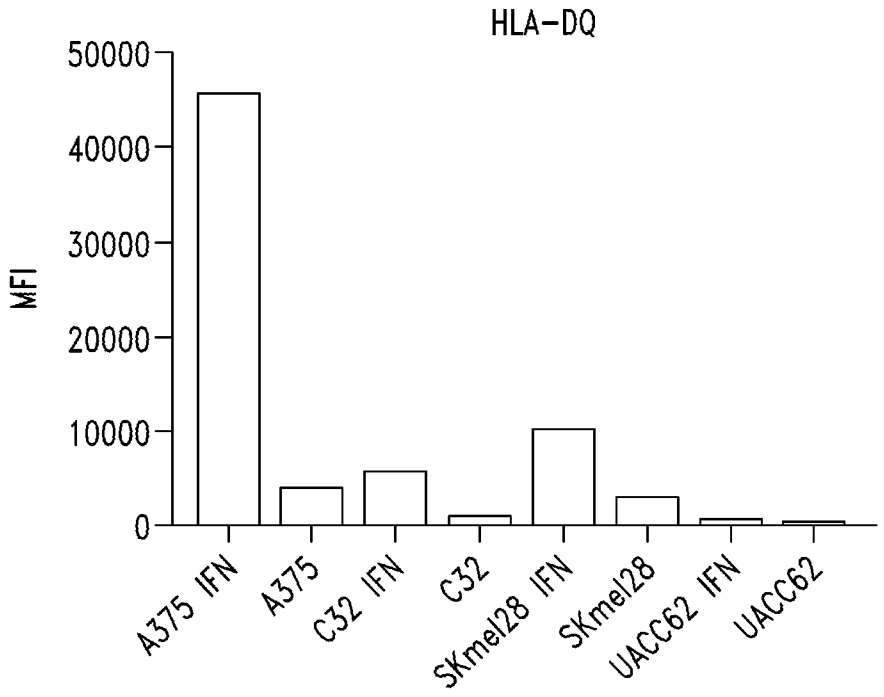

HLA-DQ
MFI
50000
40000
30000
20000
10000
0
A375 IFN
A375
C32 IFN
C32
SKmel28 IFN
SKmel28
UACC62 IFN
UACC62

FIG. 1H

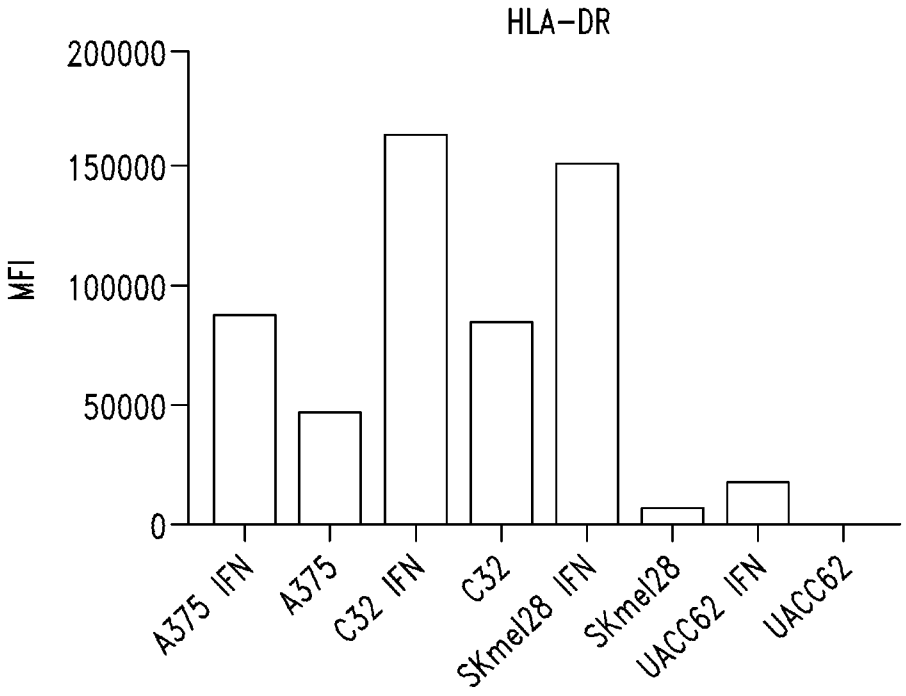

HLA-DR
MFI
200000
150000
100000
50000
0
A375 IFN
A375
C32 IFN
C32
SKmel28 IFN
SKmel28
UACC62 IFN
UACC62

FIG. 1I

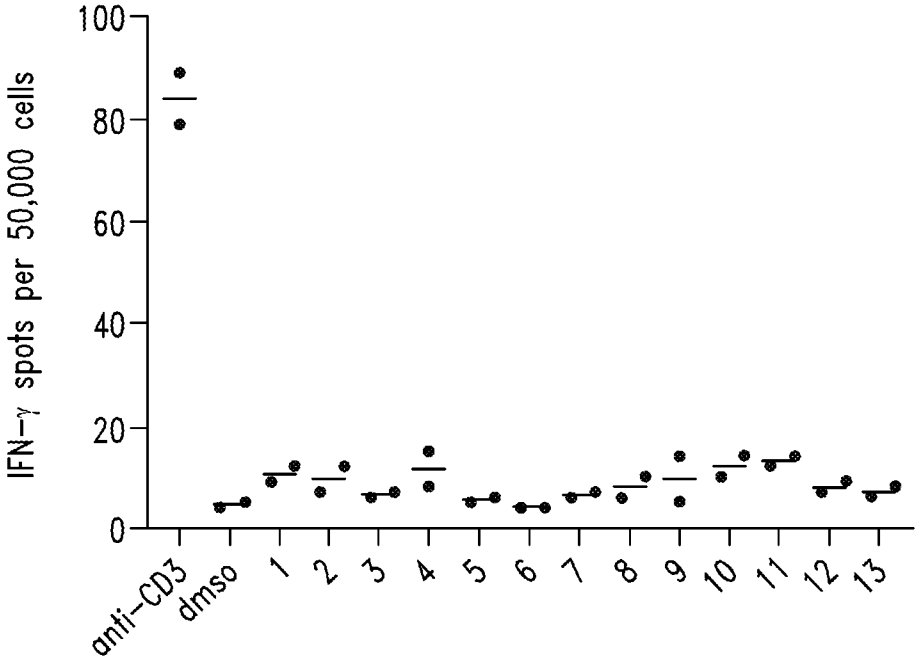

IFN-γ spots per 50,000 cells
100
80
60
40
20
0
anti-CD3
dmso
1
2
3
4
5
6
7
8
9
10
11
12
13

FIG. 2A

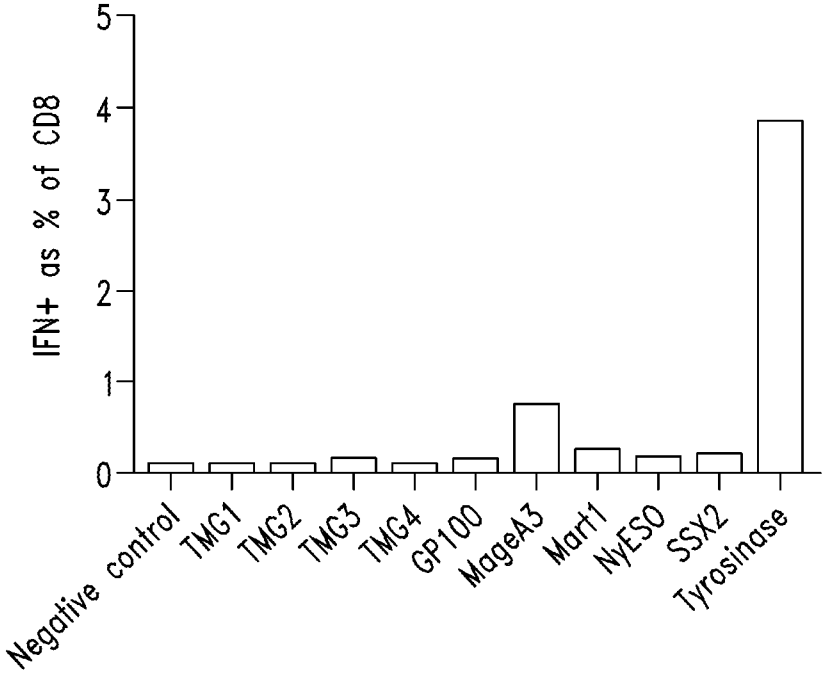

IFN+ as % of CD8
5
4
3
2
1
0
Negative control
TMG1
TMG2
TMG3
TMG4
GP100
MageA3
Mart1
NyESO
SSX2
Tyrosinase

FIG. 2B

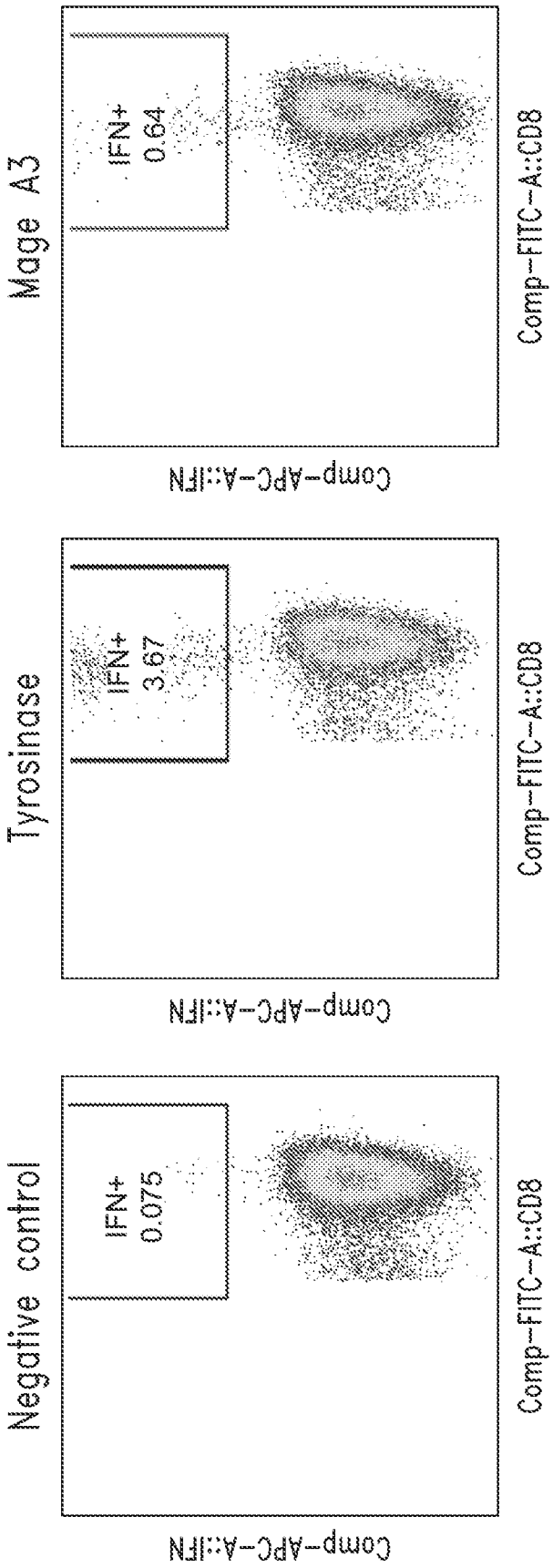

Mage A3
IFN+
0.64
Comp-APC-A::IFN
Comp-FITC-A::CD8
Tyrosinase
IFN+
3.67
Comp-APC-A::IFN
Comp-FITC-A::CD8
Negative control
IFN+
0.075
Comp-APC-A::IFN
Comp-FITC-A::CD8

FIG. 2C

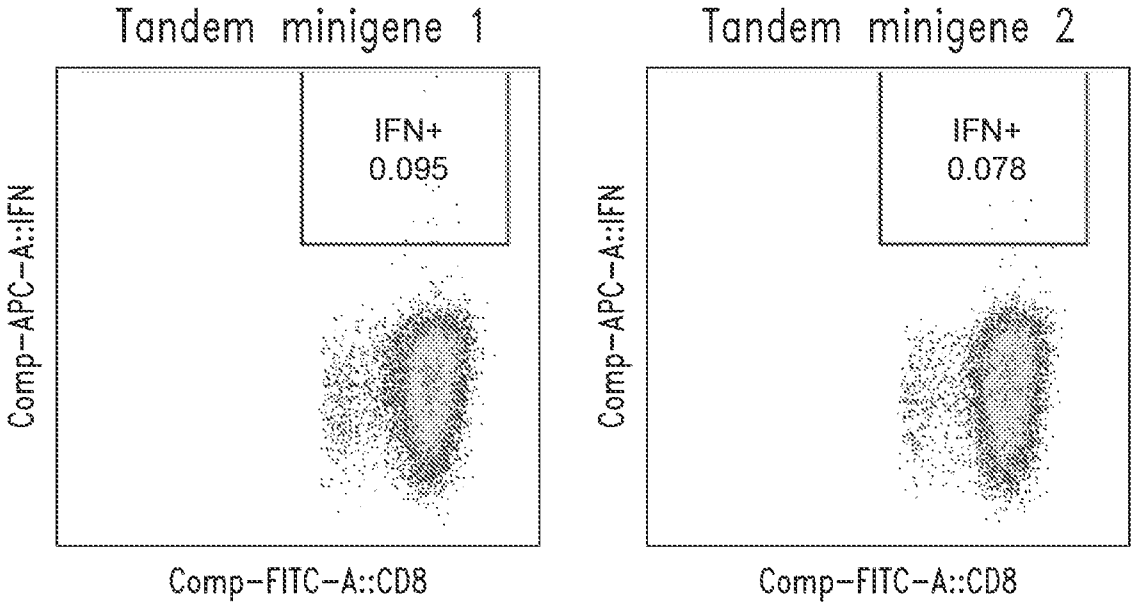

Tandem minigene 1
IFN+
0.095
Comp-APC-A::IFN
Comp-FITC-A::CD8
Tandem minigene 2
IFN+
0.078
Comp-APC-A::IFN
Comp-FITC-A::CD8

FIG. 2D

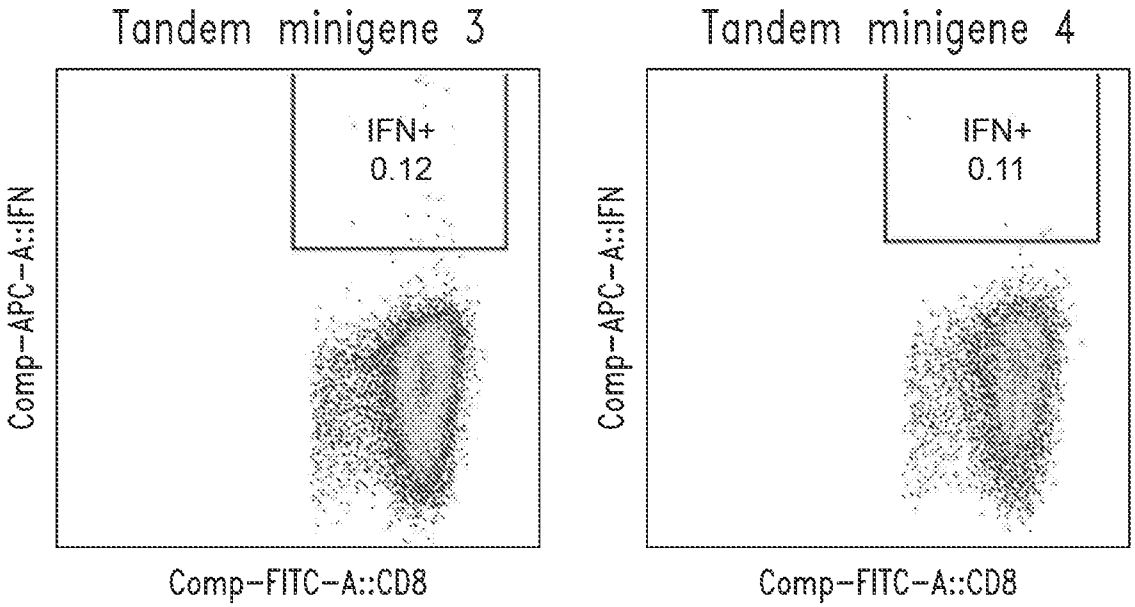

Tandem minigene 3
IFN+
0.12
Comp-APC-A::IFN
Comp-FITC-A::CD8
Tandem minigene 4
IFN+
0.11
Comp-APC-A::IFN
Comp-FITC-A::CD8

FIG. 2E

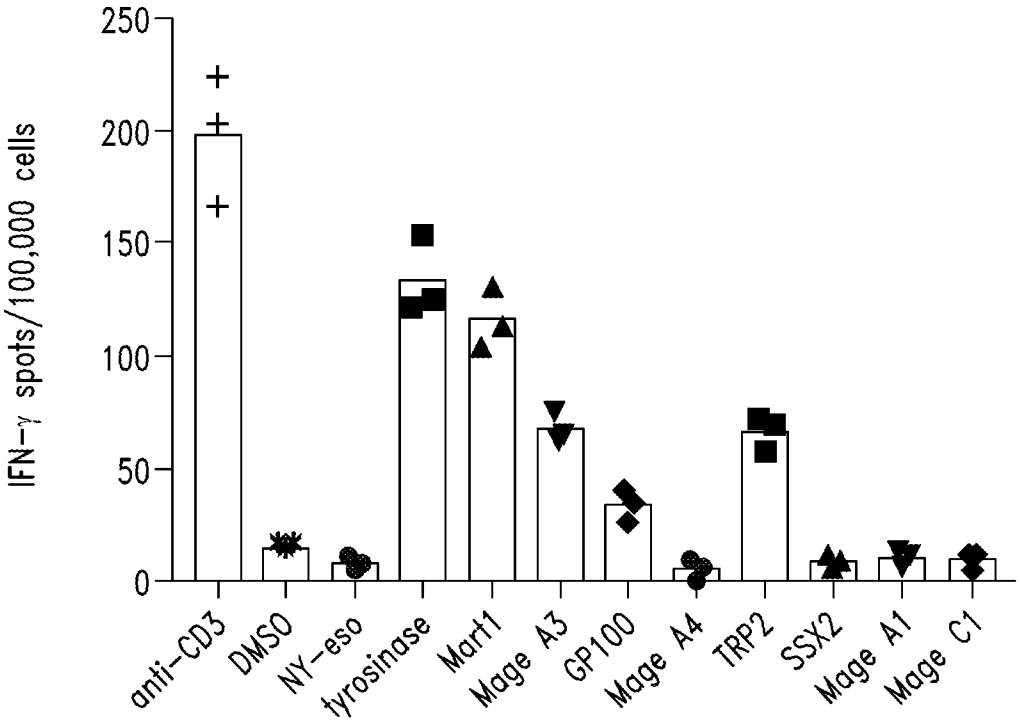

IFN-γ spots/100,000 cells
250
200
150
100
50
0
anti-CD3
DMSO
NY-eso
tyrosinase
Mart1
Mage A3
GP100
Mage A4
TRP2
SSX2
Mage A1
Mage C1

FIG. 2F

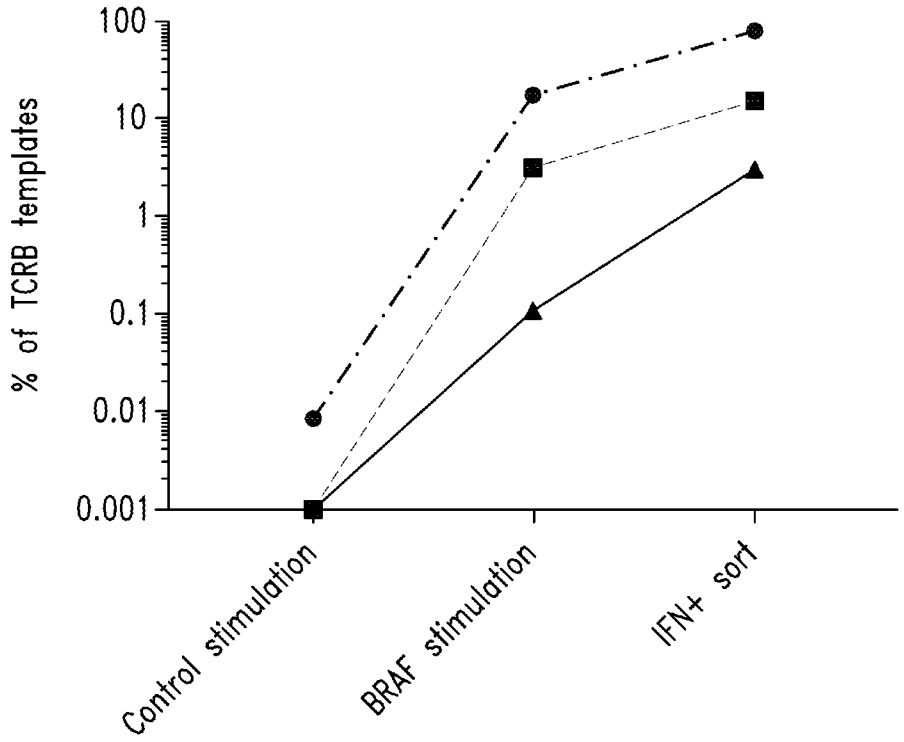

% of TCRB templates
100
10
1
0.1
0.01
0.001
Control stimulation
BRAF stimulation
IFN+ sort

FIG. 2G

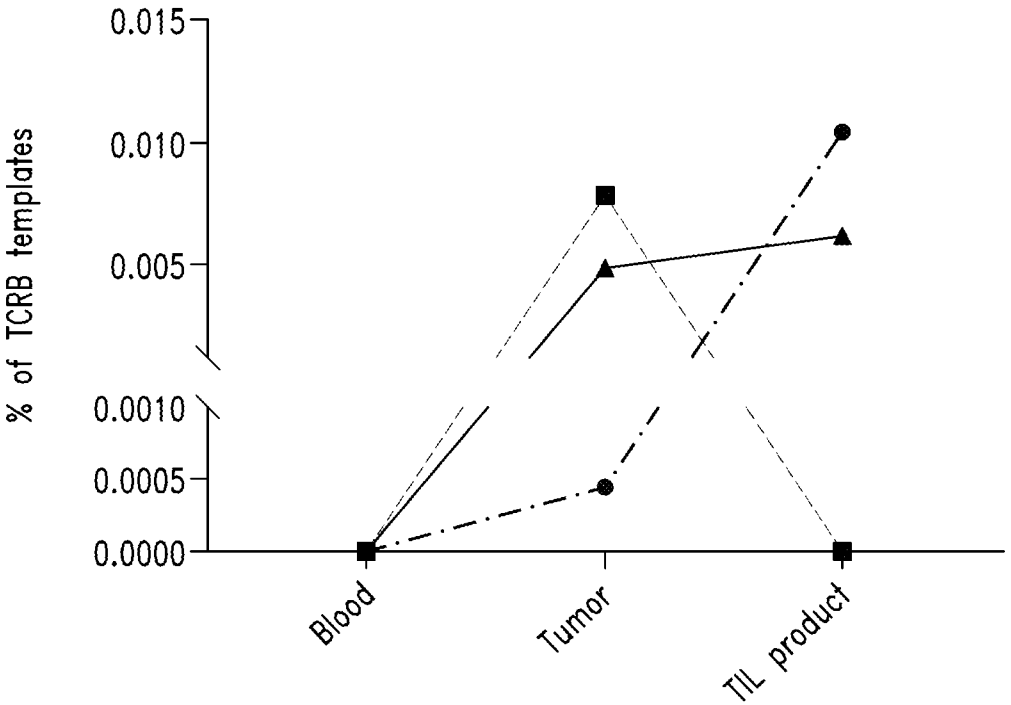

0.015
0.010
0.005
0.0010
0.0005
0.0000
% of TCRB templates
Blood
Tumor
TIL product

FIG. 2H

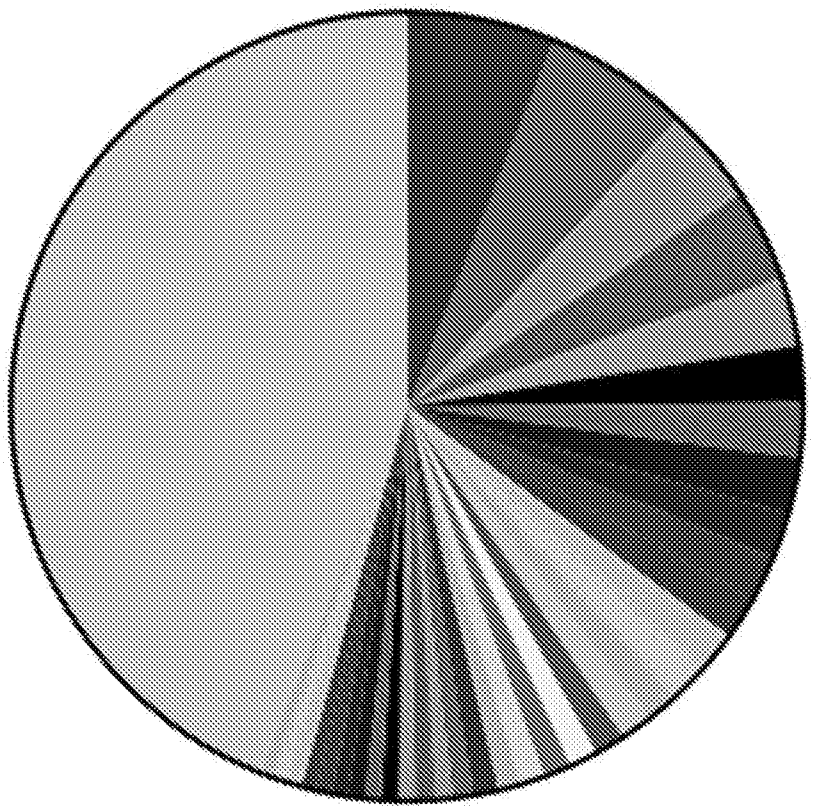


FIG. 2I

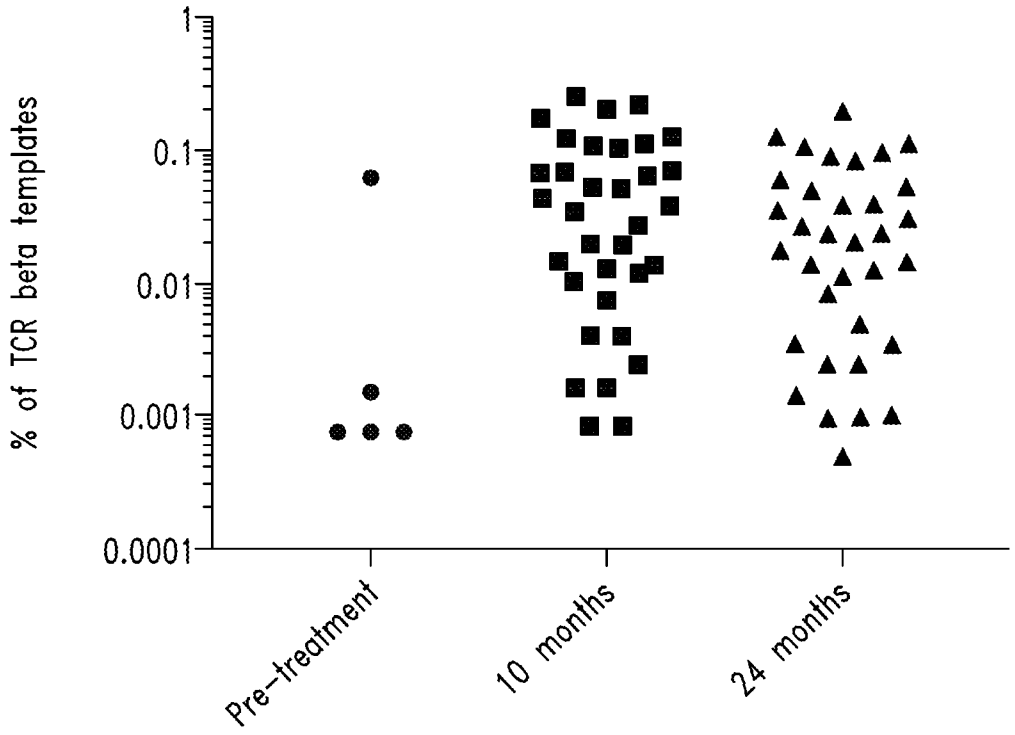

% of TCR beta templates
1
0.1
0.01
0.001
0.0001
Pre-treatment
10 months
24 months

FIG. 2J

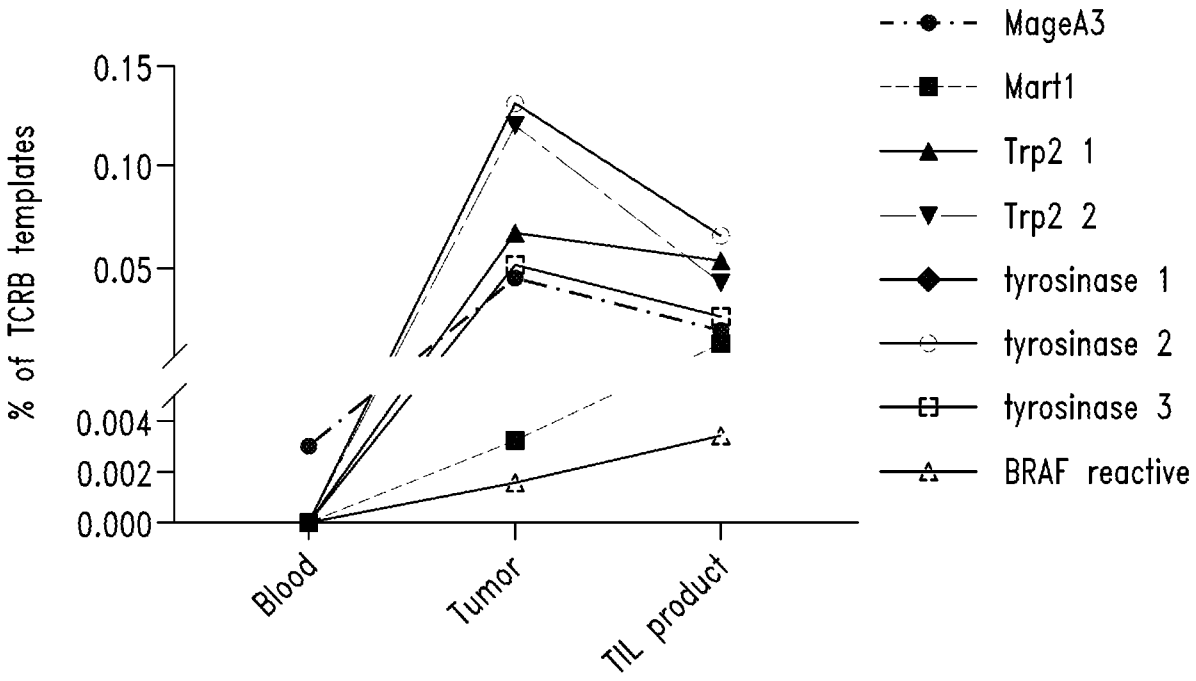

% of TCRB templates
0.15
0.10
0.05
0.004
0.002
0.000
Blood
Tumor
TIL product
MageA3
Mart1
Trp2 1
Trp2 2
tyrosinase 1
tyrosinase 2
tyrosinase 3
BRAF reactive

FIG. 2K

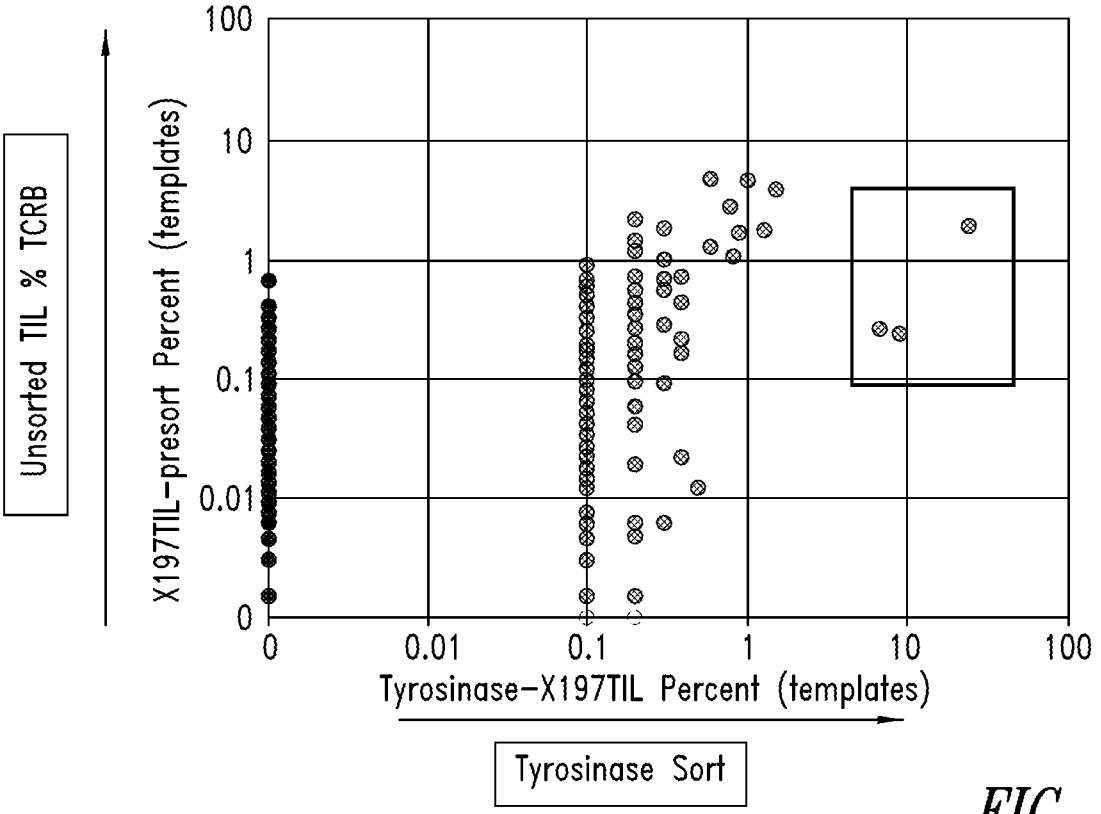

Unsorted TIL % TCRB
X197TIL-presort Percent (templates)
100
10
1
0.1
0.01
0
0
0.01
0.1
1
10
100
Tyrosinase-X197TIL Percent (templates)
Tyrosinase Sort

FIG. 2L

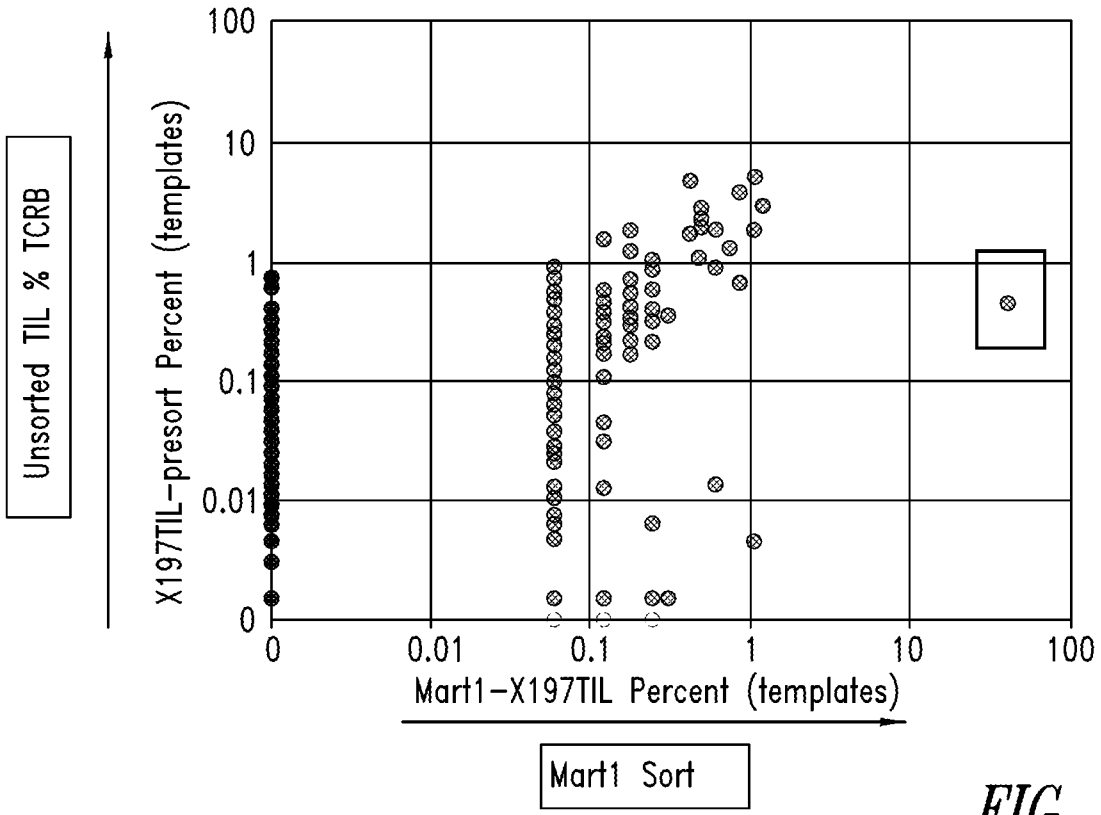

Unsorted TIL % TCRB
X197TIL-presort Percent (templates)
100
10
1
0.1
0.01
0
0
0.01
0.1
1
10
100
Mart1-X197TIL Percent (templates)
Mart1 Sort

FIG. 2M

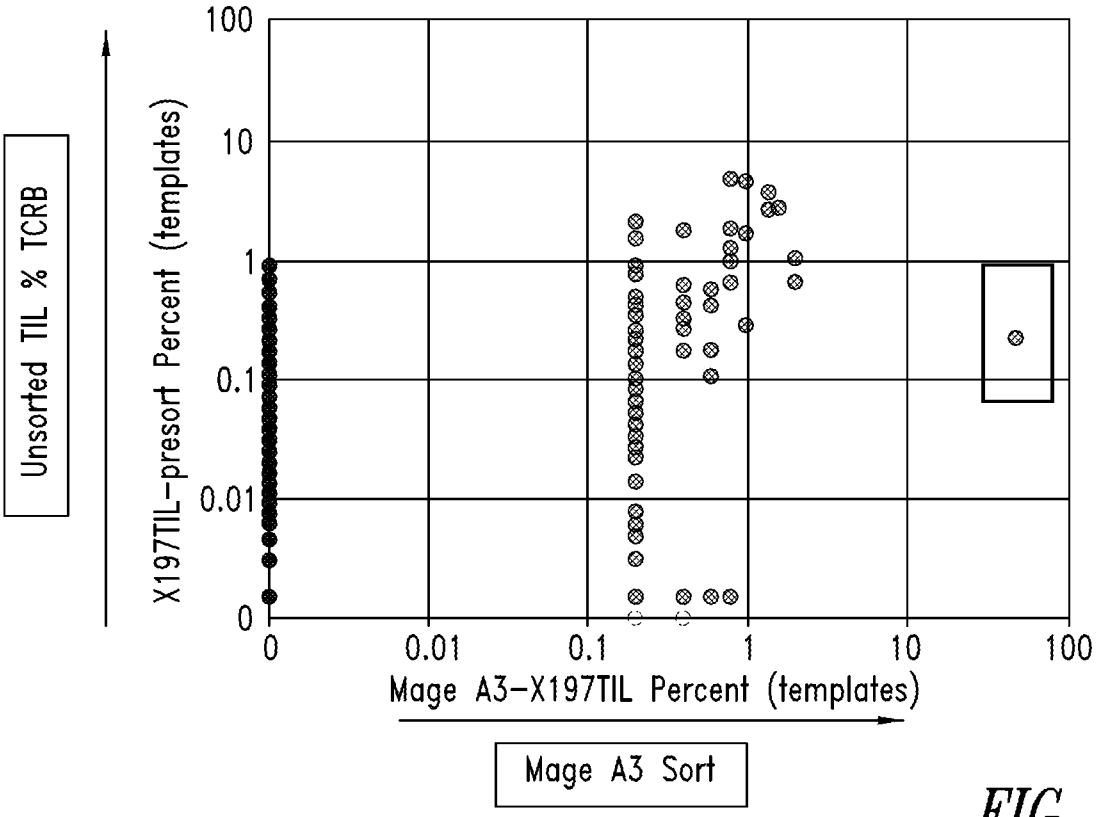

Unsorted TIL % TCRB
X197TIL-presort Percent (templates)
100
10
1
0.1
0.01
0
0
0.01
0.1
1
10
100
Mage A3-X197TIL Percent (templates)
Mage A3 Sort

FIG. 2N

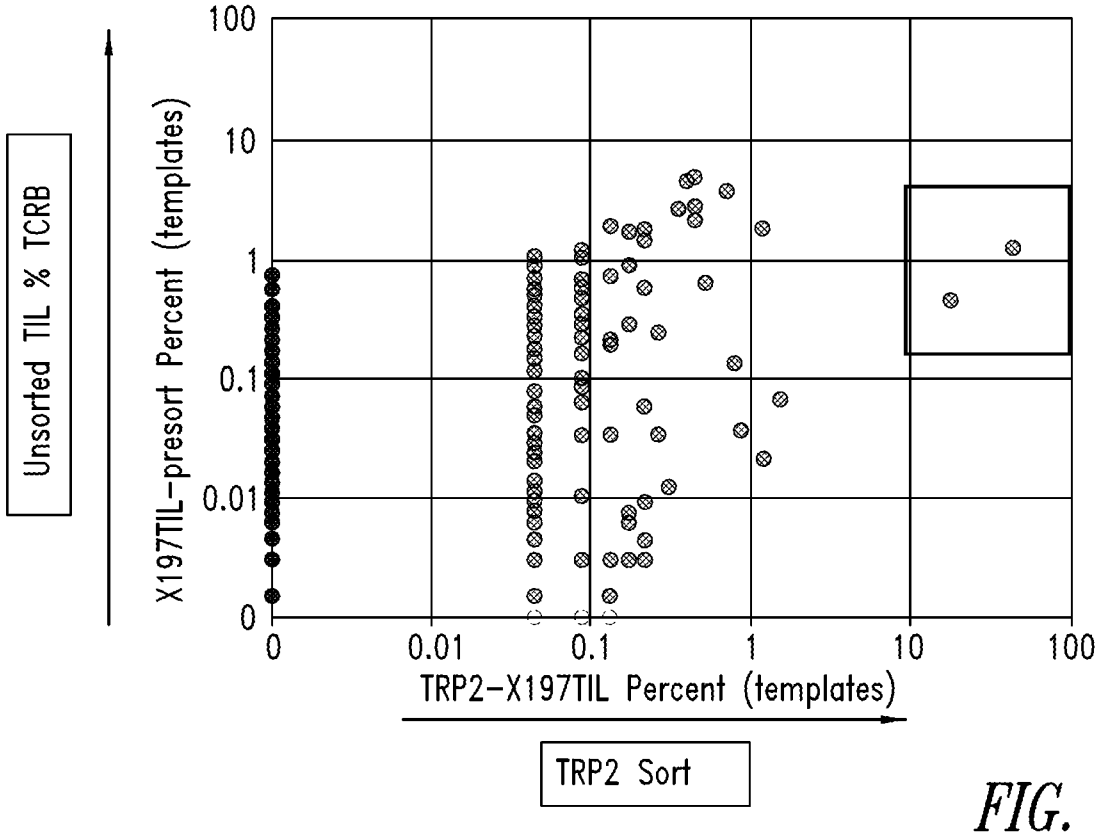

Unsorted TIL % TCRB
X197TIL-presort Percent (templates)
100
10
1
0.1
0.01
0
0
0.01
0.1
1
10
100
TRP2-X197TIL Percent (templates)
TRP2 Sort

FIG. 2O

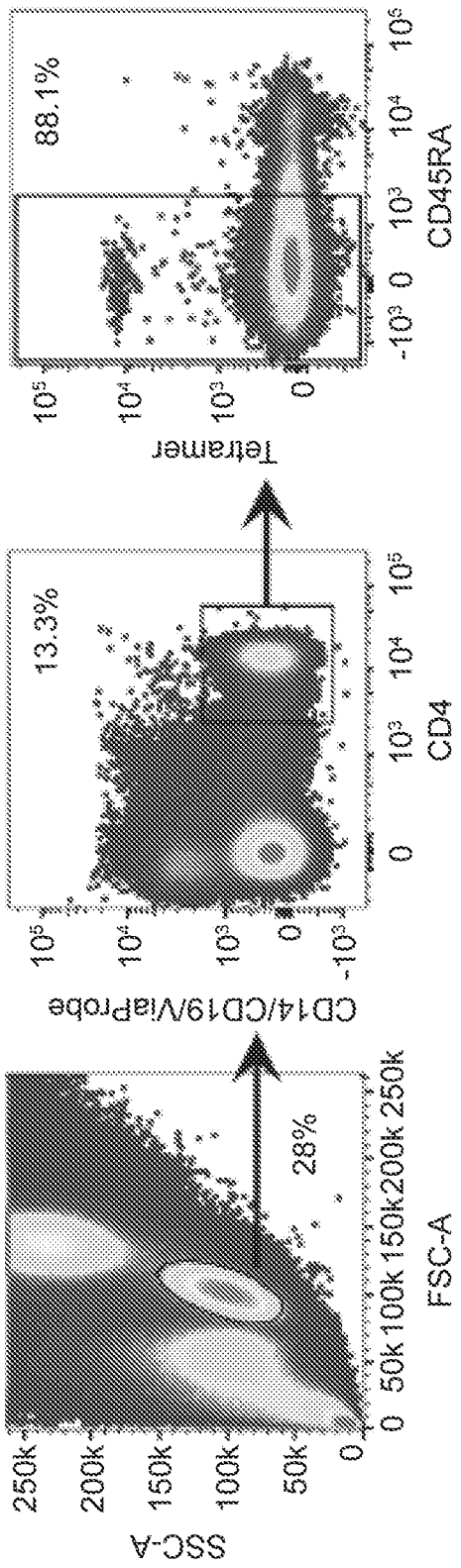

SSC-A
250k
200k
150k
100k
50k
0
0 50k 100k 150k 200k 250k
FSC-A
28%
CD14/CD19/IaProbe
10^5
10^4
10^3
0
-10^3
13.3%
CD4
Tetramer
88.1%
CD45RA

FIG. 3A

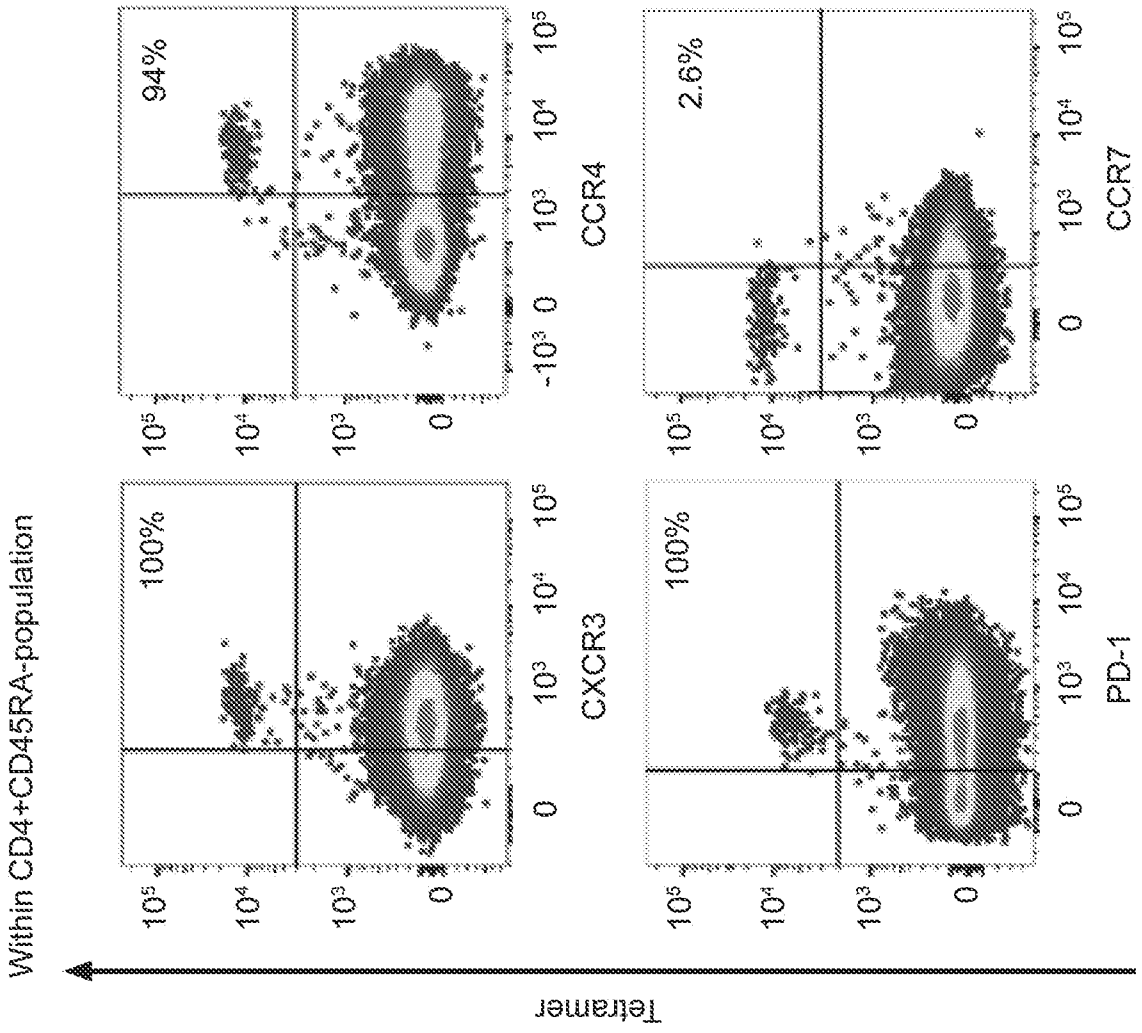

Within CD4+CD45RA-population
Tetramer
100%
CXCR3
94%
CCR4
100%
PD-1
2.6%
CCR7

FIG. 3B

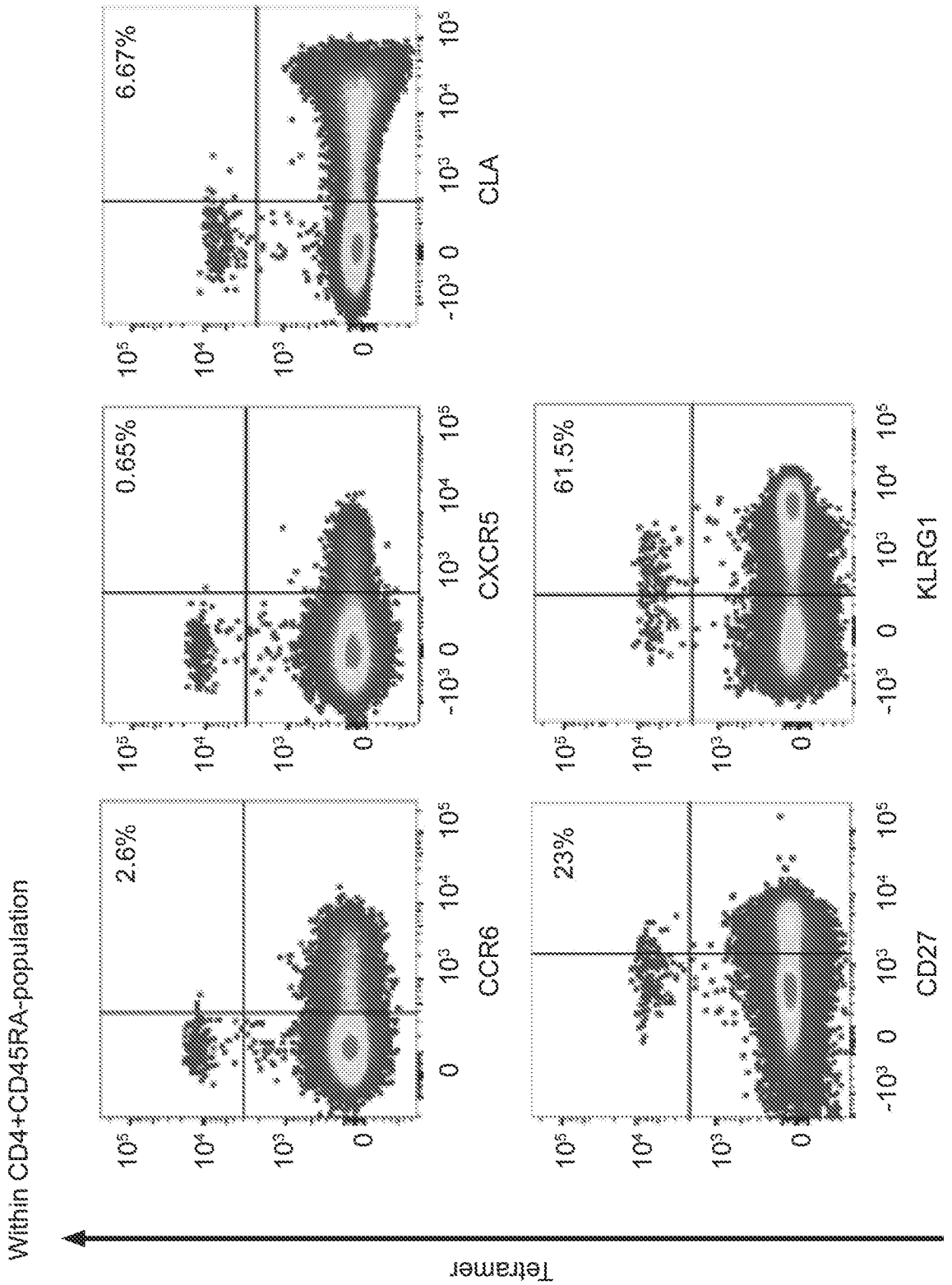


FIG. 3B (Continued)

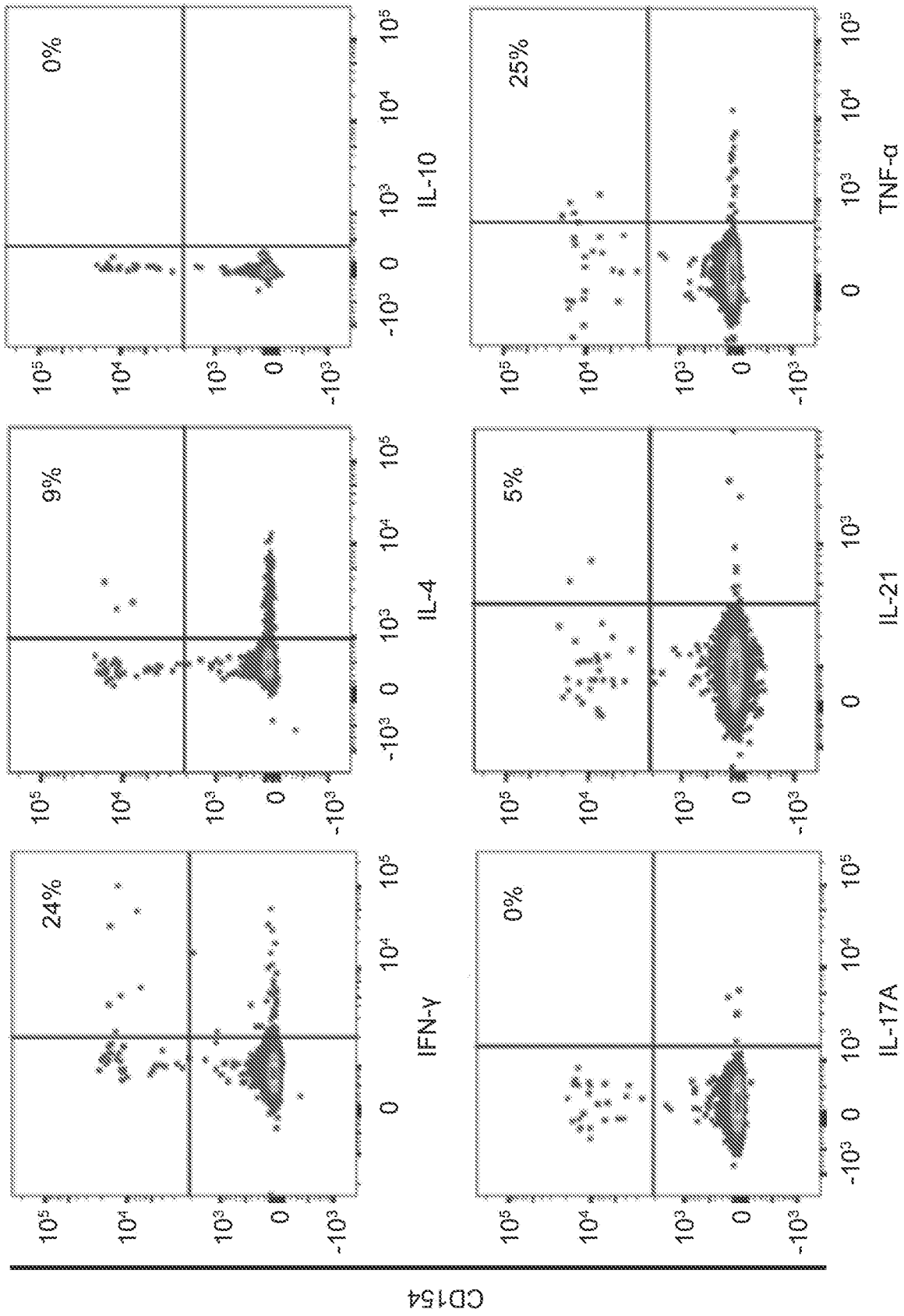


FIG. 3C

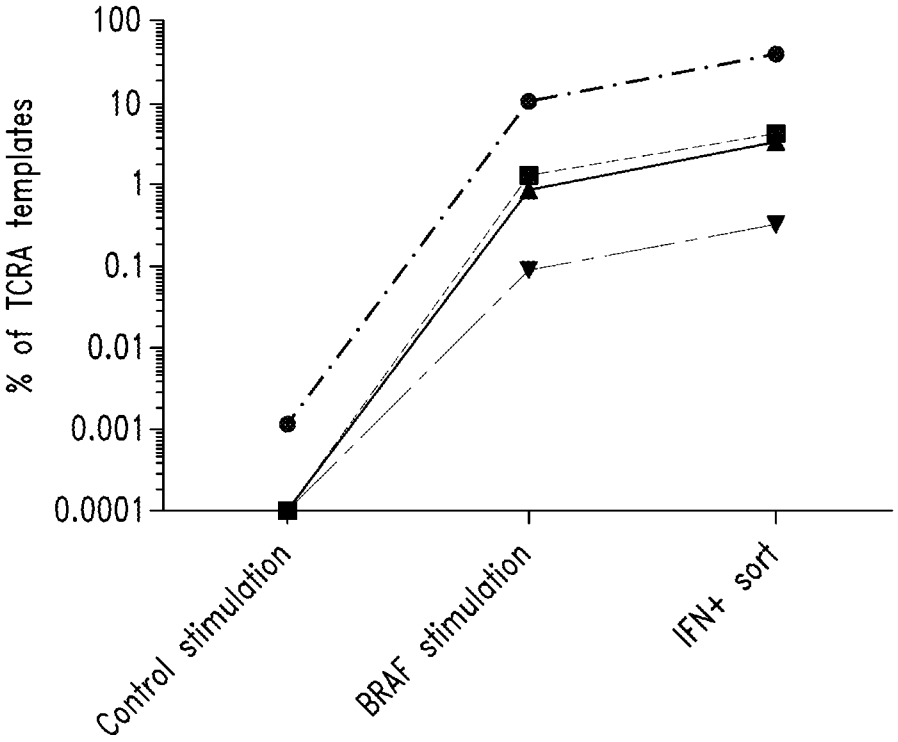

100
10
1
0.1
0.01
0.001
0.0001
% of TCRA templates
Control stimulation
BRAF stimulation
IFN+ sort

FIG. 4A

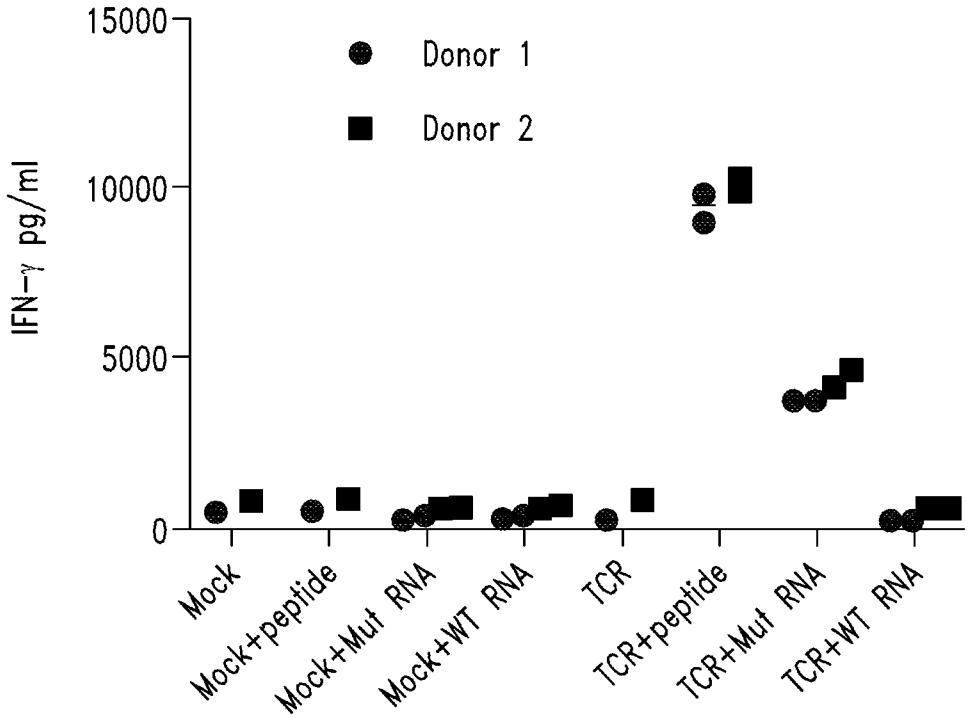

15000
10000
5000
0
IFN-γ pg/ml
Donor 1
Donor 2
Mock
Mock+peptide
Mock+Mut RNA
Mock+WT RNA
TCR
TCR+peptide
TCR+Mut RNA
TCR+WT RNA

FIG. 4B

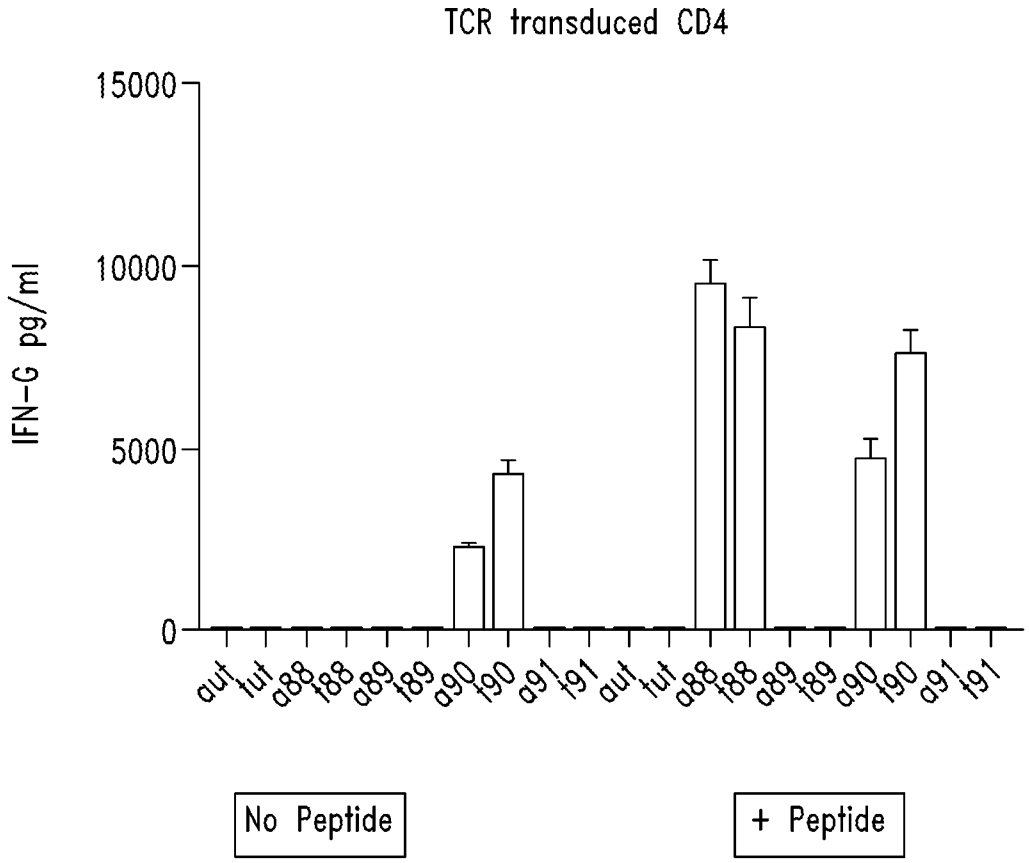

TCR transduced CD4
IFN-G pg/ml
15000
10000
5000
0
aut
tut
a88
t88
a89
t89
a90
t90
a91
t91
aut
tut
a88
t88
a89
t89
a90
t90
a91
t91
No Peptide
+ Peptide

FIG. 5

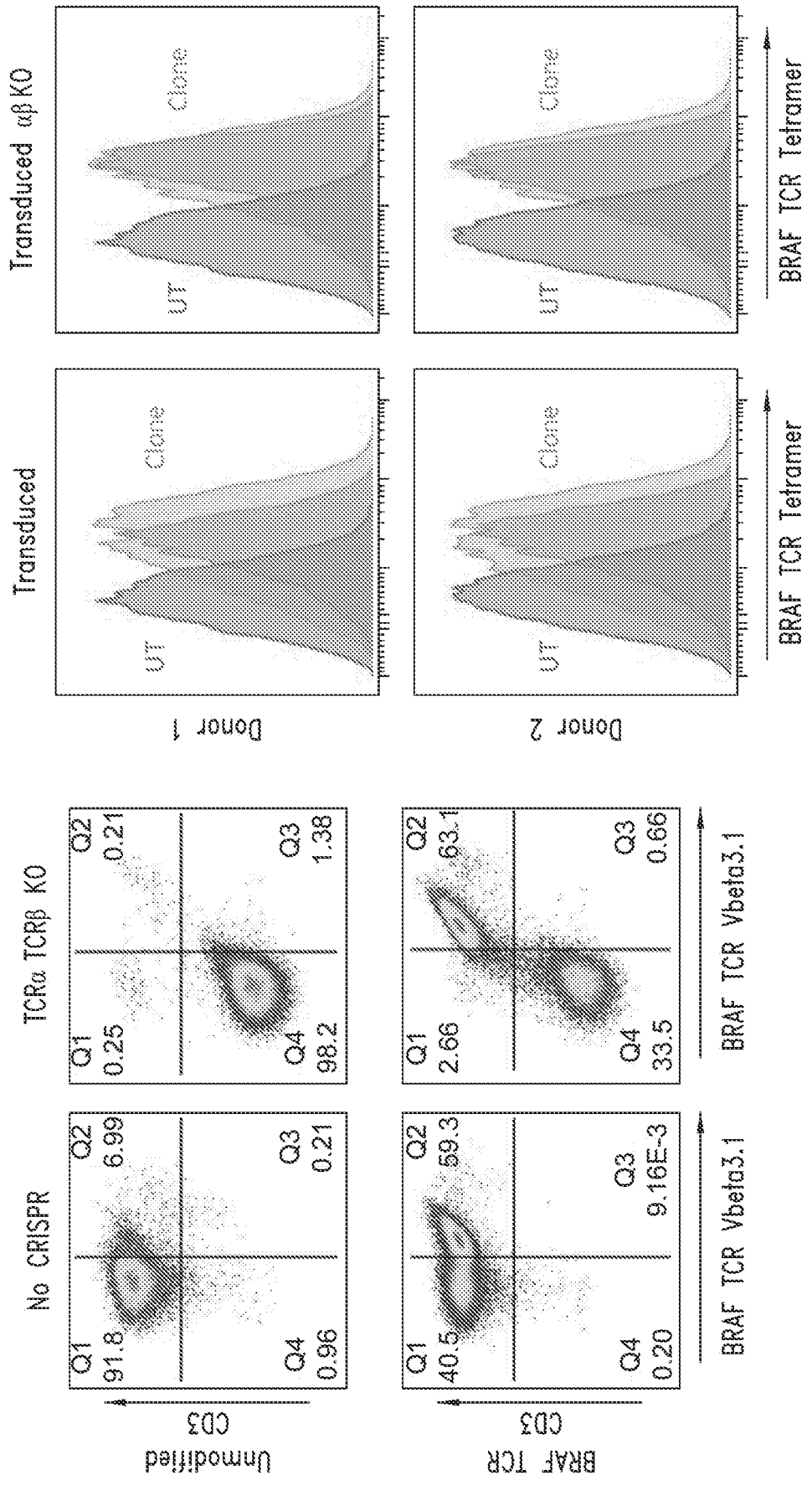

No CRISPR
TCRα TCRβ KO
Unmodified CD3
BRAF TCR CD3
Q1 91.8
Q2 6.99
Q3 0.21
Q4 0.96
Q1 0.25
Q2 0.21
Q3 1.38
Q4 98.2
Q1 40.5
Q2 59.3
Q3 9.16E-3
Q4 0.20
Q1 2.66
Q2 63.1
Q3 0.66
Q4 33.5
BRAF TCR Vbeta3.1
BRAF TCR Vbeta3.1
Transduced
Transduced αβ KO
Donor 1
Donor 2
UT
Clone
BRAF TCR Tetramer
BRAF TCR Tetramer

FIG. 6A

FIG. 6B

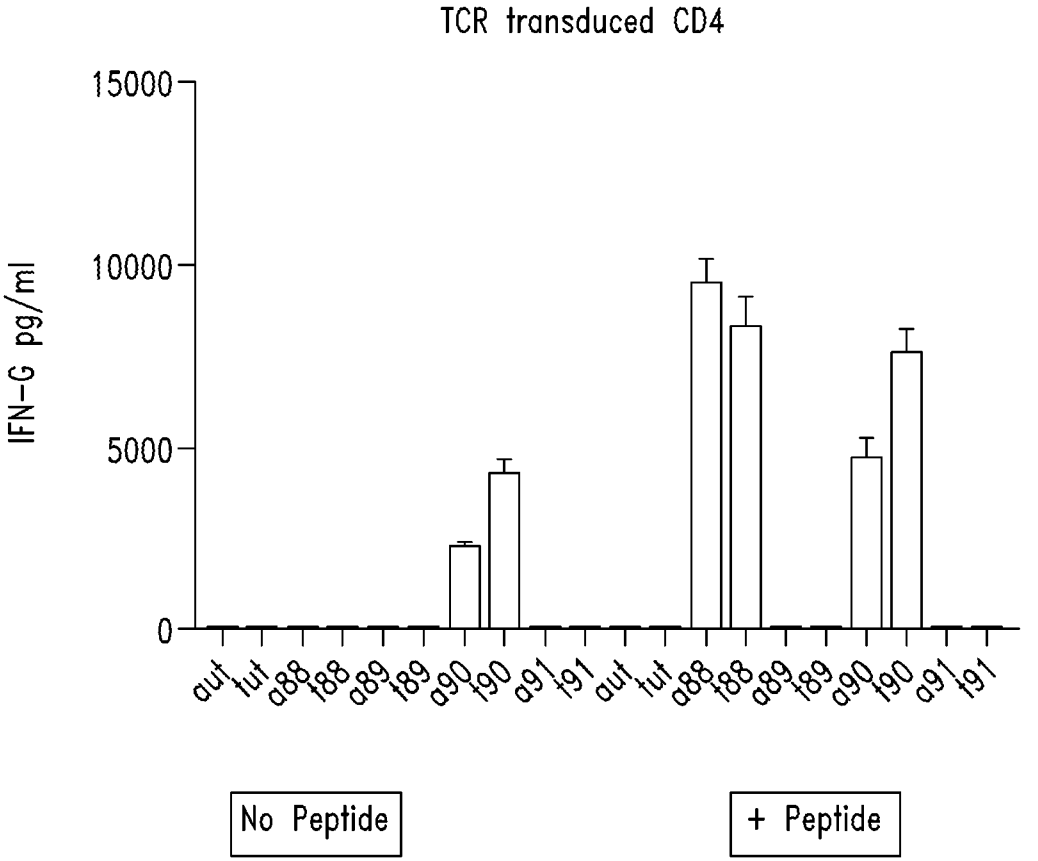

TCR transduced CD4
IFN-G pg/ml
15000
10000
5000
0
aut
tut
a88
t88
a89
t89
a90
t90
a91
t91
aut
tut
a88
t88
a89
t89
a90
t90
a91
t91
No Peptide
+ Peptide

FIG. 5